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23 & 24 December 2022

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MESSAGE FROM CONVENER

We are delighted to welcome our international presenters, delegates to 1st E-Palli International Conference (EIC) at Kuala Lumpur, Malaysia hosted by the E-Palli Publishers Delaware, USA. There are 15 different conferences going to be hosted in two major categories; Science, Technology, Engineering and Mathematics (STEM), and Business, Arts and Social Sciences (BAS).



We have observed a steady rise in the number of quality manuscripts being received in the conference. Total of 210 papers received and 692 researchers registered for the conference. We are very fortunate that all accepted papers will be published in the conference proceedings and the selected paper will be published in double-blinded peer-reviewed international indexed journals with DOI. The papers will be published in the following journals and more journals will be added in the list; American Journal of Agricultural Science, Engineering, and Technology; American Journal of Multidisciplinary Research and Innovation; American Journal of Economics and Business Innovation; American Journal of Environment and Climate; American Journal of Arts and Human Science; American Journal of Education and Technology; American Journal of Life Science and Innovation; American Journal of Interdisciplinary Research and Innovation; American Journal of Geospatial Technology; American Journal of Bioscience and Bioinformatics; American Journal of Innovation in Science and Engineering; American Journal of Environmental Economics; American Journal of Food Science and Technology; American Journal of Youth and Women Empowerment; American Journal of Chemistry and Pharmacy; American Journal of Social Development and Entrepreneurship; American Journal of Applied Statistics and Economics; American Journal of Smart Technology and Solutions; American Journal of IR 4.0 and Beyond; American Journal of Energy and Natural Resources; American Journal of Society and Law; American Journal of Financial Technology and Innovation; American Journal of Aquaculture and Animal Science; American Journal of Medical Science and Innovation; International Journal of Sustainable Rural Development; American Journal of Environmental Experience Design.

I wish that EIC will keep on growing in coming years with more impact on the International research community. It has been our privilege to convene this conference. I thank the support of all authors, reviewers, Conference Secretariat, office bearers, and everyone who make this conference successful. Our sincere thanks, to the conference organising committee and international program committee.



Professor Md Roshidul Hasan
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International Conference of Agricultural Science, Engineering (ICASE)



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KRISHOKBOT: AN INTELLECTUAL AGENT FOR FARMERS

Md. Asfaqur Rashid¹, Md. Alam Hossain^{1*}, Md. Taslim¹, and Md Nasim Adnan¹

ABSTRACT

Crop disease treatment is vital for improving agricultural production and crop yields. The early prevention and treatment of the disease is very helpful to reduce crop damages. However, traditional crop disease treatment is much costly and time consuming to consult with an agriculturist. In Bangladesh, most of the farmers are unaware of pest control and disease treatment. In order to overcome this problem, KrishokBot is deployed. It is a smart agent that makes remote interaction with farmers to provide pest and disease related solution using natural language processing. KrishokBot is a Machine Learning based virtual assistant that can respond to simple questions concerning pests and disease that affect rice production via Bengali language. The datasets have been collected from various Bangladeshi agriculture-based websites to train the KrishokBot, which includes categories, patterns, and responses. A deep neural network has been used to determine which category the user's message belongs to, and then a response is generated. For this, some tools are used such as Natural Language Tool Kit, Keras API, Tensorflow, Android SDK, Android Volley, Heroku, etc. This proposed idea offers great potential for excellent performance with approximately 85 percent accuracy, where user Interface has been developed by android application with both audio and text-based features to provide better interaction. The results prove that the bot is reliable for guiding the treatment of crop disease.

Keywords: KrishokBot, Agriculture, Android, Machine Learning.

INTRODUCTION

Rice is a primary food in Bangladesh. Nearly 160 million people in Bangladesh eat rice as their main food. The people of Bangladesh cultivate 75 percent of their land with paddy (Anon n.d.-e) (Islam et al. 2020). As paddy is the dominant food crop in Bangladesh, it contributes significantly to about 28 percent of the GDP in Bangladesh (Anon n.d.-e). As paddy becomes the staple food for our country it is necessary to produce enough paddy to fulfill the demand of our country. Every year, though, farmers lose an estimated 37 percent of their rice harvest to pests and diseases. Most of the time farmers are unaware of pests and diseases of rice plants and the treatment of these diseases. It is not possible all the time to take agro-experts advice. Agro-experts may be busy or cannot provide so many queries at a specific time or Agro-experts are so low in number in some areas where farmers do not get the proper suggestions of rice crop-related problems. So, there is a keen need for an automating system that will provide necessary queries of farmers all the time. Farmers are unaware of rice crop diseases. Krishokbot for Farmers uses a deep learning algorithm to classify rice plants' disease symptoms and can answer pesticides or prevention steps for the specific disease. As the majority of people of our country speak Bengali, so there is a keen to need to build a system that will take input and deliver output via the Bengali language.

MOTIVATION

Though Bangladesh is considered the fourth largest rice production country (Anon n.d.-e), there are some common major pests and diseases, which hampers significant damages to our rice fields. Few applications provide technological solutions to farmers but most of the time farmers need to specify disease names or need to fill a blank box of choices to forward. In country like Bangladesh where farmers are illiterate or semi-literate it becomes difficult to forward in those applications and often, they don't show enough interest in those applications. Designing user-friendly for low-literate and semi-literate populations like

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our country is a growing area of research. So, it is a good opportunity to make an AI-based Virtual Agent which will provide necessary solutions based on symptoms on the rice crop to the farmers.

LITERATURE REVIEW

FarmChat: A Conversational Agent to Answer Farmer Queries (Jain et al. 2018)

Farmer Questions to be Answered by a Conversational Agent The researchers used the KCC dataset plus information from formative interviews with smallholder farmers and agri-experts to create a knowledge base for potato production. They requested samples of frequent farmer questions from the two agri-experts who took part in the Formative Study, the follow up questions they would ask to further grasp the situation, and the final advise they would give for each of the selected themes. All of these talks were uploaded to the IBM Watson Conversation dialogue flow, and the FarmChat knowledge base was updated with the information. The knowledge base has been transformed into a SQL database with four tables, one for each of the previously mentioned topics.

AgronomoBot: a smart answering Chatbot applied to agricultural sensor networks (Mostaço, Campos, and Cugnasca 2018) A smart response data about field conditions, such as air and soil temperature, air relative humidity, soil moisture, rainfall, wind speed, and other relevant variables, must be available quickly and easily for use by farm management systems, specialists, or the farmer himself in decision-making processes for agricultural purposes.

METHODOLOGY

A. System Overview

To interact with the KrishokBot mobile app, the user clicks the microphone icon and speaks after hearing a ‘beep’ (Jain et al. 2018). Once the app detects long silence, it stops listening. Then the user needs to click a ‘Send’ button to process further. Once the ‘Send’ has been clicked the response will be delivered with audio as well as the text below. The phone receive input through Google’s Speech-to-Text API which takes input as Bengali speech and convert it into Bengali text and then through Google’s Translation API, Bengali text is then converted to English text, along with the current context of the conversation is then passed to the Heroku (provides PaaS) mobile app build with Flask API. With a successful hit on Flask API, the query

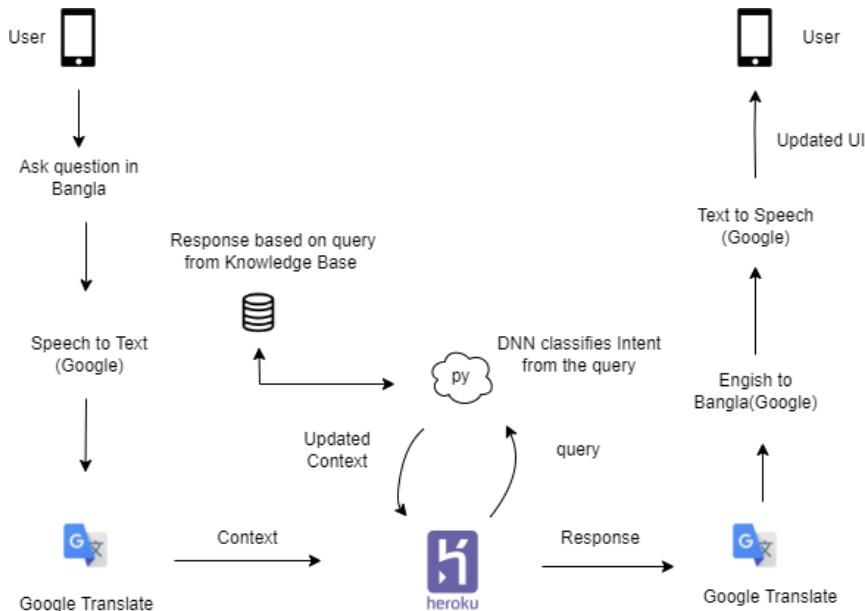


Figure 1: System Architecture of krishokbot

message then goes into a series of the data cleaning process, and after it goes into the deep neural network model. The model returns a response from the knowledge base. Responses are then translated from English to Bengali via Google’s Translation API, Bengali text is then converted to Bengali speech through Google’s Speech-to-Text API.

B. Formative Findings

For implementing KrishokBot dataset was restricted to pests and diseases of rice plants. Datasets are collected from Bangladeshi authorized agriculture-related websites such as Bangladesh Rice Knowledge Bank, Agro Knowledge Bank, Krishi Bantayan, Agricultural Information Service, and some other websites, etc. The datasets are converted into JSON format. The datasets contain the following columns (1) Categories, (2) Patterns, and (3) Responses.

C. Heroku Dashboard Interface

Heroku is a platform as a service (PaaS) that enables developers to build, run, and operate applications entirely in the cloud (Anon n.d.-b). Krishokbot application has been deployed in Heroku. As Heroku provides Platform as a Service (PaaS), Necessary libraries are provided with their corresponding version via the requirements.txt file. Files deployment size was

The screenshot shows the Heroku application dashboard for 'krishokbot'. At the top, there's a search bar with 'Jump to Favorites, Apps, Pipelines, Spaces...'. Below it, the app name 'krishokbot' is shown along with a 'Personal' dropdown and a GitHub link. On the right, there are 'Open app' and 'More' buttons. A sidebar on the left lists 'Overview', 'Resources', 'Deploy', 'Metrics', 'Activity', 'Access', and 'Settings'. The main area is titled 'Application Logs' and shows a scrollable list of log entries. One entry is highlighted in yellow:

```
2022-01-23T13:15:56.272535+00:00 app[web.1]: 2022-01-23 13:15:56.27246/: 1 TENSORFLOW/core/platform/cpu_feature_guard.cc:151] This TensorFlow binary is optimized with oneAPI Deep Neural Network Library (oneDNN) to use the following CPU instructions in performance-critical operations: AVX2 AVX512F FMA
2022-01-23T13:15:56.272535+00:00 app[web.1]: To enable them in other operations, rebuild TensorFlow with the appropriate compiler flags.
2022-01-23T13:15:56.273571+00:00 app[web.1]: 2022-01-23 13:15:56.273535: 1 tensorflow/core/platform/cpu_feature_guard.cc:151] This TensorFlow binary is optimized with oneAPI Deep Neural Network Library (oneDNN) to use the following CPU instructions in performance-critical operations: AVX2 AVX512F FMA
2022-01-23T13:15:56.273572+00:00 app[web.1]: To enable them in other operations, rebuild TensorFlow with the appropriate compiler flags.
2022-01-23T13:15:56.675442+00:00 app[web.1]: 10.1.39.247 - - [23/Jan/2022:13:15:56 +0000] "GET / HTTP/1.1" 200 111 "https://dashboard.herokuapp.com/" "Mozilla/5.0 (Windows NT 10.0; Win64; x64; rv:96.0) Gecko/20100101 Firefox/96.0"
2022-01-23T13:15:56.675442+00:00 heroku[router]: at=info method=GET path="/" host=krishokbot.herokuapp.com request_id=4f7f4c42-8613-45e0-bd68-c0e5fb5e9063 fwd="182.48.82.54" dyno=web.1 connect=3ms service=667ms status=200 bytes=164 protocol=https
2022-01-23T13:15:57.249811+00:00 app[web.1]: 10.1.39.247 - - [23/Jan/2022:13:15:57 +0000] "GET /favicon.ico HTTP/1.1" 404 232 "https://krishokbot.herokuapp.com/" "Mozilla/5.0 (Windows NT 10.0; Win64; x64; rv:96.0) Gecko/20100101 Firefox/96.0"
2022-01-23T13:15:57.249417+00:00 heroku[router]: at=info method=GET path="/favicon.ico" host=krishokbot.herokuapp.com request_id=47e65fc8-43bf-4817-ad42-40dc66bd5e3c fwd="182.48.82.54" dyno=web.1 connect=4ms service=1ms status=404 bytes=393 protocol=https
```

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Figure 2: Heroku application log files

350MB which crossed the soft limit specified in Heroku that will affect booting time.

D. Conversational Intelligence: Intent, Entity

Conversational intelligence systems recognize intents and entities from the user's inquiry to understand the user intention and the purpose of the user's inquiry. The intent of a message describes what the message is aiming for. Intent is required to understand the user's purpose. Entity of a message add value to the intention and collects numbers, time, a pattern like an email, phone number, national id, etc. like information from the user's texts. For example, "I want to book a ticket of Saturday 9:00 PM" implies "book-ticket" as intent and "Day: Saturday" and "Time: 9:00 PM" as entities (Anon n.d.-a). A conversational system can be an open domain or closed domain Open-domain refers to when the conversation system can answer anything or is ready to serve not fixed with any specific topic. Whereas, a closed domain conversation system cannot provide a response or service if the message is out of the main context. Closed domain systems are fixed with a topic and can only provide topic-related responses. KrishokBot is restricted to a closed domain system as it can only respond to pests and diseases of rice plants. For example:

Human: Hello

Bot: Hi there, how can I help? Please describe the specific symptoms or disease name of the rice plant Human: tell me how to control brown spots on the leaves? Bot: Keep the seedbed or soil wet with water. Use more organic fertilizers.

Human: thanks Bot: Anytime

Bot detects the user's purpose as 'greet' from the first message sent by the user. with the entity being the 'Hello' word. The bot then tells the user to ask specific symptoms or disease names for the query. From the third message, the bot recognizes that the intent is 'brown spot' which is one kind of rice disease name, and then recommends corresponding steps to apply.

RESULTS AND DISCUSSION

Farmers can ask for direct symptoms of diseases, names of diseases or can greet to see what KrishokBot can offer to the farmers. Initially, KrishokBot asks about the specific disease symptoms of a plant to make the conversation domainspecific. After training the model with 600 epochs, it was able to recognize the farmer queries related to the pests and diseases in rice plants. After providing training data of 800 plus intent data, it was able to get an accuracy of around 85 percent, and by raising the dataset', it is possible to achieve accuracy of 90 percent or higher.

User Interface

Most Bangladeshi Farmers are illiterate or semi-literate. Providing only text-based interaction may impact the majority of farmers in Bangladesh with low literacy levels. Also, to provide a faster and better response text- based interaction may be a good opinion for the semi-literate farmers. A solution has been proposed for both of them. A farmer with a low literacy level

has an option to interact with the system with Audio based. A microphone icon is given to take Bengali speech as a farmer query and response is delivered with Bengali Audio speech as well as Bengali text below the app. User interface with the Bengali language will make it easy to use and Farmers will be able to interact with it conveniently.

Audio Input

An audio-based input system is convenient for low-literate users. KrishokBot now supports Bengali, which is the most generally spoken the Bengali language and responds to questions concerning rice cultivation as a use case for the study. A microphone icon is given for the audio input. By clicking the microphone icon, a Google Speech recognizer will appear. Once get a long silence, it will automatically return “didn’t catch it” if there is no message was given. A given speech will be shown in real-time below to check if the message is correctly spoken. After recording the speech, the user must click the ‘send’ button to continue. Finally, the results will be displayed in the answer area below, both as text and as a speaker.

Text Input

Text-based interaction is necessary for good interaction with the mobile app. For semi-literate users, text can offer faster and unambiguous mode of interaction (Jain et al. 2018). There is a Chat box to write queries in the Bengali language. After writing a necessary query, the user needs to click the ‘Send’ button to get the response as Audio as well as Text. Sometimes noise or barrier it becomes hard for Google Speech-to-Text API to guess a perfect sentence for the input. So, for accurate user queries and better results, a text-based input system is given for the semi-literate farmers. As farmers can provide the Bengali language as text, it makes a convenient interaction with the Krishokbot app.

LIMITATIONS

A small dataset makes bot hard to guess accurate intent. The system is Retrieval in nature, providing responses based on predefined responses. It does not generate sentences. Miss classifies intents when symptoms are quite similar for different pests or diseases. There is no dialogue management system for back-and-forth conversations to identify a particular intent. As most of the core technologies provide services based on cloud services, it is needed a good internet connection to the rural area where most farmers inhabit.

CONCLUSION

Farmers are the backbone of our agriculture sector. As most of the people of our country are directly or indirectly related to farming, a large number of people's earnings and fate

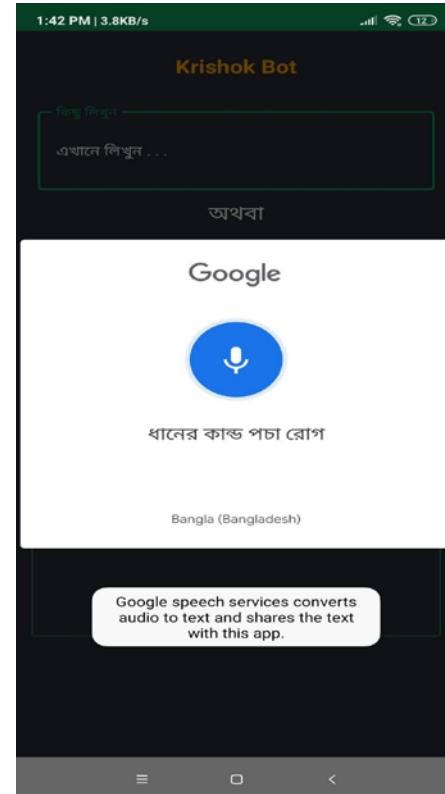


Figure 4: Audio input

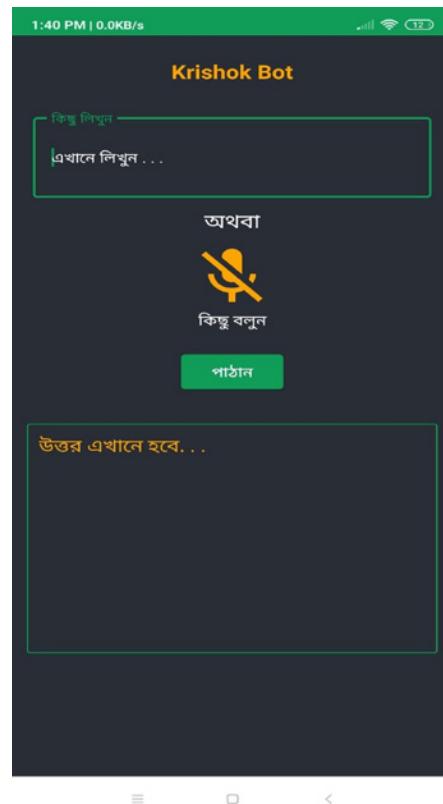


Figure 3: Android interface

makes a convenient interaction with the

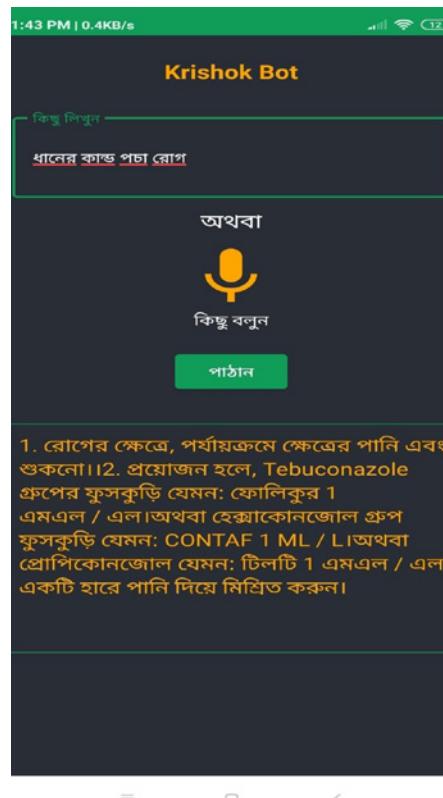


Figure 5: Text Input

are dependent on agriculture sectors. Rice is the most fundamental meal we eat every day of our life and increment of rice production is necessary according to the population increment. Pests and diseases in rice crops are a major problem of lower-yielding. ‘Krishokbot: An Intellectual Agent for Farmers’ will provide a technological solution to the farmers in answering their farmer-related queries. The conversational intelligence of the virtual assistant was trained with the pest and disease datasets collected from Bangladeshi Agriculture related websites. It can provide basic queries related to pests and diseases in rice plants. In future, ‘Krishokbot’ can be improved with the aid of latest Machine Learning algorithms such as Forest PA (Adnan & Islam, 2017) and BDF (Adnan et al., 2021).

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DECISION SUPPORT SYSTEM FOR AGRICULTURE: CROP DISEASE RECOGNITION AND CLASSIFICATION THROUGH AN OPTIMIZE CONVOLUTION NEURAL NETWORK (CNN)

Md. Taslim¹, Md Shafiuzaaman¹, Mostafijur Rahman Akhond^{1*}, and Md. Alam Hossain¹

ABSTRACT

Crop leaf diseases cause great damage to agriculture, causing significant crop losses in Bangladesh every year. Crop economic loss can be significantly reduced by accurately recognizing and classifying crop leaf diseases. This study developed an optimized Convolution Neural Network (CNN) model to recognize and classify crop leaf diseases. The proposed dataset of this study was collected from the field with the help of Bangladesh Agriculture University (BAU) and Bangladesh Agricultural Research Institute (BARI) experts. This dataset includes 5 types of crops (bean, cauliflower, paddy, potato, and tomato), 21 types of diseases, and 14624 sample images. The Adam optimizer is used as an optimizer in this study. Our developed CNN model can recognize and classify crop species and crop leaf diseases with the best accuracy of 99.67% and 96.55%, respectively. Furthermore, the proposed model is more accurate than the previous study.

Keywords: Crop Leap Disease, CNN, Crop Species, Disease Classification, Species Classification.

INTRODUCTION

Despite being the eighth most populous country in the world, Bangladesh has been plagued by a scarcity of arable land resources. According to the Ministry of Agriculture survey, less than 5% of Bangladesh's total land area is cultivated. Despite this, Bangladesh has the fastest growth rate in fruit production among the world's fruit-producing countries. It is the tenth largest producer of tropical crops, according to the United Nations Food and Agriculture Organization (FAO). According to FAO estimates (Osborne BG, 2006), the country's population has increased by 11.5 percent on average over the last 18 years. Despite this success in crop cultivation, huge amounts of crops are wasted due to a lack of timely disease identification in developing countries like Bangladesh, which is harmful to the country's economy. Natural disasters that affect a country's crop production have a negative impact on agricultural production and development. So, how to develop agriculture sustainably, particularly in a complex environment, is critical for Bangladesh. On the other hand, agricultural production is improving as science and technology advance. However, crop yield has not improved significantly due to a variety of natural and non-natural factors. Crop leaf diseases and insect problems account for the majority of the various causes. According to statistics, in Bangladesh, 20% of crops are lost due to pests and disease before they reach stable (April in Bangladesh, 15.10.22.). This problem has grown in recent years and is seriously jeopardizing the development of the plantation industry. Crop disease diagnosis and prevention have become increasingly important. Today, (Yong et al., 2020) agricultural workers often use books and networks, as well as local experts, to protect against and manage crop diseases. However, for a variety of reasons, misjudgments and other issues often arise, seriously affecting agricultural production. Crop disease research is currently divided into two areas. The first one is the traditional physical method, which detects various diseases primarily through spectral detection. Different diseases and pests cause different types of leaf damage, resulting in different spectral absorption and reflectance of diseased and healthy crops. Another is image recognition using machine learning techniques. That is, disease images are extracted using computer technology, and diseased and healthy trees are identified based on various characteristics. Some of the researchers conducted several machine learning techniques, such as (Alam et al., 2022 and Militante et al., 2019) proposed an optimized model for detecting various crop diseases. This study used 35,000 disease images, and their model achieved 96.5% accuracy, as well

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as 100% accuracy in detecting crop species. This paper (Hu WJ et al., 2020) addresses the issue of crop fine-grained disease. They built an IoT system using IoT technology and deep learning to identify this disease. They proposed a multidimensional feature compensation residual neural network (MDFC-ResNet) as a model, and their model identifies coarse-grained disease, species, and fine-grained disease. Their proposed model performed well in recognizing and detecting crop fine-grained disease. The authors of this study (Zhou et al., 2021) proposed a restructured residual dense network to detect tomato leaf disease. This hybrid deep learning model combines dense and deep residual networks to improve training performance and solve the vanishing gradient problem. Initially, residual deep networks are used to increase image resolution and then use the combined model to classify the disease. Finally, in the testing phase, their model achieves a top-1 average accuracy of 95%. In this study, we developed an optimized deep-learning solution for detecting crop species and diseases. Our model efficiently detects crop species and diseases within less training time. We build an efficient model to reduce model complexity and overfitting issues. In addition, we found the misclassification reason with misclassified images from our model. In this research, we conducted five species and twenty-one diseases with 14,624 sample images.

The rest of the paper is organized as follows: Section 2 Related works. Section 3 Structure of our model. Section 4 Methodology, Section 5 Result and discussion, and Section 6 Conclusions.

LITERATURE REVIEW

Crop leaf disease is a concerning issue all over the world. Researchers all over the world are working to solve this problem in order to reduce crop losses caused by leaf diseases. In this section, we review some related work on leaf disease recognition and classification.

This research (Roy et al., 2021) proposed a novel model based on state-of-the-art computer vision techniques to classify apple plant diseases. Their model addresses the existing model classification problem of apple disease. In addition, their model increases both the detection speed and the accuracy of classifying the disease. As a result, the model finds the F1-score and mean average precision (mAP) at 91.2% and 95.9%, respectively, and at a detection rate of 56.9 FPS. Moreover, their model increases the precision and the F1-score by 9.05% and 7.6%, respectively.

In addition, the authors (Cap et al., 2018) of this paper proposed a leaf localization method using on-site wide-angle images and a deep learning approach to detect plant diseases in their early stages. Their proposed model received the highest F1 score of 78% at 2 fps.

Additionally, this (Türkoğlu et al., 2019) study conducts numerous studies to assess the effectiveness of the model utilizing diverse methodologies. They identified the crop disease using nine widely used deep learning architectures. They also used transfer learning and deep feature extraction techniques, followed by classification using extreme learning machine (ELM), K-nearest neighbor (KNN), and support vector machine (SVM). For model evaluation accuracy, F1-score, specificity, and sensitivity are considered for F1-score. Finally, SVM/ELM performs better than transfer learning.

In this paper (Militante et al., 2019), they propose an optimized model for detecting various crop diseases. This study used 35,000 disease images, and their model achieved 96.5% accuracy, as well as 100% accuracy in detecting crop species.

Furthermore, this (Hu WJ et al., 2020) paper addresses the issue of crop fine-grained disease. They built an IoT system using IoT technology and deep learning to identify this disease. They proposed a multidimensional feature compensation residual neural network (MDFC-ResNet) as a model, and their model identifies coarse-grained disease, species, and fine-grained disease. Their proposed model performed well in recognizing and detecting crop fine-grained disease.

The authors of this study (Zhou et al., 2021) proposed a restructured residual dense network to detect tomato leaf disease. This hybrid deep learning model combines dense and deep residual networks to improve training performance and solve the vanishing gradient problem. Initially, residual deep networks are used to increase image resolution and then use the combined model to classify the disease. Finally, in the testing phase, their model achieves a top-1 average accuracy of 95%.

Also, the author of this (Ahmed et al., 2021) paper proposed a dual-phase CNN strategy in which small crops, including diversity, could be used to effectively analyze disease data. First, a faster RCNN method is used to remove a significant portion of the image (rice husk) and generate a secondary dataset of rice husks with no distinguishing background. CNN Architecture divides diseases into two categories: developing infectious diseases and general models. The proposed method, when combined with CNN's dual phase method, achieves an accuracy of 88.92% when applied directly to small grain datasets, which is five times better with mutual efficiency.

METHODOLOGY

In this research, our main goal is to develop an efficient CNN model to recognize and classify crop diseases and crop species using crop leaf images. For that crop, leaf images are used as the input of our model. Then the convolution operation extracts the feature and it is classified by the fully connected layer. Our entire experiment is depicted in Fig.1.

Data Description

The proposed dataset is collected from the field with the help of Bangladesh Agriculture University (BAU) and Bangladesh Agricultural Research Institute (BARI), Gazipur, Bangladesh. There are 14624 sample images of five types of crops with 21 disease classes. Five crops are Bean, Cauliflower, Paddy, Potato, and Tomato. In addition, twenty-one disease classes are Bean Angular Leaf Spot, Bean Rust, Cauliflower Alternaria Leaf Spot, Cauliflower Cabbage Aphid Colony, Cauliflower Ring Spot, Paddy Bacterial Leaf Blight, Paddy Brown Spot, Paddy Leaf Smut, Potato Early Blight, Potato Late Blight, Potato Healthy, Tomato Bacterial Spot, Tomato Early Blight, Tomato Late Blight, Tomato Leaf Mold, Tomato Septoria Leaf Spot, Tomato Spider Mites, Two-Spotted Spider Mite, Tomato Target Spot, Tomato Yellow Leaf Curl Virus, Tomato Mosaic Virus, Tomato Healthy. Fig. 2 and Fig. 3 show some sample images for both crop species and crop diseases.

Data Preprocessing

Data preprocessing is the crucial stage of any deep learning task. Preprocessing was done on the images before putting them into the model to make it easier to extract features. The pixel value of the magnified image is a single integer in the range of 0-255 that represents the brightness of the pixel. A pixel with a value of 0 is considered black, and a pixel with a value of 255 is considered white. All input images were resized into a 256x256@3 dimension. Scaling every image to the same range [0,1]. Augmentation of images is done by the random flip with horizontal and vertical flip and random rotation with 0.2 factor. Then the preprocessed image is used as input to fit the proposed CNN model.

Classification By CNN

The color of crop leaf disease contributes significantly to need-based nitrogen management (Torikul et al., 2020). The convolutional layers in the proposed model are responsible for extracting optimal features. We demonstrated that CNN can train for classifications based

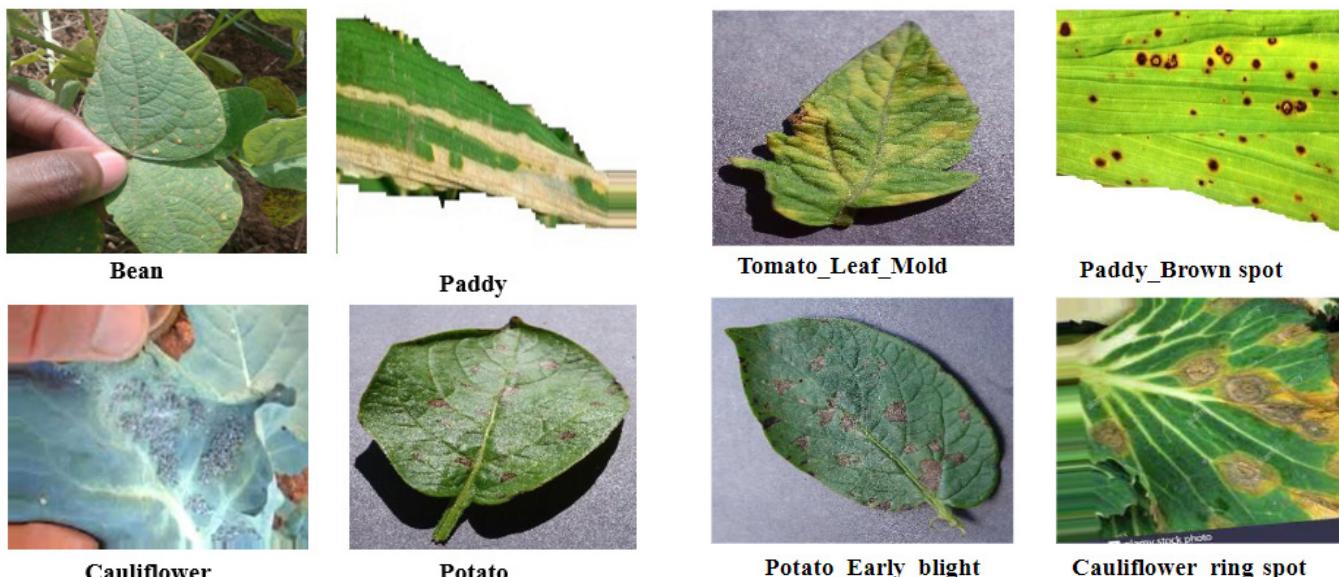


Figure 2: Crop Species

Figure 3: Crop Disease

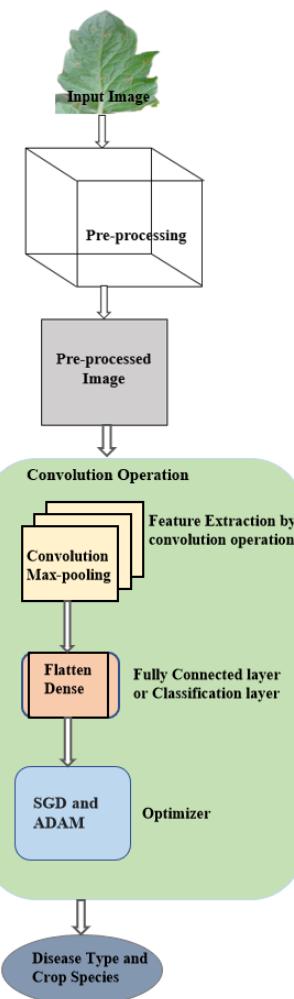


Figure 1: Proposed CNN Model

on shape information as well as classifications based on color. We developed an optimized CNN model for crop leaf disease and species classification.

Six convolutional layers, six pooling layers, two fully connected layers, and one SoftMax layer make up our CNN architecture. Convolution is the first layer in our CNN architecture. after the convolution process pooling processes are performed. In Fig. 4, our CNN model's architecture is shown.

In the CNN, the convolution operation extracts the feature from the image. This process is done based on the given eq. (1). If P_i is the input image, k is the kernel of convolution then the output of the convolution operation P_0 can be written as,

$$P_0[x, y] = \sum_{n=-\infty}^{\infty} \sum_{m=-\infty}^{\infty} P_i[n, m] \cdot k[x, y] \quad (1)$$

Where $P[x, y]$ is the pixel value of the P coordinate. In neural networks (NN), the activation function is used to provide the non-linearity of the hidden layers. The Activation function activates the network between hidden layer nodes. Different activation functions range differently. So, the choice of the specific activation function has a vital task for the NN. In this research, Rectified Linear Unit (ReLU) activation functions are used. The ReLU activation function range is 0 to x , where x means input value. ReLU activation functions are used in all hidden layers, and SoftMax activation functions are used in the output layer. ReLU activation functions are used to solve the gradient problem. Then the normalization process is done by the eq. (2).

$$P_{a,b}^i = \frac{h_{a,b}^i}{(1 + \frac{\infty}{n} \sum_{j=i-\frac{n}{2}}^{i+\frac{n}{2}} (h_{a,b}^j)^2)^\beta} \quad (2)$$

Here, the output of the normalization process is $P_{(a,b)}^i$ and $h_{(a,b)}^i$ is the output of the activation function at (a, b) coordinate. After the first convolution layer, pooling operations are performed. There are two types of pooling, namely max pooling and mean pooling. Max pooling performs based on the sharp edge's techniques. In this research, max pooling is used with size 2×2 and stride 1. After the first pooling process, the second convolution layer is performed, and the second pooling, and so on. After six convolutions and pooling, layer features are extracted, and then these features are provided as the fully connected layer for classification.

In addition, our developed CNN architecture consists of six convolution and pooling layers. The first layer consists of 32 kernels with a 3×3 kernel size, and the second to sixth layer consists of 64 kernels with a 3×3 kernel size. Additionally, the pooling size of 2×2 is used in those layers. Finally, the flatten and output layer consists of 16448–21 neurons for disease classification and 16448–5 neurons for crop species classification.

Our CNN model is trained by two optimizers, namely stochastic gradient descent (SGD) and adaptive moment estimation (Adam). The batch size is 64, and three channels are used with $256 \times 256 @ 3$ image resolution. The SGD is used to find the maximum or minimum value of some function. It will work with the gradient dissent for all functions. In addition, SGD is used for minimizing loss or error. The weight loss function updates are done by the eq. (3). While Adam optimizers are used for solving non-convex issues.

$$W_i = W_{i-1} - \alpha * \Delta l(p, W_{i-1}) \quad (3)$$

Here, W_i is the current weight, $W_{(i-1)}$ is the previous weight, α is the learning rate and $\Delta l(p, W_{(i-1)})$ is the gradient of loss function of $l(p, W_{(i-1)})$.

In this experiment, we used 14624 sample images with 21 class for disease and 14624 sample images with 5 class for crop species. For training and testing split the dataset into 80% training, 10% for testing and 10% for validation and before train the model to reduce the bias set the shuffle is true.

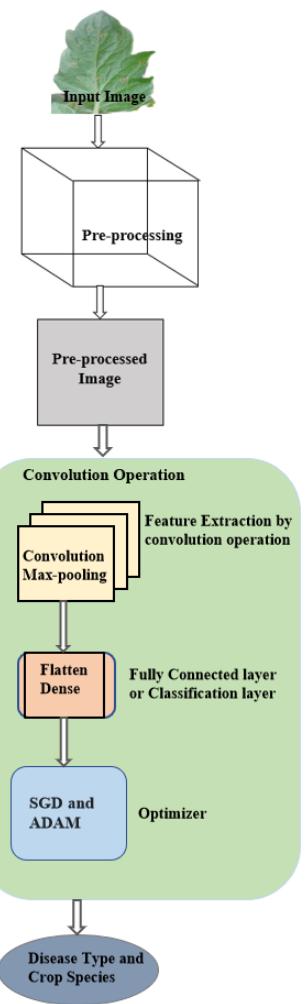


Figure 4: Proposed CNN Model Architecture.

Experimental Analysis

In this section, we describe our experimental process and model evaluation criteria. For model performance evaluation, consider accuracy and loss of both train and validation accuracy. In this experiment, 100 epochs are used for both crop species and disease classification. After completing the training process get a 96.66% accuracy for disease classification and a 99.01% accuracy for crop species classification in the validation phase. In addition, in the testing phase, we get a 96.42% accuracy for disease classification and 99.67% for crop species classification. Furthermore, training and validation loss are low, which means the model is less overfit. All experimental outcomes are listed in table 1.

Table 1: CNN model performance comparison for Adam optimizer.

Parameter	Crop Disease	Crop Species
Training Accuracy	96.97%	99.31%
Validation Accuracy	96.66%	99.01%
Training Loss	9%	1.88%
Validation Loss	9.95%	2.17%
Testing accuracy	96.55%	99.67%

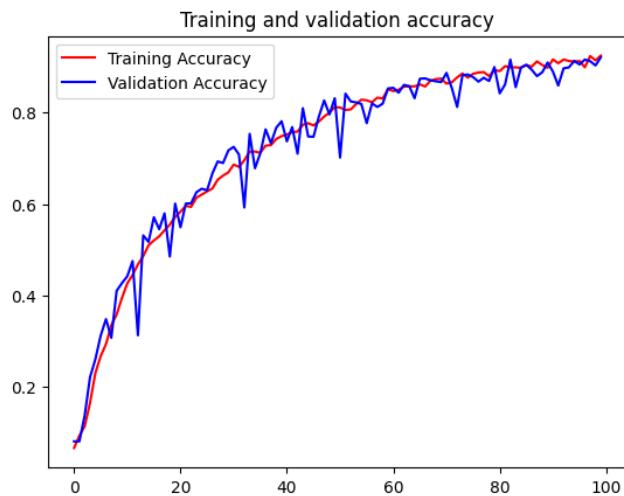


Figure 5: Crop Disease Accuracy

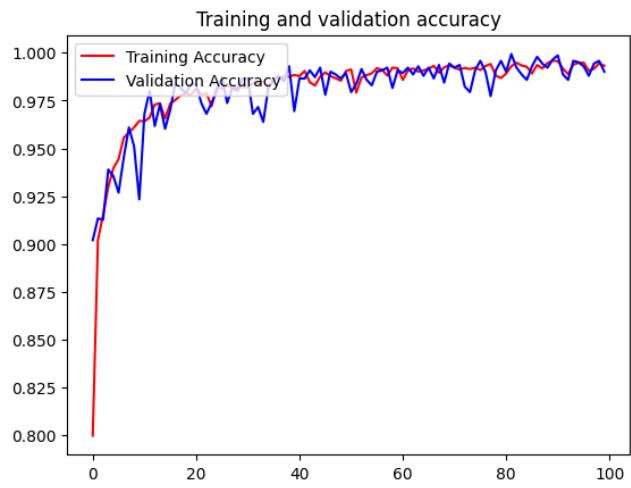


Figure 6: Crop Species Accuracy

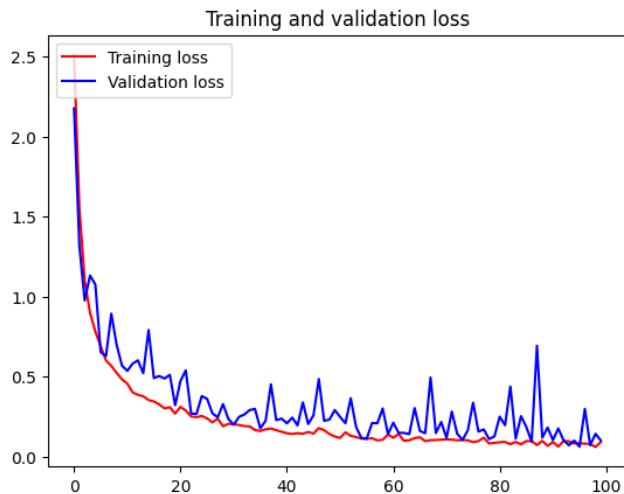


Figure 7: Crop Disease Loss

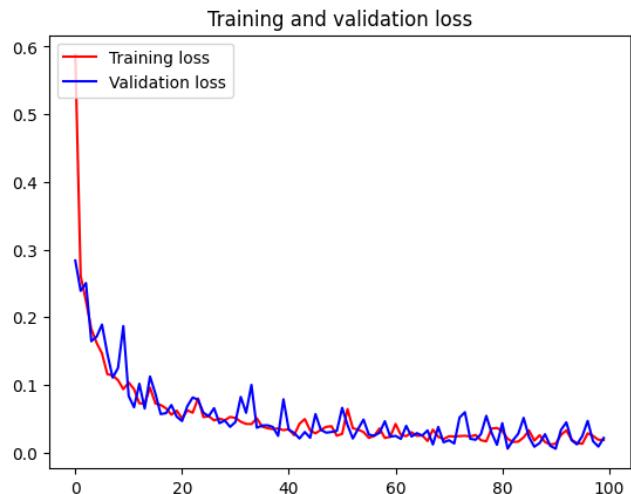


Figure 8: Crop Species Loss

In figs. 5 and 6, the training and validation accuracy is shown. Here, it is clear that in both figures, increasing the number of epochs means the proposed model learns better and almost the same as the validation set, which means the proposed model is less overfit.

On the other hand, fig.7 and fig.8 represent the model loss on the training and validation sets. Also, here we see that the proposed model produces less error and loss when going to converge.

RESULT ANALYSIS

In this section, we discuss mechanism of our develop CNN model. We see the experimental section that CNN model achieved the 96.42% and 99.67% accuracy disease and species classification respectively. Due to provide good result of our proposed CNN model that our proposed model kernels and filter work coherent way. As a result, misclassification rate is reduced. The proposed CNN model learns in better way than raw CNN model and recognized the crop disease and crop species in better way.

Misclassification

In this section, we present misclassification issues with misclassified images. The proposed CNN model misclassifies those images which are affected by the sunlight. In sunlight-affected images, pixel values change, so the proposed CNN model cannot recognize the correct diseases and species in those images. In addition, in the proposed datasets, some images have low pixel values, and those images increase the chance of misclassification. Fig.9 represents some misclassified images with their actual and predicted levels.

Here, actual level of first image is Tomato_Target_Spot but predicted level is Potato_Late_blight, actual level of second image is Tomato_Spider_mites_Two_spotted_spider_mite but predicted level is Tomato_Leaf_Mold, third image actual level is Tomato_

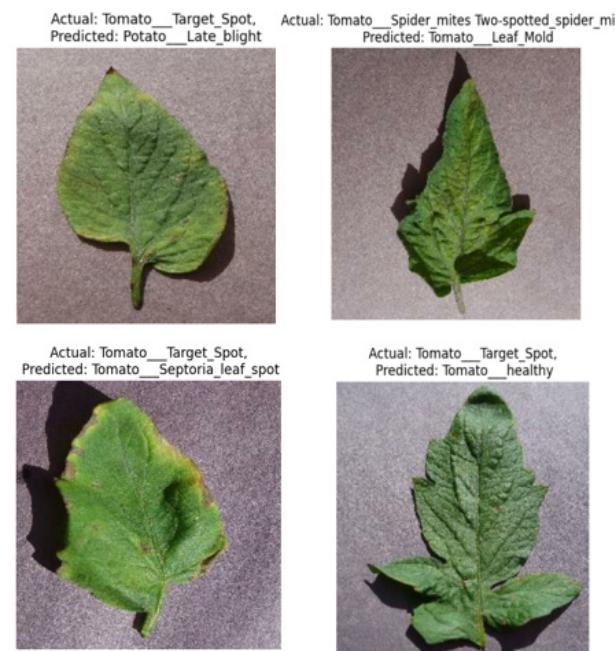


Figure 9: Misclassified Image

Table 2: Represents the comparison with the previous study.

Ref.	Previous Study	Proposed Study (testing accuracy)
(Adedamola et al., 2019)	93.82%	
(Adesh et al., 2021)	81.4%	
(Sambasivam et al., 2021)	93%	
(JANARTHAN et al., 2020)	95.04%	96.55%
(QINGMAO et al., 2020)	92.60%	
(Siddharth et al., 2018)	86.21%	

Target_Spot but predicted level is Tomato_Septorial_leaf_spot and also fourth image actual level is Tomato_Target_Spot but predicted level is Tomato_healthy.

Comparison Of Performance Proposed CNN Model with Previous Study

The proposed model efficiently recognizes the crop species and crop disease with good accuracy. The proposed model provides 96.42% accuracy for disease classification and 99.67% accuracy for species classification on the test dataset. whereas other previous studies found less accuracy than our proposed model. Table 2 represents a comparison with the previous study.

CONCLUSION

In this research, we mainly developed a CNN model to accurately recognize the crop species, crop disease, and classification. The whole work divides into three-part (1) pre-processing (2) feature extracting (3) model training and disease recognition and classification. This process is done by the Convolution Neural Network. To reduce the sunlight effect of image data acquisition techniques are used. This work performs on five crops Bean, Cauliflower, Paddy, Potato, and Tomato. In addition, we have

implemented an optimized CNN model to classify the crop species and crop disease with the best accuracy of 99.67% and 96.55% respectively.

This study helps the farmers to detect the automatically of disease class and crop species using leaf images. This implementation of the smart device can monitor the automatically of crop fields that helps the village farmer. In the future, researchers improved the data collection process to remove the sunlight effect on the image. Our study will bring unprecedented success to the farmers as well as the economy of Bangladesh.

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ISOLATION AND IDENTIFICATION OF PUTATIVE PROBIOTIC BACTERIA FROM FISH GUT AND EVALUATION OF THEIR ANTIBIOTIC PROPERTIES

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ABSTRACT

Probiotic bacteria play a vital role in the host animal's growth acceleration, enhancing digestion capabilities, and defense against diseases. Though the study of gut probiotic bacteria is a trending topic in Biology, research on fish gut probiotic bacteria has not flourished. In this regard, the present study was intended to isolate and identify putative probiotic candidate isolates from the fish gut and evaluate the isolates' inhibitory effects against various fish pathogens. The bacterial isolates were isolated from the gut of four Indian Major Carp fish species namely Rohu (*Labeo rohita*), Catla (*Catla catla*), Mrigal (*Cirrhinus cirrhosus*), and Bata (*Labeo bata*) collected from natural water bodies. Among the collected isolates, thirty isolates were randomly selected from MRS Agar (De Man, Rogosa, and Sharpe agar) and then cultured in NA (Nutrient Agar) culture plates for further studies. Then the preliminary phenotypic characterization of the isolates was done. In vitro antimicrobial activity of these isolates against ten fish pathogenic strains belonging to four genera viz., *Aeromonas* spp., *Pseudomonas fluorescens*, *Enterococcus faecalis*, and *Stenotrophomonas maltophilia* were done following the agar well diffusion assay. Nineteen out of thirty isolates showed antimicrobial activity against the fish pathogens. Among these, isolates C102L, R102L, M102L, M101L, and M201L showed remarkable antibacterial activity against most of the fish pathogens whereas isolate B102L showed the highest antibiotic activity. The 16S rRNA gene sequence homology of three selected bacterial isolates was done for molecular identification, on which the isolates M201L, R102L, and C102L were identified as *Lactococcus lactis*, *Lactococcus garvieae*, and *Kurthia zopfii*, respectively. Identification of these probiotic bacteria will contribute to understand the role of probiotics in host physiology and feed industries. Hence, further study on the gut probiotic bacteria of other common fishes of Bangladesh should be done.

Keywords: Fish Gut Probiotic, Antimicrobial activity, *Lactococcus lactis*, *Lactococcus garvieae*, *Kurthia zopfii*.

INTRODUCTION:

The favorable geographic position of Bangladesh blessed with various types of waterbodies comes with a large number of aquatic species and provides plenty of resources to support the fisheries sector of the country. Fish supply about 60% of Bangladeshi people's daily animal protein intake (DoF, 2017). The application of probiotics is one of the various biotechnological approaches that have appeared behind the scene in case of increasing fish production. Probiotics are defined as microorganisms that are believed to provide health benefits when consumed (Hill et al., 2014). As implicitly noted in 2001 by an expert panel commissioned by the Food and Agriculture Organization of the United Nations and the World Health Organization, probiotics are nonpathogenic organisms that have no necessary phylogenetic relation to one another and are therefore best defined functionally rather than structurally. This led the panel to characterize probiotics very broadly as 'live microorganisms which, when administered in adequate amounts, confer a health benefit on the host' (The World Health Organization, 2001). Probiotics ostensibly fulfill this definition through a variety of somewhat disparate, somewhat overlapping mechanisms. These include the regulation of intestinal microbial homeostasis, the interference with the ability of pathogens to colonize and infect the mucosa, the modulation of local and systemic immune responses, the stabilization or maintenance of the gastrointestinal

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barrier function, the inhibition of pro-carcinogenic enzymatic activity and the induction of enzymatic activity that favors good nutrition (Boirivant & Strober, 2007).

On the other hand, in the last several years, evidence has been obtained that the introduction of probiotics into the intestine can lead to the inhibition of the growth of conventional organisms or potential pathogens through a variety of mechanisms. These include their capacity to decrease luminal pH, secrete bactericidal proteins (bacteriocins), and inhibit bacterial adhesion to epithelial cells (Boirivant & Strober, 2007).

Like other countries, in Bangladesh, the application of antibiotics is generally used as the most familiar technique for dealing with the incidence of bacterial diseases. But the indiscriminate use of these antibiotics for maintaining bacterial infection has been accountable for the development of antibiotic-resistant bacteria that has a significant effect on the reduction of the efficiency of a treatment option and may be liable for long term unpleasant impacts in the aquaculture environment (Defoirdt et al., 2007). With the growing claim for eco-friendly aquaculture, alternate sources of harmless therapeutics rather than chemicals has become demand of time. Due to the threat related with the application of antibiotics, use of probiotics are increasing and are becoming popular amongst the farmers (Nahid Akter Hajee Mohammad et al., 2016). Preceding studies have suggested varied bacterial species from the GI tract of Indian major carps, exotic carps and other cultivable teleosts, and apparent beneficial functions of the gut microbiota pertaining to nutrition of the host fish have been emphasized (Ray et al., 2012).

Reports on the antibacterial efficiency of the extracellular enzyme producing gut bacteria isolated from the Indian major carps are scarce (Ghosh et al., 2007). Apart from the functional role that a putative probiotic bacterium might play, viability within the host gut is often believed to be one of the main selection criteria for prospective probiotics (Nayak, 2010).

The probiotic market of Bangladesh fully depends on imports from neighboring countries like India, Thailand, China, and so on. Hence, available probiotic formulations are very costly. In most cases, the performance of the presently marketed commercial probiotics is not up to the mark. The reason may be due to the change in the climatic and geographical conditions of the manufactured countries. Moreover, the *in vivo* performance is different from the *in vitro* assay due to culture conditions and fluctuating water parameters. In this regard, the use of isolated probiotic bacteria from indigenous sources may ease this problem. Besides, Bangladesh has a great mass of inland and marine water which are abundant with fisheries resources so, there is a vast resource of indigenous probiotics waiting to be discovered.

The freshwater aquaculture of Bangladesh is one of the world's best which is rendered by high production of carps species contributing 35.1% in total annual production (freshwater and marine water) followed by pangasius hypophthalmus (8.71%), and Oreochromis nilotica (8.48%) (DoF, 2021). And amongst the carp species, Indian Major Carp (IMC) is the most popular and most cultured species. As a result, isolation and application of probiotics from local sources have a greater chance of better performance than imported probiotics with a guaranteed reasonable price. Moreover, it is well established that probiotics have inhibitory activity against fish pathogens. So, the indigenous probiotics isolated from local sources should perform better against the pathogens which cause different diseases among the locally cultured species.

The identification of indigenous putative probiotic bacteria inhibiting fish different pathogens will flourish local probiotic industries and contribute as an eco-friendly way to cure pathogenic diseases, ultimately increasing fish production of Bangladesh.

METHOD

Collection of Samples

Wholesome and fresh specimen fishes of Rohu (*Labeo rohita*), Catla (*Gibelion catla*), Mrigal (*Cirrhinus circhosus*), Bata (*Labeo bata*) were collected from two beels in Modhupur, Tangail and Gazipur district. The samples were packed in separate polybag in aseptic condition then transported in an icebox to the biotechnology laboratory at Bangabandhu Sheikh Mujibur Rahman Agricultural University.

Sterilization of glass and plastic wares

Glass materials like Petri dishes, test tubes, conical flasks, stock bottles etc. were washed with detergent, dried at 70°C in oven drier and sterilized at 170°C for 1.5 hour in a hot air sterilizer. The tips and media were autoclaved at 121°C (15 lb/inch⁻²) for 21 minutes. After autoclaving, tips were again put into a drier at 70°C to evaporate the moisture. Autoclaving at 115°C (10 lb/inch⁻²) for 10 minutes was used for sterilization of sugar containing media. Most of the sterilization processes were followed according to the instructions described by Phillips (1993).

Isolation and identification of bacterial strains

Isolation and maintenance of bacteria

The fish body surface was sterilized with 70% ethanol to avoid bacterial contamination. Under sterile condition, fish gut was removed and homogenized with 5 ml of 0.9% physiological saline. The GI tracts were divided into proximal (PI) and distal (DI) parts and processed according to Mandal & Ghosh (2013) for isolation of autochthonous microorganisms. Homogenates of the pooled intestinal segments of the two regions were serially (1:10) diluted and each diluted sample (0.1 mL) was poured aseptically onto MRS medium which is a special media for the culture of lactic acid bacteria and also in Nutrient Agar media, followed by incubation under aerobic conditions at 28oC for 24 hrs. to determine the autochthonous culturable lactic acid bacterial population. After 24 hours of incubation the colony characteristics of the bacterial isolates grown on the agar plates were observed carefully to choose desired colonies. Several bacterial colonies representing different types of colonies were randomly selected from each plate and inoculated on Nutrient Agar (NA) media. Discrete bacterial pure colonies were obtained by streak culture method. The isolates were routinely sub-cultured on NA plates and incubated at 28oC and stock cultures were maintained in NB (Nutrient Broth) supplemented with 10% glycerol and stored in a freezer at -20oC.

Table 1: Source of bacterial isolates from different fish and different organs

Source Fish	Source Organ	Isolate Name
<i>Labeo rohita</i>	Gut	R101N
		R202N
		R301N
		R302N
	Stomach	R303N
		R101L
		R102L
	Gut	R103L
		R104L
<i>Catla catla</i>	Gut	C101L
<i>Cirrhinus cirrhosus</i>	Hind gut	M202L
		M201L
	Mid gut	M101N
		M102L
	Fore gut	M301N
		M302N
	Stomach	M101L
<i>Labeo bata</i>	Fore gut	B301N
		B302N
	Mid Gut	B102N
		B201N
		B101N
		B202N
	Hind Gut	B105L
		B102L
		B106L
		B101L
		B104L
		B103L
		B103N

Phenotypic identification of bacterial isolates

i. Colony characteristics

Individual colonies grown on MRS agar media or NA plates were carefully observed and colony characteristics such as colony

size, shape, color, type and elevation were recorded.

ii. Morphological and physiological characterization

The shape of bacterial isolates was observed under a compound microscope after Gram's staining. Motility of the isolates was also studied. A brief description of Gram's staining and motility test is given below.

a. Gram's staining Method

Gram's staining procedure was performed with fresh sub-culture of 24 h. In order to carry out Gram's staining, a smear of the isolate was prepared on a clean glass slide and the smear was allowed to air-dry and then heat fixed. The heat-fixed smear was flooded with crystal violet solution for one minute and then it was washed with water and flooded with mordant Gram's iodine and wait for one minute. After one minute it was washed with water and decolorized with 95% ethyl alcohol for five seconds, washed again with water and then counter-stained with safranin for 45 seconds. After washing with water, the smear was dried in air and examined under a microscope (100X) with immersion oil. The bacteria were considered Gram positive if it was stained with violet color whereas Gram negative bacteria stained pink or red color.

b. Motility test

For motility test dilute suspension of fresh bacterial culture was suspended in physiological saline (0.9%NaCl). A drop of the suspension was taken on a clean glass cover slip. Petroleum jelly was used at the four end of the cover slip and placed carefully on to a clean glass slide. A drop of immersion oil was added on the opposite side of the cover slip and then placed under a microscope.

iii. Biochemical characterization

Several biochemical tests were performed to identify the bacterial isolates. Brief description of the biochemical tests perform for the present study is given below:

a. Gram's test

To perform Gram's test, a drop of 3% NaOH solution was taken on a glass slide and then a small amount of sample was sink into it through a sterile inoculating loop. Smeared the sample with loop and observed either it was sticky or not. If sticky, it was considered as negative and if not, it was considered as positive.

b. Catalase test

To perform catalase test, two or three drops of hydrogen peroxide (H₂O₂) were dropped on a glass slide. A loop full fresh culture of bacteria was taken and mixed with H₂O₂. When bubbles of oxygen were released from the surface of the colony immediately the bacteria was considered as catalase positive. When no bubble was formed, the bacteria was considered as catalase negative.



c. Oxidase test

To perform oxidase test, a fresh solution of the reagent was prepared each time of use by adding a loop full of NNNN-tetramethyl-p-phenylene di-amine dihydrochloride to about 3 ml of sterile distilled water. A sterile filter paper was wetted in a sterile Petri dish with a few drops of the indicator solution and smear the culture (grown on a medium free from glucose and nitrate) across the moist paper with a platinum loop. The appearance of a dark purple color on the paper within 30 seconds denoted a positive reaction (Steel, 1961).

d. Oxidative-Fermentative (O-F) test

The purpose of this test was to determine whether an organism utilize sugars (in this case glucose was used) by fermentation or oxidation process. Two tubes of Hugh and Leifson's O-F medium (Himedia, India) were used. Bacterial isolates were inoculated into each of the two tubes of O-F basal medium containing 1% glucose by stabbing the inoculums from a fresh plate culture by the help of a straight wire. The surface of one tube was covered with autoclaved liquid paraffin and incubated at 28°C. Fermentative organisms produced acid throughout both tubes. Oxidative organisms produced acid only in the open tubes which was not covered with paraffin. No reaction was implicated when the change of color occurred in both tubes. Some aerobic bacteria may use the peptone in the medium, producing ammonia, with resulting alkalinity (blue) in the top part of the open tube.

In Vitro Inhibitory Activities of Putative Probiotic bacterial isolates Antibacterial activities of cultured bacteria were evaluated using agar well diffusion method on Nutrient agar (NA). The inhibition zones were reported in millimeter (mm). Strains of

Aeromonas sp., Enterococcus sp. and Stenotrophomonas sp. were used as pathogens for the antibacterial assay of the collected bacteria. Briefly, NA agar plates were inoculated with pathogen strain under aseptic conditions and wells (diameter=9mm) were filled with 50 µl of the test samples and incubated at 37°C for 24 hours. Antimicrobial test or Zone of Inhibition Testing was conducted by the protocol discovered by Kirby- Bauer method described by Hudzicki (2009).

Protocol for Antimicrobial Test:

1. A pathogenic bacterial strain of interest was grown in pure culture.
2. A suspension of the pure pathogen (50 µl) culture was spread evenly over the face of a sterile agar plate.
3. The probiotic strains were applied to the center of the agar plate (in a fashion such that the antimicrobial doesn't spread out from the center). A hole was bored in the center of an agar for holding the probiotic broth.
4. The agar plate was incubated for 18-24 hours at a temperature of 28oC.
5. If probiotic leached from the object into the agar and then would exert a growth inhibiting effect, then a clear zone (the zone of inhibition) would appear around the test product.
6. The size of the zone of inhibition was related to the level of antimicrobial activity present in the sample or product - a larger zone of inhibition usually means that the antimicrobial is more potent.

All the probiotic strains were examined in this method against three pathogens.

Enzymatic Assay:

Specific activity of the enzymes Protease, Lipase and Cellulase were determined of the primarily selected probiotic isolates.

Protease activity test:

Protease activity of the selected isolates were determined according to the method stated by SÖDERHÁLL & UNESTAM (1975). The protocol is given below:

Fifty milliliters of Nutrient broth were prepared in conical flask and sterilized at 121oC for 20 minutes for each isolate. After cooling of the broth, 5ml of the probiotic suspension was added. Then the broths were incubated at 30oC with shaking at 180rpm for 5 days. After the incubation period, the content of each conical flask was filtered through Whatman no. 2 filter paper. 1ml of filtered broth were transported into test tubes. Next the test tubes were centrifuged at 12000rpm for 20 minutes (4oC) and then they were incubated in water bath at 30oC. After 5 minutes, 1ml of a 2% vitamin free casein solution in 0.5M Tris- HCl buffer, pH 8.3 which was of the same temperature was added to each test tubes. The total mixtures were incubated at 30oC for 15 minutes without shaking. Next the reaction was stopped by the addition of 5ml of a 10% (w/v) trichloroacetic acid (TCA) and the samples were then allowed to stand for 1 hr at 25oC.

Then the samples were centrifuged at 3000 rpm for 15 mins. Next the supernatants were filtered and their absorbance were checked at 280 nm in spectrophotometer. There was a control sample in which TCA was added to the filtration before adding the substrates and prepared in parallel for all treatments. A mixture containing 1ml buffer was used as blank and was treated in the same manner. One unit (U) of enzyme activity was defined as the amount of enzyme that, under the assay conditions described, gives rise to an increase of 0.1 units of absorbance in 1 h at 30 °C (Tremacoldi et al., 2006).

Lipase Activity:

Lipase activity of probiotic bacteria were determined as described by Cordenons et al., (1996). After incubation period, the substrate emulsion was prepared as a 1:1 mixture of olive oil (50 ml) and gum arabic (50 ml, 10% w/v). The test tube contained 1 ml of the probiotic suspension (107cell/ml), 5 ml substrate emulsion, and 2 ml of 50 mM phosphate buffer (pH 6.8) and was incubated for 1 h at 37°C with shaking. The reaction was stopped with 4 ml of acetone-ethanol (1:1) containing 0.09% phenolphthalein as an indicator. The contents were titrated with 0.05 N NaOH using a burette until a light blue color appears. Quantity of fatty acids liberated in samples was determined by equivalents of NaOH used to reach the titration end point, accounting for any contribution from the reagent, using the following equation:

$$\text{mol fatty acid/ml subsample} = \frac{(\text{ml NaOH for sample} - \text{ml NaOH for blank}) \times N \times 1000}{5 \text{ ml}}$$

Where, N is the normality of the NaOH titrant used (0.05 in this case). Lipase activity (U/ml) was calculated by determining the amount of supernatant that produces 1 mol of fatty acid per minute under the specified assay conditions.

Cellulase Activity Test:

Cellulase activity of the probiotic bacteria were determined according to the protocol described by Ghose, (1987). The isolates were cultured in CMC (Carboxymethyl Cellulase) media. 0.5 ml enzyme sample was added for each isolate, diluted in citrate buffer (0.05 M sodium citrate buffer, pH 4.8.), to a test tube of volume of 25 ml. Then 3 ml DNS sample were added to each

sample. Next the samples were boiled for exactly 5.0 mins in a vigorously boiling water bath containing sufficient water. All samples, enzyme blanks, glucose standards and the spectro zero were boiled together. After boiling, all the samples were transferred immediately to a cold-water bath. After that, 20 ml deionized or distilled water was added to each sample. The samples were mixed by completely inverting the tube several times so that the solution was separated from the bottom of the tube at each inversion. Finally, the absorbance of the samples was measured in spectrophotometer at 540nm and the color was compared against the spectro zero. And the absorbance of the black sample was subtracted from probiotic sample absorbance. The absorbance data was translated using glucose standard curve.

Detection of probiotic strains:

Probiotic strains which showed the maximum inhibiting activity were collected for identification.

Table 2: Bacterial isolates used in molecular identification

Sl. No	Isolates
01.	R102L
02.	C102L
03.	M201L

Molecular identification:

Isolation of genomic DNA:

Three probiotic isolates showing highest antimicrobial activity were selected for molecular identification.

Genomic DNA extraction

At first the selected bacterial isolates were cultured in nutrient broth at 28oC and 120 rpm in a shaker incubator. About 24 hours old cultures were subjected for genomic DNA isolation. Genomic DNA was extracted by using Gene JET Genomic DNA Purification Kit (Thermo Scientific Corp.) following the manufacturer's protocol. The steps for extraction of genomic DNA is briefly described below:

Bacterial cells were harvested up to 2×10^6 cell/ml in a 1.5 ml micro-centrifuge tubes by centrifugation for 10 minutes at $5000 \times g$ and discarded the supernatant. The pellet was re-suspended in 180 μ l of Digestion solution and added 20 μ l of Proteinase K solution and mixed thoroughly by vortexing to obtain a uniform suspension. The sample was incubated at 56o C while vortexing occasionally until the cells were completely lysed for 30 minutes. Exactly 20 μ l of RNase A solution was added and mixed by vortexing and incubated the mixture for 10 minutes at room temperature. Lysis solution of 200 μ l was added to the sample and mixed thoroughly by vortexing for about 15 seconds until a homogenous mixture was obtained. Then 400 μ l of 50% of ethanol was added and mixed by vortexing. The prepared lysate was transferred to a Gene JET Genomic DNA Purification Column inserted in a collection tube. The column was centrifuged for 1 minute at $6000 \times g$ and discarded the collection tube containing the flow-through solution. Then the Gene JET Genomic DNA Purification Column was placed into a new 2ml collection tube. Exactly 500 μ l of Wash Buffer I was added and centrifuged for 1 min at $8000 \times g$. Discarded the flow-through and placed the purification column back into the collection tube. Exactly 500 μ l of Wash Buffer II was added to the Gene JET Genomic DNA Purification Column and centrifuged for 3 minutes at $13000 \times g$. Discarded the collection tube containing the flow-through solution and transferred the Gene JET Genomic DNA Purification Column to a sterile 1.5ml micro centrifuge tube. Elution Buffer of 200 μ L was added to the center of the Gene JET Genomic DNA Purification Column membrane to elute genomic DNA and incubated for 2 minutes at room temperature and centrifuged for 1 minute at $8000 \times g$. The purification column was discarded and purified DNA was immediately stored at -20o C for further use.

Quantification and storage of DNA

Preparation of TBE

For preparation of 1 liter of 5 X TBE, 54 g Tris base was taken in 800 ml of sterile ddH₂O. Then the mixture was stirred for 30 minutes. Following that 27.5 g boric acid was added and again stirred for several hours. Twenty milliliter of 0.5 M EDTA (3.7224 g) was added and finally water was added for making the volume to 1000 ml. The pH was strictly adjusted at 8.3.

Preparation of Agarose Gel

For genomic DNA quantification 0.4g of agarose powder was dissolved in 50 ml of 0.5 X TBE buffer in a conical flask by heating and poured into the gel tray with comb. After 5 min 50 μ l of ethidium bromide (EtBr) solution was added to the gel solution in the gel tray and mixed it properly by slightly moving the gel tray and waited till it became cool and appeared as

thick gel.

DNA Quantification

DNA quality and quantity were checked by electrophoresis on agarose gel comparing with the 1 Kb DNA ladder marker (AccuLadder, Bioneer Corporation, Taiwan). In Eppendorf tube 0.5 μ L of loading dye and 2.5 μ L of DNA samples were taken and mixed gently by pipetting. Sample mixture was then loaded into each of the well in the agarose gel created by the comb. 2.5 μ L of 1 Kb DNA ladder marker was also taken aside near the samples. The chamber was fulfilled with 0.5X TBE buffer until the maximum line and covered with the lid. Gel was run at 70 volts for 45 min. Then the gel was transferred to the gel documentation system and visualized under UV light to observe the DNA band. The DNA was stored in a freezer at -20°C for future use.

PCR for amplification of 16S rRNA

PCR Primers

The polymerase chain reaction (PCR) for amplification of the targeted 16S rRNA of six representative isolates was performed with the universal primer sets (27F and 1492R). These primers (bioneer ltd.) are routinely used for molecular identification of fish pathogenic bacteria.

Table 3. Primer sequences used for PCR amplification and the expected amplicon size:

Primers	Sequence (5'- 3')	Primer Size (bp)	GC Content (%)	PCR Amplicon Size (bp)
27F	AGAGTTGATCCTGGCTCAG	19	50	
1492R	GGATACCTGTTACGACTT	20	42.1	1465

Table 4. Concentration of PCR mixture:

Reagents	Final concentration	Final volume (100 μ L)
25mM MgCl ₂ (Promega)	1.5 mM	6
Reaction buffer (Promega)	1X	10
10mM dNTP (Promega)	200 μ M each dNTP	2
F-Primer (SigmaTM)	0.1-1.0 μ M	5
R-Primer (SigmaTM)	0.1-1.0 μ M	5
DNA template	100-200 ng/100 μ L	3-7
Taq polymerase (Promega)	0.05 U	1
Sterile deionized water	-	64- 68

PCR Master Mixture Preparation

Individual PCR mixture contained 6 μ L of 25 mM MgCl₂, 10 μ L of 10 \times PCR buffer, 2.0 μ L of 10 mM deoxyribonucleotide tri-phosphate mix, 5.0 μ L of a 100 μ M solution of each primers, 100-200 ng of DNA template, 0.5 μ L of TaqDNA polymerase (Promega) at 5U/ μ L and sterile double-distilled water in a total volume of 100 μ L (Table 4). A negative control (without template DNA) was included in every PCR run.

Thermal Profile for PCR Amplification

The PCR amplification was performed in a PCR Thermocycler (Eppendorf Ltd.). The optimum condition for PCR was set as follows: an initial denaturation step at 94°C for 5 min; 35 cycles of a denaturation step at 94°C for 1 min, an annealing at 57°C for 40 sec and an extension at 72°C for 1 min and a final extension step at 72°C for 10 min.

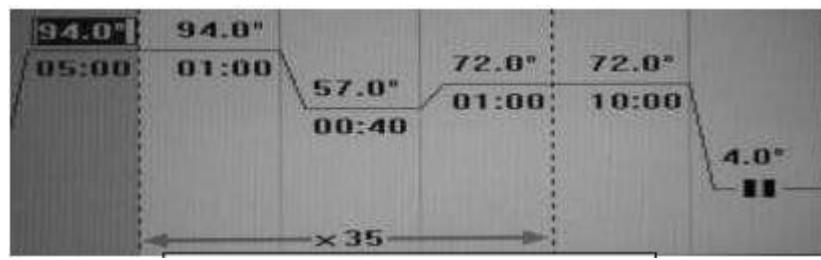


Figure 01: PCR Thermal Profile.

Agarose Gel Electrophoresis:

A small portion (usually 5 μ L) of the PCR amplicons were mixed with 1-2 μ L of 6 \times loading dye (0.25% bromophenol blue

and 40% sucrose in double-distilled water) and loaded in a 1.5% agarose gel with a molecular weight standard marker (1 Kb ladder marker, Promega). Then electrophoresis was performed in $0.5 \times$ Tris-Borate-EDTA (TBE) buffer for 40 min at 70 volts. Amplicons were visualized with UV light in a gel doc system (Weltec KETA G, Weltec Corporation, USA).

2.7.5 Purification of PCR product

The PCR product was purified by using a commercial Gene JET PCR Purification Kit (Thermo Scientific Corp.) following the manufacturer's protocol. The steps for purification of PCR products were as follows:

A 1:1 volume of Binding Buffer was added to completed PCR mixture and mixed thoroughly. The color of the solution was checked and a yellow color indicated an optimal pH for DNA binding. Transferred up to 800 μ l of the solution from step 1 to the Gene JET purification column and centrifuged for 1 minute. Then the flow-through was discarded. Exactly 700 μ l of Wash Buffer was added to the Gene JET purification column and centrifuged for 1 minute. The flow-through was discarded and placed the purification column back into the collection tube. The empty Gene JET purification column was centrifuged for an additional 1 minute to completely remove any residual wash buffer. The Gene JET purification column was transferred to a clean 1.5 ml micro centrifuge tube. Fifty micro liter of Elution Buffer was added to the center of the Gene JET purification column membrane and centrifuged for 1 minute. The Gene JET purification column was discarded and purified DNA was stored at -20°C.

DNA Sequencing of Probiotic Bacteria

The purified PCR products of the pathogenic bacteria were sent along with sequencing primer to the Centre for advance studies and research, University of Dhaka for sequencing of 16S rRNA gene. The sequence was extracted by using BIOAD software as a FASTA format.

Phylogenetic Analysis

DNA sequences (FASTA format) of the isolates were then used for DNA sequence homology study and phylogenetic analysis using the BLAST and Phylogeny.fr web based software respectively.

Table 5. Colony, morphological, physiological and biochemical characterization of bacterial isolates.

Name	Color	Colony Shape	KOH Test	Gram Staining	Shape	Catalase Test	Oxidase Test	OF Test	Motility
R101 N	Off White	Spreading	-	+	Cocci	-	+	F	+
R302 N	Off White	Round	-	+	Rod	+	-	O	-
R202 N	Creamy	Spherical	-	+	Cocci	+	-	O	-
R301 N	Yellowish Brown	Round	-	+	Cocci	-	-	O	-
R303 N	Creamy	Spherical	-	+	Cocci	+	-	O	-
B301 N	Creamy	Spherical	+	-	Cocci	-	+	F	-
B302 N	Off White	Spreading	+	-	Cocci	+	+	O	+
B102 N	Yellowish Brown	Round	+	-	Rod	-	+	O	-
B201 N	Off White	Spherical	-	+	Rod	-	-	F	+
B101 N	Creamy	Spherical	-	+	Cocci	+	+	O	-

B202 N	Creamy	Spherical	-	+	Cocci	+	+	O	-
M301 N	Off White	Round	-	+	Cocci	+	-	O	+
M101 N	Off White	Round	+	-	Cocci	+	-	F	-
M302 N	Yellowish Brown	Spherical	-	+	Rod	+	+	O	-
R101 L	Off White	Spherical	+	-	Cocci	-	+	F	-
R103 L	Off White	Spreading	+	-	Cocci	-	+	F	+
R102 L	Milkytype White	Spherical	-	+	Rod	-	+	F	-
R104 L	Milkytype White	Round	-	+	Cocci	+	+	F	-
B103 L	Creamy	Spherical	-	+	Cocci	-	-	F	-
B104 L	Off White	Round	-	+	Rod	-	+	O	-
B105 L	Creamy	Spherical	+	-	Cocci	-	+	F	-
B102 L	Off White	Spreading	-	+	Cocci	-	-	F	+
B101 L	Milkytype White	Round	-	+	Cocci	-	+	F	-
B106 L	Off White	Spreading	+	-	Rod	+	+	O	+
M101 L	Milkytype White	Round	-	+	Cocci	-	+	F	+
M102 L	Creamy	Spherical	-	+	Rod	-	+	F	-
M201 L	Creamy	Spherical	-	+	Cocci	-	+	F	-
M202 L	Off White	Round	-	+	Rod	-	+	F	-
C102 L	Off White	Round	-	+	Cocci	-	+	O	-
C101 L	Milkytype White	Spreading	+	-	Cocci	-	-	F	+

RESULT

Characterization of Bacterial Isolates:

Four carp species Rohu (*Labeo rohita*), Catla (*Catla catla*), Mrigal (*Cirrhinus cirrhosus*), Bata (*Labeo bata*) were collected from different natural sources without any deformities. A total of 14 isolates were taken from the MRS media and 16 isolates from NBA media.

Among 30 isolates 14 isolates were collected from NBA media and 16 isolates were collected from MRS media. Among the isolates 21 isolates were Gram positive, 21 isolates were cocci shaped 9 were rod shaped, 17 were fermentative bacteria.

Most of these isolates were also oxidase negative and catalase positive, 21 isolates were non motile, showed growth at 35°C temperature. Most of the isolates exhibited whitish and a creamy transparent color when grown on nutrient agar media. Among the isolates 9 were collected from L. rohita, 2 from C. catla, 7 from C. cirrhosus and 12 from L. bata

Table 6. Inhibitory activity of the isolates collected from carp fishes (Note: A22, A27, B19, B55- Aeromonas sp., F1B1, FF11- Enterococcus faecalis., FxRhG2, FxRhG3, FxRhG4, Tela1- Stenotrophomonas maltophilia. Inhibition zone > 3 = +++, >2 = ++, >1 = +)

Isolate S	FxR hG 2	F x R h G 3	F x R h G 4	B1 9	B5 5	F1 1	F1B1	A2 2	A2 7	Tela 1
R101N	-	-	+	++	-	-	-	-	++	-
			+	+					+	
			+							
R30 2N	-	-	-	-	-	-	-	-	-	-
R20 2N	-	-	-	-	-	-	-	-	-	-
R30 1N	-	-	-	-	-	-	-	-	-	-
R30 3N	-	-	-	-	-	-	-	-	-	-
B30 1N	-	-	+	-	-	-	-	-	+	-
			+						+	
B30 2N	-	-	-	-	-	-	-	-	-	-
B10 2N	-	-	-	-	-	-	-	-	-	-
B20 1N	-	-	-	-	-	-	-	-	-	-
B10 1N	-	-	+	-	-	-	-	-	+	-
			+						+	
B20 2N	+	-	+	+	-	-	-	-	+	-
	+		+	+					+	
			+							
M301N	-	-	-	-	-	-	-	-	-	-
M101N	-	-	-	-	-	-	-	-	-	-
M302N	-	-	-	-	-	-	-	-	-	-
R10 1L	+	-	-	-	-	-	-	-	-	-
	+									
R103L	-	-	-	-	-	-	-	-	-	-
R10 2L	+	-	+	++	-	-	-	++	++	+
	+		+	+				+	+	+
			+							
R10 4L	+	-	-	+	-	-	-	-	++	+
	+			+					+	+
										+
B10 3L	-	+	+	+	-	-	+	-	+	-
		+		+			+		+	
B10 4L	+	-	+	+	-	-	+	-	-	-
	+			+			+			
B10 5L	-	-	+	+	-	-	-	-	-	-
			+							

B10 2L	+	-	+	+	-	-	-	+	+	+
	+		+	+						+
B10 1L	+	-	+	+	-	-	-	+	+	+
	+		+	+				+	+	+
	+		+							+
B10 6L	+	-	+	++	-	-	-	+	+	+
	+		+	+						+
	+		+							+
M10 1L	+	+	+	+	-	-	+	-	-	-
	+		+				+			
M10 2L	+	-	+	++	-	-	-	++	+	+
	+		+	+				+	+	+
	+		+							+
M201L	+	-	+	++	-	-	-	++	+	+
	+		+	+				+	+	+
	+		+							+
M20 2L	-	+	+	+	-	-	-	-	++	+
	+		+						+	+
C10 2L	+	-	+	++	-	-	-	+	+	+
	+		+	+				+	+	+
	+		+							+
C10 1L	+	-	+	-	-	-	-	-	-	-
	+		+							
Control	-	-	-	-	-	-	-	-	-	-

Pathogen Inhibition

In vitro inhibitory activity of the fish gut isolates were evaluated against different fish pathogens following the agar well diffusion method. Ten fish pathogenic strains. belonged to four genera viz., *Aeromonas* spp., *Pseudomonas fluorescens*, *Enterococcus faecalis* and *Stenotrophomonas maltophilia* were used for this purpose. Nineteen out of thirty isolates exhibited inhibitory activities against the fish pathogenic strains. Most of the isolates inhibited the growth of fish pathogenic strains *S. maltophilia* FxRhG3 and *Aeromonas* sp. B19. Among the fish gut isolates, six isolates C102L, R102L, B102L, M101L, M102L and M201L showed higher inhibitory activities against the pathogens. Among them B102L showed highest inhibitory activity against most of the fish pathogens.

Enzyme Activity:

Six isolates which showed highest anti-microbial were used for enzymatic activity assay. In this study, the highest protease activity was found for the isolate C102L. Both M201L and M102L showed highest lipase activity and C102L secreted highest cellulose enzyme. Boirivant and Strober (2007) reported that the probiotics with anti-pathogenic activity would secret considerable enzymes to destroy the cell membrane of the pathogen. Three enzyme tests were done for each of the isolates. The results of the enzyme activity assay are given below in Table 7.

Table 07: Enzyme activity of the bacterial isolates.

Isolates	Protease activity	Lipase activity	Cellulase Activity
B102L	7.606U	0.90U	6.28U
C102L	9.326U	1 U	10.65U
R102L	5.275U	4.8U	8.87U
M101L	8.573U	1.8U	9.2U
M102L	6.695U	5.2U	7.54U
M201L	7.57U	5.2U	9.85U

Here, one unit of protease activities was equivalent to μg of tyrosine liberated ml-1of enzyme extract mg-1protein min-1. Similarly, Lipase and cellulase activity is equivalent to μg of free fatty acid and μg of glucose liberated ml-1of enzyme extract mg-1protein min-1

DNA sequencing and molecular characterization:

DNA extraction and PCR purification of six bacterial isolates B102L, M101L, C102L, R102L, M201L and M102L were completed for 16S rRNA gene sequencing for identification and phylogenetic analyses of those bacteria.

Partial sequence of 16S rRNA of the selected bacterial isolates C102L, R102L and M201L were analyzed using web based Basic Local Alignment Search Tool (BLAST) program of National Centre for Biotechnology Information (NCBI).

CONCLUSION

19 out of 30 isolates isolated from fish gut showed inhibitory activity against 10 fish pathogens belonged to four genera. Among these, six isolates higher inhibitory activity against most of the pathogens. Further studies on in vivo infection studies are suggested to know the potentials of the isolates as candidate probiotics for carp fish.

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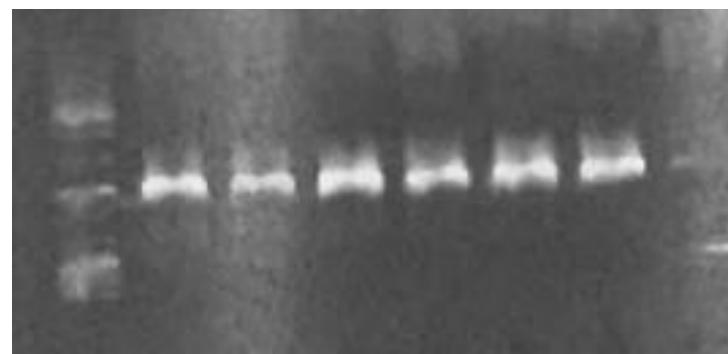


Figure 02: Gel electrophoresis photograph of purified PCR products of bacterial isolates (2 $\mu\text{l}/\text{lane}$, 0.5% agarose, 0.05% TBE, electrophoresis time= 25 min; M= Lamda DNA, 1) B102L, 2) M101L, 3) C102L, 4) R102L, 5) M201L and 6) M102L

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PERFORMANCE EVALUATION AND OPTIMIZATION OF VACCUM MARINATION PROCESS FOR FRESH AND FROZEN SOLE FISH

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ABSTRACT

The major problem facing the fish processing industry is reducing the marination process time and increasing the uptake of marinade. Previously, a marination bath has been utilized for this purpose with different combinations of acid and salt, which causes physiochemical changes in fish texture. The vacuum marination method seems to be a good way to solve this problem. In this study, the effect of the vacuum marination process was studied on the marination of sole fish. Two varieties of fish (fresh and frozen) and three levels of time (10, 20 and 30 minutes) were studied for their effect on marinade uptake. The marinade was used in the ratio of 1.2:10 spices to meat, respectively. Fresh samples were weighted, vacuum packed, and stored at 4–7 °Celsius, whereas frozen samples were stored at -20 °C for 24 hours prior to marination and thawed at room temperature. After marination, percentage uptake, retention, and cooking loss of the marinade were calculated. It was concluded from the yield of marinated products that about 80% of marination takes place in the first 10 minutes of the process. After 10–20 minutes, there was no significant difference ($p>0.05$), and only 1–2% of the marinade was consumed. The frozen samples show significantly ($p\leq 0.05$) higher yields and marinade uptake. After marination, the samples were stored at 0–4 °C and fried in vegetable oil until the center reached 70 °C. Cooking loss for this process was non-significant for both varieties of fish as it depends on the water-holding capacity of the meat, which remains the same for both cases. The use of vacuum marination reduced the marination time significantly from 4–6 hours to 10 minutes, and marinade uptake was improved. This process can be used as an alternative to traditional marination techniques.

Keywords: Vacuum-marinator, Fish, Frozen, cooking loss, marinade uptake

INTRODUCTION

Most of the people in Pakistan like to eat fish in its fresh and firm form after frying, grilling, salting and marination. The methods like salting, smoking, and fermenting are not much used for fish products in Pakistan. A large quantity of fish is usually consumed in winter season all over Pakistan. The products of fish marinades are obtained from different kind of whole fish, through different process like frozen products, salted products and fresh fish. The meat for these products is prepared through different acid and salt concentration to develop a better taste in the products. At industrial level, it is important to reduce the marinating time to obtain the same final characteristics of pH, aw, and sensorial attributes. This can be done by increasing either the salt and acid contents of the marinating solution or the marinating solution fish ratio, or by agitation during the marinating process. Thawing after freezing changes both the substance and the moisture of meat tissue. Moisture content as a quality trademark in meat can be assessed in a few different ways, including trickle misfortune, defrost misfortune, cooking loss, water restricting limit and aggregate dampness content. In any case, since the techniques used to decide dampness misfortune and changes in meat are not set by a global standard, usually hard to straightforwardly look at and reach inferences from considers in the writing that have utilized diverse strategies for such purposes. Marination plays a vital role in meat processing at different level of processing as marination helps in decreasing microbial and enzymes load. Marination also provides a value addition in fish. Marinated fish fillets are preserved by the concurrent action of natural acids, for example, acetic acidic and salt. The joined additive activity keeps the development of pathogenic microscopic organisms and most deterioration microorganisms. The aims of this research were to determine the effect of subfreezing and thawing conditions on the marination uptake and the salt and

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acid, immersion time, and sensorial characteristics during the marinating process of sole fish. Fresh and frozen samples were investigated for uptake time of marinade within same conditions.

MATERIAL AND METHODS

Sample and Marinade Preparation

Sole fish used with maximum acceptance was procured from local market of Faisalabad after sensory evaluation by the team of experts. After washing and cutting the whole fish, its skin was removed for better marination. Removed skin helps in taking maximum marinade, reduce cooking time, good color, and sensory characteristics. After size reduction, the fish pieces were packed in polythene bags for both frozen and fresh samples. Fresh samples were weighted and vacuumed packed until the marination starts and kept at 4-7 0C. Samples were weighted before freezing and each sample was separately frozen (-20 0C) in vacuumed packaging. All samples of fish both fresh and frozen were independently labeled before marination to so that their identification remains same throughout the process of marination. Primary ingredients in marinade were salt, chili, phosphate, and water. The concentration of each ingredient in the marinade formulation can vary according to the product desired. The major concern of this study was to investigate the salt uptake that enriches the taste of product and sodium tri poly phosphate which increases the water holding capacity, marinade uptake and retention of marinade. The marinade used in this experiment was 1.2:10 water and meat respectively.

Vacuum Marination of samples

The reverse process of freezing is called thawing. The process used in this experiment was still air thawing at ambient conditions. The samples were placed on the mesh so that the water released from the meat did not interact with the fish. Marinating was performed for period of 10, 20, 30 minutes interval for both fresh and frozen sample at same ambient condition. The process of marination was performed each time at 8 rpm and 5 in Hg. After each sample treatment, sample were weighted separately as tagged and the final weight of sample was recorded.

Analysis and Calculations

Fresh and frozen samples were weighted after marination to find the percentage uptake of each sample

$$\% \text{ Uptake} = (\text{final weight}(Y) - \text{initial weight}(X)) / \text{initial weight } X \times 100$$

The samples were stored after marination at 4 0C for 24 hours to calculate the marinade retention

$$\text{Marinade retention (\%)} = (\text{Weight after marination}-\text{Weight after 24 hours})/\text{Weight after marination} \times 100$$

After 24 hours the marinated fish was cooked until the center of the sample reached to 75 0C

$$\text{Cooking Loss (\%)} = ((\text{Wt.}_{\text{precook}} - \text{Wt.}_{\text{post cook}}) / \text{Wt.}_{\text{precook}}) \times 100$$

To find the overall efficiency of the process was calculated as final yield

$$\text{Yield (\%)} = (\text{Wt.}_{\text{post cook}} / \text{Wt.}_{\text{pre marination}}) \times 100$$

RESULT

Effect Of Variety and Time on Marinade Uptake

Two varieties of fish (V, fresh and frozen) and three levels of time (T, 10, 20 and 30 minutes) were studied for their effect on marinade uptake. The results obtained by the data showed that the effect of T, V and interaction V*T was highly significant. The effect of T on marinade uptake was significant showing that in first 10 minutes marinade uptake is maximum later, it is in very small amount (< 5%MU).

Table 1: Effect of V and T on Percentage Marinade Uptake

Marinade Uptake (MU)					
Source	df	SS	Mean Square	F Value	Pr > F
V	1	17.7488	17.748	112.37	0.0001**
T	2	1.723	0.867	5.45	0.0207*
V x T	2	37.369	18.687	118.29	0.0001**
Error	14	11.2619	0.8044		
Corrected Total	17	51.8992			

Table 2: Comparison of Means of MU (%) Under the Effect of V and T

VARIETY	MU		
	1	2	3
1	83.300±1.538	90.367±1.057	84.900±0.700
2	92.567±1.257	86.123±0.646	90.150±0.489

Effect of Variety and Time on Marinade Retention

Two varieties of fish (V, fresh and frozen) and three levels of time (T, 10, 20 and 30 minutes) were studied for their effect on marinade retention. The results obtained by the data showed that the effects of V and interaction V*T were highly significant. The effect of T on marinade retention was non-significant.

Table 3: Effect of V and T on Marinade Retention (%)

Marinade Retention					
Source	df	SS	Mean Square	F Value	Pr > F
V	1	52.776	52.779	65.81	0.0001**
T	2	1.565	0.787	0.98	0.4050 ^{NS}
V x T	2	144.388	72.197	90.04	0.0001**
Error	14	12.00	0.8571		
Corrected Total	17	12.00			

Table 4: Comparison of Means (%) of Marinade Retention Under the Effect of Interaction V And T

VARIETY	T		
	1	2	3
1	1.487±0.078	5.370±0.607	2.716±0.088
2	6.013±0.676	3.326±0.138	6.185±0.318

Effect of Variety and Time on Yield

Two varieties of fish (V, fresh and frozen) and three levels of time (T, 10, 20 and 30 minutes) were studied for their effect on marinade yield. The results obtained by the data showed that the effect of V and T was non-significant. The effect of V*T on marinade yield was highly significant.

Table 5: Effect of V and T on Yield (%) of the Process

YIELD					
Source	df	SS	Mean Square	F Value	Pr > F
V	1	0.379	0.3189	0.67	0.427 ^{NS}
T	2	1.563	0.787	1.66	0.239 ^{NS}
V x T	2	22.275	11.117	23.60	0.0001**
Error	14	18.6211	1.3300		
Corrected Total	17	20.16			

Table 6: Comparison of Mean Percentage Yield Under the Interactions of V And T

VARIETY	YIELD		
	1	2	3
1	95.167±0.919	93.200±0.487	94.867±0.377
2	93.267±0.997	96.077±0.389	93.033±0.836

Effect of Variety and Time on Cooking Loss

The results obtained by the data showed that the effects of V and interaction V*T were highly significant. The effect of T on cooking loss was also highly significant showing that in first 10 minutes marinade uptake is maximum.

Table 7: Effect of V and T on Cooking Loss (%)

Cooking Loss					
Source	df	SS	Mean Square	F Value	Pr > F
V	1	0.749	0.749	18.28	0.001**
T	2	1.658	0.829	20.40	0.0001**
V x T	2	15.428	7.729	190.43	0.0001**
Error	14	4.4657	0.3189		
Corrected Total	17	7.099			

Table 8: Comparison of Mean Percentage Cooking Loss Under the Effect of V and T

VARIETY	CL		
	1	2	3
1	10.467±0.157	11.567±0.152	9.277±0.173
2	11.657±0.123	9.417±0.325	11.453±0.259

DISCUSSION

It was concluded that about 80% of marination take place in first 10 minutes of marination at the condition that were provided in this experiment. Time plays an important role in explain the uptake of marinade. Time from 10-20 minutes plays very minute role in up taking time of marinade. As this experiment were conducted to minimize the process optimization of meat marination while considering next ten minutes are not beneficial for 1-2 % of marination process. Rather than increasing time experiment should be conducted on marination ratio or vacuum control as this experiment were taken out at 5 inches of Hg. Increasing rpm during vacuum marination above 8 results in damaging the meat fillets as during these experiments 2 fillets were broken. The stirrer design was also very important meat can stick and marination of these samples were seems stopped about one and other which result in reduced uptake. Agitation can increase the temperature of process temperature that can directly affect the marinade retention of meat. It was concluded increasing temperature above certain range resulted in decrease of marinade retention and meat started releasing the marinade. After marination of meat samples meat store at 0-4 0C retained the maximum marinade. Cooking loss were seeming not different from each other as cooking loss depend upon the water holding capacity of the meat in both cases the thawing losses were same. The above result concluded that using vacuum marination reduced the time marination but after freezing and thawing the process time decreased significantly. The parameter of vacuum marinade ratio rpm and time plays a significant role. According to this study marinade uptake were improved in the marinating solution, the water contents were decreased after thawing and the average thawing loss during all these experiments were remain 2.22% and while comparing with the fresh sample these thawing loss help to gain more marinade uptake during vacuumed marination process and the texture and water content of the sole fillet were reliant on the levels of salt and acid that the fish tissue takes.

CONCLUSION

The results reduced the time of marination to only 10 minutes as compared to traditional 3 hour with increased tenderness better and enriched taste. The core purpose of marination is to absorb the flavors of the marinade for the food like meat, to tenderize it. Polyphosphates changes the microstructure of the meat by solubilizing salt soluble proteins. In addition, polyphosphates improve tenderness of chicken breast, reduce cooking and frying losses. The frozen samples showed significant improvement in marinade uptake and its retention due to broken structure of cell during freezing and thawing which helped the meat to gain more marinade. The vacuum marination process seems to be a future promising approach which can reduce the time of marination as well as provide the good quality meat due to no use of chemicals and additives to enhance the uptake and retention.

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BIOCONTROL OF FOOT AND ROOT ROT DISEASE OF GROUNDNUT (*Arachis hypogaea*) BY DUAL INOCULATION WITH RHIZOBIUM AND ARBUSCULAR MYCORRHIZA

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ABSTRACT

The present study was carried out to investigate the potential of AM (Arbuscular mycorrhiza) fungi alone and in combination with bioinoculants i.e., Rhizobium to find out the best combination on dry biomass, nodulation, colonization, and yield, along with their biocontrol against groundnut foot and root rot caused by Sclerotium rolfsii. The study was carried out under pot culture conditions in the net house of the Soil Science Division, Bangladesh Agricultural Research Institute, Joydebpur, Gazipur in 2020 and 2021. The experiment was designed in RCBD with eight treatments and four replications. Peat-based rhizobial inoculum (BARI RAh-801) was used in this experiment. Soil-based AM inoculum containing approximately 252 spores and infected root pieces of the host plant was used in pot-1. The treatments were Arbuscular mycorrhiza (AM), Rhizobium, AM+Rhizobium, Sclerotium rolfsii, Sclerotium rolfsii+AM, Sclerotium rolfsii+Rhizobium, Sclerotium rolfsii+AM+Rhizobium and Control. Dual inoculation (AM+Rhizobium) significantly increased dry biomass, nodulation, colonization, yield, and yield attributes of groundnut compared to single inoculation or other treatments. The result showed that dual inoculation (AMF+Rhizobium) increased nut yield (59.61% in 2020 and 26.32% in 2021) and stover yield (23.21% in 2020 and 33.74% in 2021) compared to control. On the contrary, Sclerotium rolfsii+AMF+Rhizobium increased nut yield (65.50% in 2020 and 52.94% in 2021) and stover yield (36.45% in 2020 and 99.35% in 2021) compared to only Sclerotium rolfsii treatment. Therefore, AMF species and its combination with rhizobial inoculum were significant in the formation and effectiveness of AM symbiosis. They also increased yield and reduced the incidence of foot and root rot disease in groundnut plants.

Keywords: Biocontrol, biomass, nodulation, nut yield and root colonization

INTRODUCTION

Foot rot (caused by *Fusarium oxysporum* and *Sclerotium rolfsii*) is considered an essential and destructive disease of pulses and oilseeds in almost all countries of the world (Anonymous, 1986). *Sclerotium rolfsii* are soil-borne pathogens that commonly occur in the tropics and sub-tropic regions of the world, causing root and foot rot in many crops (Aycock, 1966). It causes early seedling death, resulting in an inferior plant stand, producing a meager yield. Though chemical pesticides can control this disease, it causes environmental pollution and health hazards and is not economical. Hence, biological control agents like arbuscular mycorrhizal fungi and Rhizobium can be used for green, safe, sustainable agriculture.

Arbuscular mycorrhizal fungi (AMF) that form symbiotic relationships with the roots of most terrestrial plants are known to improve the nutritional status of their host and protect plants against several soil-borne plant pathogens. (Smith & Read, 1997; Bi et al., 2007). The significant effect of mycorrhizal fungi in undisturbed ecosystems is to improve the growth of mycorrhizal plants compared to non-mycorrhizal plants (Planchette et al., 1983). It covers the root of plants, making it a protective physical

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barrier against diseases (McAllister et al., 1997; Karagiannidis et al., 2002). Induce local and systemic resistance against pathogens using a variety of mechanisms, including increased mineral nutrition and the expression of plant genes related to resistance or direct anti-fungal effects (Aghighi et al., 2004). AMF are currently studied as biological control agents against soil-borne diseases (Hooker et al., 1994). Their impact on plant-pathogen interactions ranges from disease reduction (Smith & Read, 1997; Rahman et al., 2017a) to a neutral action (Baath and Hayman, 1984). In this way, using AMF as inoculants to benefit plant growth and health could contribute to reducing the inputs of pesticides and other environmentally harmful agrochemical products currently required for optimal plant growth and health (Barea et al., 1997).

Many disease management methods exist, such as crop rotation use of resistant varieties, and chemical pesticides. However, frequent and indiscriminate use of these pesticides affects the Soil's physical, chemical, and biological properties. It also affects non-target organisms and has developed resistance among pathogens against these chemicals (Arwry & Quandt, 2003). The Biocontrol potential of AM fungi against various phytopathogens is well documented (Xavier & Boyetchko, 2014; Rahman et al., 2017b). Arbuscular Mycorrhizal Fungi (AMF) are the major component of the rhizosphere of most plants and play a significant role as a biocontrol agent and help decrease plant disease incidence (Akthar & Siddiqui, 2008). The combined use of AMF and Rhizobium can improve plant growth and protection against the pathogen Sclerotium rolfsii within a sustainable soil-plant system. Keeping in view the above information, the present investigation was undertaken to investigate the potential of AM fungi alone and in combination with bioinoculants i.e., Rhizobium to find out the best combination on dry biomass, nodulation, colonization, and yield, along with their biocontrol against groundnut foot and root rot caused by Sclerotium rolfsii.

MATERIALS AND METHODS

Seed collection and Soil Preparation

The experiment was carried out during the rabi season from December 2019 to April 2020 and December 2020 to May 2021 in the net house of Soil Science Division, BARI, Joydebpur, Gazipur (23° 59' 378" N latitude, 90° 24' 886" E longitude and 8.4 m elevation). Seeds of groundnut (BARI Chinabadam-8) were collected from RARS, Jamalpur. The silted (sandy clay loam) soils were collected from the bank of Turag river at Kodda, Gazipur, mixed with cow dung at a 5:1 ratio, and were used as the potting media. Each pot (25 cm in diameter and 21 cm in height) was filled with approximately 6 kg soil leaving the upper 3 inches of the pot vacant to facilitate watering. The pH of cow dung was 6.7, and the nutrient contents were: organic matter 14.1%, N 0.8%, P 1.26%, K 0.88%, Ca 1.55%, Mg 0.82%, S 0.62%, Fe 0.25%, and Mn 0.112%. The physical and chemical properties of the Soil are presented in Table 1. The Soil contained 12 AM (100-1 g soil) spores of indigenous mixed AM fungal species, and the experiment was conducted under sterilized soil conditions.

Table 1: Initial fertility status of the soil and cowdung used in the investigational pot

Samples	Texture	pH	OM (%)	Ca	Mg	K	Total N (%)	P	S	B	Cu	Fe	Mn	Zn
				meq 100 g ⁻¹				μg g ⁻¹						
Soil	Sandy clay loam	7.1	0.51	7.2	2.5	0.11	0.026	9.9	21.1	0.22	1.8	15	1.1	0.38
Cowdung	-	6.7	14.1	1.55	0.82	0.88	0.84	1.26	0.62	0.02	0.01	0.25	0.11	0.02
Critical level	-	-	-	2.0	0.5	0.12	-	10	10	0.20	0.2	4.0	1.0	0.60

Methods of chemical analysis

Soil pH was measured by a combined glass calomel electrode (Jackson, 1958). Organic carbon was determined by the Wet Oxidation Method (Walkley & Black, 1934). Total N was determined by the modified Kjeldahl method (Jackson, 1962). Calcium, K, and Mg were determined by the NH4OAc extraction method (Black, 1965). Copper, Fe, Mn, and Zn were determined by DTPA extraction followed by AAS reading. Boron was determined by the CaCl₂ extraction method. Phosphorus was determined by the Modified Olsen method (Neutral + Calcareous soils) according to Olsen et al. (1954). Sulfur was determined by CaH₄(PO₄)₂.H₂O extraction followed by turbidimetric turbidity method with BaCl₂.

Chemical fertilizers were applied as soil test basis according to the method described in the fertilizer recommendation guide (BARC, 2018). Half of N and all of P, K, S, Mg, Zn, B, and Mo were applied as basal during final land preparation, and the remaining N was used as top dressing at the flowering stage.

Collection of pathogens Sclerotium rolfsii and Rhizobium inoculum

Pathogen Sclerotium rolfsii was collected from Plant Pathology Division, BARI, Gazipur, which was grown on non-seed barley. Non-seed barley collected from Plant Breeding Division, BARI, Gazipur. Pathogen Sclerotium rolfsii and non-seed barley 50 g were used per Sclerotium treatment pot. After disease development, pathogen sclerotia is mixed with Soil. Rhizobium strain BARI RAh-801 was collected from Soil Microbiology Division, BARI, Gazipur, and mixed correctly with the seed before sowing.

Preparation of mycorrhizal inoculum

The arbuscular mycorrhizal inoculum was prepared from Sorghum's roots and rhizosphere soils. Mycorrhizal species were initially isolated from different AEZ regions using the wet sieving and decanting method. A mixture of infected sorghum root and Soil, which contained spores, was used as mycorrhizal inoculum. The spores were left to multiply for six months on sorghum plants using unsterilized Soil collected from the same site in the net house of the Soil Science Division, BARI. Plants were rinsed with tap water as needed. The soil-based AM fungal inoculum having approximately 252 spores and colonized sorghum root fragments with a minimum colonization level was inoculated to each mycorrhizal pot. The mycorrhizal inoculum was placed in each pot at a 3-5 cm depth. It was covered with a thin soil layer of 1 cm immediately before the seed sowing of groundnut to facilitate fungal colonization of plant roots.

Experimental design

The experiment was designed in RCBD with eight treatments and four replications. Fifteen seeds were sown in each pot at 1 cm soil depth. The eight (08) treatments were: T1: Arbuscular mycorrhizal fungi (AMF), T2: Rhizobium (R), T3: AMF+Rhizobium, T4: Sclerotium rolfsii, T5: Sclerotium rolfsii+AMF, T6: Sclerotium rolfsii+Rhizobium, T7: Sclerotium rolfsii+AMF+Rhizobium and T8: Control. The treatment was sustained with 06 vigorous seedlings in pot 1, and the other seedlings were removed. Three plants each were collected for nodulation (Khanam et al., 2005) and colonization data, and rest three plants were kept finally in each pot for yield and yield contributing measurements.

Determination of germination percentage

The germination test was carried out according to ISTA rules (ISTA, 1976). For each treatment, 100 seeds were put into Petri dishes. The Petri dishes were put on a laboratory table at room temperature ($25 \pm 2^{\circ}\text{C}$). Germination of groundnut seed in the laboratory table was 95-100%.

Plant harvest

Groundnuts were harvested after maturity, and yield parameters were measured.

Assessment of spore population density and root colonization

The spore population was assessed following the Wet Sieving and Decanting Method (Gerdemann & Nicolson, 1963). All the AM spores were isolated from the extract with the help of fine forceps into a watch glass with little water. The extract, with AM spores, was observed under a stereomicroscope, and the number of spores was counted. Spore numbers from the three replicates per sample were averaged, and the result was expressed as a number per 100 g of dry soil basis. The root slide technique estimated the percentage of AM colonization (Read et al., 1976). A root segment was considered positively infected if it showed mycelium, vesicles, arbuscules, or any other combination of these structural characteristics of AM colonization. The presence or absence of colonization in the root pieces was recorded. The percent colonization was calculated by dividing the number of AM-positive segments by the total number of segments scored and multiplying this value by 100.

Statistical analysis

Data were statistically analyzed using Analysis of Variance (ANOVA) following the Statistics 10 package.

RESULTS AND DISCUSSION

Nodulation, fresh and dry biomass, and growth parameters

Results on the effect of inoculation of AMF, Rhizobium, and Sclerotium rolfsii on nodulation, fresh and dry biomass, and growth parameters of groundnut are presented in Table 2. Significant differences were found in the case of nodulation, fresh and dry biomass, and growth parameters of groundnut in both of the years.

In the year 2019-2020, the highest nodule number (30.58 plant-1), nodule weight (75.83 mg plant-1), fresh root weight (0.64 g plant-1), fresh shoot weight (16.92 g plant-1), dry root weight (0.19 g plant-1), dry shoot weight (3.71 g plant-1), plant height (18.11 cm), and no. of branch plant-1 (6.17) were observed in AM+Rhizobium treatment. The lowest nodule number (16.17 plant-1), nodule weight (36.67 mg plant-1), fresh root weight (0.44 g plant-1), fresh shoot weight (11.63 g plant-1), dry root

weight (0.14 g plant-1), dry shoot weight (2.23 g plant-1), plant height (15.48 cm), and no. of branch plant-1 (4.25) were observed in Sclerotium treatment. In all cases, Sclerotium+AM+Rhizobium treatment produced higher parameters compared to Sclerotium treatment.

In the year 2020-2021, the highest nodule number (13.13 plant-1), nodule weight (80.00 mg plant-1), fresh root weight (1.42 g plant-1), fresh shoot weight (46.42 g plant-1), dry root weight (0.49 g plant-1), dry shoot weight (12.14 g plant-1), plant height (41.00 cm), and no. of branch plant-1 (5.50) were observed in AM+Rhizobium treatment. The lowest nodule number (6.38 plant-1), nodule weight (31.25 mg plant-1), fresh root weight (0.97 g plant-1), fresh shoot weight (35.34 g plant-1), dry root weight (0.32 g plant-1), dry shoot weight (8.71 g plant-1), plant height (36.75 cm), and no. of branch plant-1 (4.13) were observed in Sclerotium treatment. In all cases, Sclerotium+AM+Rhizobium treatment produced higher parameters compared to Sclerotium treatment.

Table 2: Effect of inoculation of AMF, Rhizobium and Sclerotium rolfsii on nodulation, fresh and dry biomass, and growth parameters of groundnut during 2020 and 2021

Treatments	Nodule number plant-1	Nodule weight (mg plant-1)	Fresh weight (g plant-1)		Dry weight (g plant-1)		Plant height (cm)	No. of branch plant-1
			Root weight	Shoot weight	Root weight	Shoot weight		
2019-2020								
AM	24.58bc	59.17bc	0.57ab	15.27a	0.16bc	3.55ab	17.43ab	5.92abc
Rhizobium	25.17bc	63.33ab	0.61a	15.88a	0.17ab	3.61ab	17.83a	6.00ab
AM+Rhizobium	30.58a	75.83a	0.64a	16.92a	0.19a	3.71a	18.11a	6.17a
Sclerotium	16.17d	36.67e	0.44c	11.63c	0.14c	2.23d	14.70c	4.25d
Sclerotium+AM	21.42c	47.50cde	0.47c	12.79bc	0.15bc	2.58d	16.92ab	5.17bc
Sclerotium+Rhi.	21.67c	54.17bcd	0.57ab	14.93ab	0.15bc	2.69cd	17.41ab	5.58abc
Scle.+AM+Rhi.	27.25ab	66.67ab	0.59a	15.94a	0.17ab	3.16bc	18.03a	6.00ab
Control	16.50d	40.83de	0.50bc	12.93bc	0.15bc	2.45d	16.00bc	5.08cd
SE (\pm)	1.40	4.60	0.03	0.78	0.01	0.17	0.63	0.59
F test	**	**	**	**	*	**	**	**
CV (%)	12.19	16.56	9.38	10.68	12.52	11.09	6.94	10.93
2020-2021								
AM	10.38b	61.50b	1.10bcd	37.69d	0.43ab	10.08bcd	40.15a	5.25a
Rhizobium	11.00b	65.00b	1.22b	40.94c	0.45a	11.36ab	40.25a	5.25a
AM+Rhizobium	13.13a	80.00a	1.42a	46.42a	0.49a	12.14a	41.00a	5.50a
Sclerotium	6.38d	31.25e	0.97d	35.34d	0.32c	8.71e	36.75b	4.13b
Sclerotium+AM	10.13b	42.50cd	1.08bcd	36.88d	0.36bc	9.00de	39.50a	5.13a
Sclerotium+Rhi.	10.25b	45.00c	1.14bc	42.95bc	0.43ab	10.91abc	39.50a	5.25a
Scle.+AM+Rhi.	13.00a	62.50b	1.21b	45.43ab	0.47a	11.57a	40.63a	5.38a
Control	8.13c	33.75de	1.06cd	36.51d	0.34c	9.87cde	39.75a	5.25a
SE (\pm)	0.49	3.00	0.05	0.91	0.03	0.44	0.81	0.17
F test	**	**	**	**	**	**	*	**
CV (%)	9.53	11.37	8.97	4.51	13.23	8.49	4.07	6.24

AM: Arbuscular Mycorrhiza, Rhi.: Rhizobium; Scle.: Sclerotium. The values represent means of 04 replicates. Different letters within each column indicate significant differences between treatments. Test Statistix 10. **Significant P≤0.01. *significant P≤0.05.

Root colonization, spore population, yield, and yield attributes

Results on the effect of inoculation of AMF, Rhizobium, and Sclerotium rolfsii on root colonization, spore population, yield, and yield attributes of groundnut are presented in Table 3. Significant differences were found in all the parameters mentioned

above.

In the year 2019-2020, the highest root colonization (45.00%), spore population (109.00, 100 g-1 soil), nut (18.42 plant-1), kernel (26.08 nut-1), kernel weight (11.50 g plant nut-1), nut yield (16.52 g plant-1) and stover yield (16.88 g plant-1) were observed in AM+Rhizobium treatment. The lowest root colonization (00.00%), spore population (57.00, 100 g-1 soil), nut (09.17 plant-1), kernel (12.83 nut-1), kernel weight (5.98 g plant nut-1), nut yield (8.84 g plant-1) and stover yield (12.21 g plant-1) were observed in Sclerotium treatment. In all cases, Sclerotium+AM+Rhizobium treatment produced higher parameters compared to Sclerotium treatment. Dual inoculation (AMF+Rhizobium) increased 59.61% nut yield and 23.21% stover yield compared to control. On the contrary, Sclerotium rolfsii+AMF+Rhizobium increased 65.50% nut yield and 36.45% stover yield compared to only Sclerotium rolfsii treatment.

In the year 2020-2021, the highest root colonization (45.00%), spore population (61.50, 100 g-1 soil), nut (14.58 plant-1), kernel (20.67 nut-1), kernel weight (8.09 g plant nut-1), nut yield (12.24 g plant-1) and stover yield (27.71 g plant-1) were observed in AM+Rhizobium treatment. The lowest root colonization (00.00%), spore population (41.50, 100 g-1 soil), nut (6.75 plant-1), kernel (9.25 nut-1), kernel weight (3.64 g plant nut-1), nut yield (7.65 g plant-1) and stover yield (13.75 g plant-1) were observed in Sclerotium treatment. In all cases Sclerotium+AM+Rhizobium treatment produced higher parameters compared to Sclerotium treatment. Dual inoculation (AMF+Rhizobium) increased 26.32% nut yield and 33.74% stover yield compared to control. On the contrary, Sclerotium rolfsii+AMF+Rhizobium increased 52.94% nut yield and 99.35% stover yield compared to only Sclerotium rolfsii treatment.

Table 2: Effect of inoculation of AMF, Rhizobium and Sclerotium rolfsii on root colonization, spore population, yield and yield attributes of groundnut during 2020 and 2021

Treatments	Root colonization (%)	Spore population (100 g-1 soil)	Nut plant-1	Kernel plant nut-1	Kernel weight (g plant nut-1)	Nut yield (g plant-1)	Stover yield (g plant-1)
2019-2020							
AM	40.00ab	93.50b	16.83ab	24.67ab	10.29ab	15.77ab	16.20a
Rhizobium	0.00d	77.00cd	17.17ab	25.25ab	10.56ab	16.36a	16.45a
AM+Rhizobium	45.00a	109.00a	18.42a	26.08a	11.50a	16.52a	16.88a
Sclerotium	0.00d	57.00e	9.17c	12.83c	5.98c	8.84c	12.21c
Sclerotium+AM	35.00b	82.50bc	14.75b	21.25b	9.23b	13.42b	15.80ab
Sclerotium+Rhi.	10.00c	75.00cd	16.33ab	22.58ab	9.42b	13.58b	15.08ab
Scle.+AM+Rhi.	35.00b	94.50ab	17.33ab	25.75a	10.68ab	14.63ab	16.66a
Control	5.00cd	67.50de	10.75c	13.67c	6.41c	10.35c	13.70bc
SE (±)	2.67	5.00	1.12	1.39	0.64	0.90	0.76
F test	**	**	**	**	**	**	**
CV (%)	25.15	12.20	14.83	12.90	13.78	13.10	13.33
2020-2021							
AM	40.00ab	55.00ab	11.75bc	15.42b	7.21ab	10.04bcd	21.03bc
Rhizobium	15.00c	53.00b	11.83b	15.92b	7.47ab	10.18bc	22.59b
AM+Rhizobium	45.00a	61.50a	14.58a	20.67a	8.09a	12.24a	27.71a
Sclerotium	0.00d	41.50c	6.75d	9.25c	3.64c	7.65e	13.75e
Sclerotium+AM	35.00b	52.50b	9.83c	10.08c	4.63c	8.31de	18.10d
Sclerotium+Rhi.	15.00c	51.50b	10.92bc	14.17b	6.25b	9.11cde	20.67c
Scle.+AM+Rhi.	35.00b	61.00a	14.25a	19.50a	7.97a	11.70ab	27.41a
Control	0.00d	43.50c	11.50bc	15.17b	6.85ab	9.69cd	20.72c
SE (±)	2.56	2.45	0.66	0.76	0.42	0.63	0.63
F test	**	**	**	**	**	**	**
CV (%)	22.13	9.36	11.50	10.09	13.03	12.85	5.88

AM: Arbuscular Mycorrhiza, Rhi.: Rhizobium; Scle.: Sclerotium. The values represent means of 04 replicates. Different letters within each column indicate significant differences between treatments. Test Statistix 10. **Significant P≤0.01.

CONCLUSIONS

The findings of this study suggest among all treatments, the dual combination of AMF plus Rhizobium was most effective in increasing biomass, nodulation, colonization, yield, and yield attributes in rhizosphere soils of groundnut. Dual inoculation (AMF+Rhizobium) increased nut yield (59.61% in 2020 and 26.32% in 2021) and stover yield (23.21% in 2020 and 33.74% in 2021) compared to control. On the contrary, Sclerotium rolfsii+AMF+Rhizobium increased nut yield (65.50% in 2020 and 52.94% in 2021) and stover yield (36.45% in 2020 and 99.35% in 2021) compared to only Sclerotium rolfsii treatment. Thus, AMF and Rhizobium combinations could control groundnut foot and root rot disease more effectively than either biocontrol agent applied alone, which would be the vital basis of sustainable agricultural systems.

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ANALYSIS OF JAPA INTEREST AMONG CSC PG STUDENTS OF UNIVERSITY OF IBADAN NIGERIA

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ABSTRACT

Japa means to leave an environment quickly. There is a long tradition of people moving from one location to other for reasons best known to each individual, such as people commuting from one location to other which can be within the same nation or internationally. This has made some nations to be more populated than the other, some have increased in power, some increased in number. The increase in the population of a community can either add positively or negatively to the economy, crime rate etc of that community. It is important to know the interests in the heart of the masters' students of University of Ibadan to consider leaving Nigeria as quick as possible especially after the long strike by the Academic Staff Union of Universities (ASUU), Nigeria and the hardship in the country. Through the use of Microsoft Power BI software, this article analyzed and evaluated the interest rate of Japa among the current final year Computer Science Masters students of University of Ibadan Nigeria with sample of the students in the class. From the available clustered data through interviews and survey questionnaires, the analysis addressed the interest rate of the students to quickly leave the country, the reason they want to leave Nigeria and the effects on the country Nigeria.

Keywords: Commuting, Japa, Microsoft Power BI analytics, University of Ibadan students.

INTRODUCTION

According to newspaper publication by Chidirim & Njideka, 2022, "Japa" is a Yoruba popular slang that can be translated into English words as: "to run, flee, or escape". The word "Japa" is recently used in Nigeria as a vernacular to means migration from Nigeria to other countries, especially the United Kingdom, the United States, and Canada, Australia, Malaysia and other economic viable countries. But what do Nigerians really see in those countries that are not in their country and what brought the interest of them being eager to leave Nigeria? This is what we analyzed in this research work to mine the interest rate of Nigerian students using the current final year Computer Science Masters' students of University Ibadan as at the period of this research work.

One of the blessings Nigeria being the largest country in West Africa have is oil, and it is the largest country in West Africa. Nigeria has large lands, distinct talent in arts, in entertainment, media and technology. Though Nigeria have resources and riches, the problems facing Nigeria are insecurity, improper way of management of funds, poverty, education and medical facilities not properly funded, bad roads and a lot more.

The hardship experienced by Nigerians forces them to find a better place to stay. According to Chiamaka & Caleb, 2020, migration of workers with high skills from nations that are still underdeveloped has both positive and negative impact on the country. A quite large number of Nigerians mainly professionals like Health workers, Engineers, Software Developers and many others left Nigeria for other countries. By so doing, it is termed that they have "japa". Some of the reasons why Nigerians flee from their country is to have a better life, education, job, exposure, change career, flee from incessant strikes, finding greener pastures, secure good future and so on.

Apart from the hardship Nigerian students face outside academics, they still face the issue of Academic staff union of universities (ASUU) strikes. Strike is a practice by ASUU which is used as a tool for the Federal Government to honor agreement between

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both parties.

According to Ogunode & Agbaje, 2021, inadequate funding of educational administration and planning programme, inadequate academic staff, lack of laboratory, poor research funding of educational administration and planning programme, inadequate infrastructural facilities and poor capacity development of lecturers in educational institutions were identified as some of the challenges that faced by higher institutions in Nigeria. These are part of the reasons why Nigerian students are leaving the country to other countries to run away from such hardship. There is need for all these problems to be seriously tackled so that Nigerian students can learn with some comfort. The way forward as stated by the Ogunode & Agbaje, 2021, are: government should increase the funding of educational administration and planning programme, employment of more academic staff, provision of adequate infrastructural facilities, effective staff development, provision of research grant for the academic staff, implement all agreement signed with union groups and motivation of lecturers.

This paper seeks to evaluate and analyze the interest rate of the Masters students of Department of Computer Science University of Ibadan Nigeria using the current final year as at the time of this study as a case study. By so doing, we compared the percentage of those looking out for ways to flee from Nigeria to those that were still okay with staying in the country. The reasons why they wanted to leave the country was also analyzed and the effects of students fleeing from Nigeria from their own perspective were analyzed. We analyzed the percentage of the students that has already left the country and the country that they went to. All the analysis provided statistics on students thought patterns and the country they intended to flee to.

This research is significant because it will help the school authorities of the University of Ibadan to know the students' motivation in continuing with the current Masters program or not now that the ASUU strike has been called off after 8 months of students staying at home despite the hardship experienced in the country. This will help the school authorities, the country and the world at large to know how the students of Nigeria can be supported psychologically and otherwise.

The remaining part of this paper is organized as follows: Section two contains a brief review of related literature necessary to establish adequate knowledge of the work and establish the research gap. Section three gives the materials and methods adopted in this work. Section four shows the results obtained from the test implementation and the discussion of the various results. Section five conclude the paper and give insight into future work that may be necessary.

LITERATURE REVIEW

Jahnea, 2022 explained that education and economical opportunities outside the country are the reasons why Nigerians migrate. Though during the time between 1950s and 1980s, emigration was a result of building a nation and decolonization projects. The author also explained that the movement of Nigerians to other countries like United State was a temporal stay. But now, Nigerians are only interested in moving permanently to other countries unlike before that it was temporal. According to the author, from the late twentieth century and the early twenty-first century, the migration of Nigerians was as a result of the instability in politics and also the ambitions of the various individuals to go after opportunities in education and economic. Opportunities in academics and economically abroad are other reasons that Nigerians migrate to other countries. The major reason for some people is the state of the insecurity ongoing in the country.

Jahnea, 2022 also stated that the United State is actually one of the countries that are at the top list of the countries that Nigerians migrate to because of stability in US economy and educational opportunities. This has birthed high population of Nigerian-American society in the United State. The author reported the increment by 79% of the population of Nigerian immigrants in US from 2010 to 2019. This shows that aside United Kingdom and Canada, United State is a country in which Nigerians develop interest day and night to flee to.

Ojoko, 2022, one of the many websites that provides news about various issues going on in Nigeria, highlighted the surge in the number of people traveling out of the country and the danger it can bring to Nigeria in the future. The author made a list of the different jobs that help people get out of Nigeria. These jobs include software and hardware engineers, system integrators, digital marketers, accountants, auditors, etc. In addition to what has been stated previously, school is still another means of people fleeing out of Nigeria. In the research, Nigerians flee from their country mostly to Canada which made Canada to have the highest percentage of Nigerians. Places like United Kingdom, United State, France and some other European countries are countries of interests where people flee to also. The similarity that can be observed in the interest of Nigerians in fleeing abroad is channeled to Canada than other countries. By so doing, we can assume that Canada will be highly populated with Nigerians than other countries. The author pointed out the fact that even before now, students used to go abroad to school but still return back to the country to become established but now it is a different story as people are looking for ways to flee without returning

back to the country. Nigerian students now have reasons to travel out of the country without coming back due to insecurity in Nigeria, hardship and ASUU strike.

The author gave a statistic that showed the number of Nigerians that were granted visas to study in the United Kingdom (UK) in May, 2022 which increased for over 500 percentage within two years. There were increase from the number of students granted visas from 9,355 in March 2020 to 58,887 in March 2022. The increase in September, 2022 is 70,000. This is a whole lot which shows that the interests of Nigerians fleeing abroad builds up geometrically daily.

The increment for that of Canada according to the author is from 10,550 in March 2020 to 13,745 in March 2022 which is an increment by 30.0 percent.

From the above statistics, it shows that Nigerians are fleeing from Nigeria to other countries like Canada, UK etc daily. Nigerians are known to be hardworking, the countries they flee to can experience good economy since Nigerians will be there to help in building the economy.

A research conducted by Adeyanju & Olatunji, 2021 looked what motivated the Nigerian to emigrate from Nigeria to Canada for undergraduate study while receiving sponsorship from their parents. The students of this century were researched to be interested in fleeing from Nigeria without receiving help from their parents. Though postgraduate students might have reached the stage of sponsoring themselves to study in Nigerian Universities but the large sum of money for payment of fees for school fees abroad is enough to have Nigerian students to depend on sponsors like their parents to be able to cope outside Nigerians but the Nigerian students are mainly interested in fleeing out of the country without bothering about survival. The author pointed out the fact that the Nigerian parents that belongs to the middle or upper class are those that sends their children to Canada to study. It is actually easy for respected families' children to be admitted in Canadian schools because during or after study, they can become citizens of Canada. By so doing, it boosts the economy of the country because educated students joining the workforce of the country can add positively to the development of the country.

The author pointed out the global interests in international education showing that not only Nigeria students are interested in schooling abroad, other countries too are interested in schooling abroad not minding if the school is a public school or not.

According to Ogunode, et al., 2022, the factors and reasons that made Nigerians to patronize foreign higher education are: Inadequate funding of higher education, inadequate higher institutions, unstable academic calendar, admission problems, poor quality of higher education, inadequate infrastructural facilities, insecurity problem, un conducive learning environment, scholarships opportunities, and competitive higher education using secondary and primary data which were sourced from print and online resources. The author gave comparison between Motivators factors which makes employees to have internal motivations within them and Hygiene factors which makes the employees to be unhappy and not satisfied but they cannot be motivated. Motivators factors were stated to be inclusive of provision of adequate and modern higher institutions which is across the country that have adequate staff and good facilities. While the hygiene factors are: inadequate staff, shortage of facilities, inadequate funding of the higher institution and so on. The author also stated that the Governments are not looking at the ways to motivate its people by the Government tackling issues related to Hygiene factors. If those problems were being solved, this will reduce the rate at which Nigerians are fleeing to other counties and this will help in the development of the country. One of the factors as mentioned by the author is inability for Nigerians to secure admission because the facilities in the higher institutions are limited but so doing few Nigerians are being admitted into Nigerians' higher institution. Between the students that are admitted into Nigerian higher institutions and those that were refused to be admitted. The percentage of those that their admission was refused should overweighs those that their application became successfully but admission is not the only reasons why Nigerian students are fleeing from the country. In addition, the author stated that instability in the academic calendar is another strong reason why the Nigerian students looked for a way to study in foreign countries. Recurrent strike by different union groups in the Nigerian higher institutions also makes the students of Nigerians to consider schooling in foreign countries.

According to Ahaotu & Ogunode, 2021, there many challenges facing Nigerian higher institutions administrators in Nigeria. Part of the problem as stated by the author they face are: inadequate funding, inadequate infrastructural facilities, inadequate personnel, brain-drain, corruption, incessant strike actions, political influence and insecurity. All of the problem mentioned by the author are not just faced by Nigerian higher institutions alone like but the entire Nigeria itself. We can assume that to solve problems that Nigerian' students are going through; the problem of Nigeria has to be addressed which will help to have governing rules that can be applied to all bodies in Nigeria. The issue of insecurity needs urgent attention because higher institution students travel everyday which expose them to high risk. Some left the country to more secured countries in order

to protect themselves. Another problem higher institution faces as stated by the author is incessant strike action by the various unions in higher institutions. Most of the higher institutions conduct masters for two years, from statistics only students from private institutions can finish up within the two years. Also, some state-owned institutions but public schools owned by Federal Government, a master's program can take a student to complete in four years. Part of the reasons for delay is strike. Students can decide to flee to countries that less than one years to run masters' program. Comparing such an international program with the local program, it can be noticed that students learn more in such well structured environment than that of Nigeria.

MATERIALS AND METHODS

A survey method was used as according to Esiefarienrhe & Mokeresete, 2022, it involves the use of questionnaires and interviews. The survey consists of 2 multiple-choice questions, 7 option-choice questions where only one response can be chosen from the list of options and 4 open-ended questions where the participants were allowed to freely express themselves. These amounted to a total of 13 questions in the questionnaires. The aim was to conduct the survey with the entire final year Masters student of Computer Science Department at University of Ibadan, Oyo State Nigeria. The students received lectures remotely during the first year due to guiding rules applied during pandemic. The participants were reached through WhatsApp by sending the link of the survey created using Google form to them, constant follow up was applied to remind them in attending to the questionnaires because majority of them were busy with work, some even schooled outside the country during the eight months ASUU strike. It was discovered that some of the students dropped out and wished not to be contacted for any academic work. There was no way to get the list of those that couldn't continue with the program but we were able to get 33 students to fill the survey.

Before analysis, the dataset was checked for missing data and outliers. There were absolute no much missing data because since we dealt with the class of Computer professionals, less work was needed to put them through on how to go about the filling of the survey. The datasets were then been analyzed using Microsoft Power BI tool after downloading the online responses from Google account to Microsoft Excel.

In order to gain better insight into the study range, semi-structured interviews were conducted with 4 of the students which were identified to had left for study in United Kingdom and United State.

They were contacted because they left in the period when schools were on strike. We wanted to be sure that our study questions were enough to get the right data extracted and they were also asked few questions on the research topic.

The surveys were used to further select participants for further interviews. The interviews were conducted through WhatsApp video calls and lasted approximately 10 minutes each in other not to take much of their time since they were still trying to settle down in the new countries they flee to. Answers were recorded by note-taking.

Some of the responses were in sentences, the visualization tool for theming related text were used to analyze them. While the rest were analyzed and visualized using other tools used for quantitative analysis in Microsoft Power BI.

We chose to use Microsoft Power BI because it is an analytics tool that is powerful for analysis. It was flexible in term of use for the research team, help in analysis of data of all sizes and can easily share insights. It helped us to monitor the data closes because as we were getting updated data, instant update was done on the dashboard. The rich dashboards helped us to get instant answers and quickly derived meaning from the data set. We were also able to work both online and locally on our system.

To be able to analyze the data quantitatively, some responses that were not numeric in nature like Yes, No, Maybe. The replace tool in Microsoft Power BI was used to replace Yes with 1, No with 0 and

Figure 1: Questionnaire's Sample for Data Gathering

Maybe with 2.

Microsoft Power BI was a good tool for this research as it was very simple for the entire team to be able to learn and use within few hours. It requires less coding, only few lines of codes were written to calculate the percentage for proper analysis of some of the datasets. The click and drop/apply feature made the process of the analysis to be easier for us because we could feel the touch of what we were doing instantly and we were able to make some instant changes and decisions for better analysis of the data. Figure 1 shows the sample of the questionnaire used in the collection of data.

RESULTS AND DISCUSSION

The case study, as stated earlier, was the present final years Computer Science Masters' student at the University of Ibadan as at the period of this research. After the formatting of the data gathered and using of Microsoft Power BI tools for analysis, below contains the screenshot of the result of the visualized data.

From figure 2 above, the number of female participants outweighs that of male participants. That is, 58% of the participants are female while 42% are male.

From figure 3 above, 70% of the class wants to leave the country and presently, only 9% of the students in the class had already left the country for other countries.

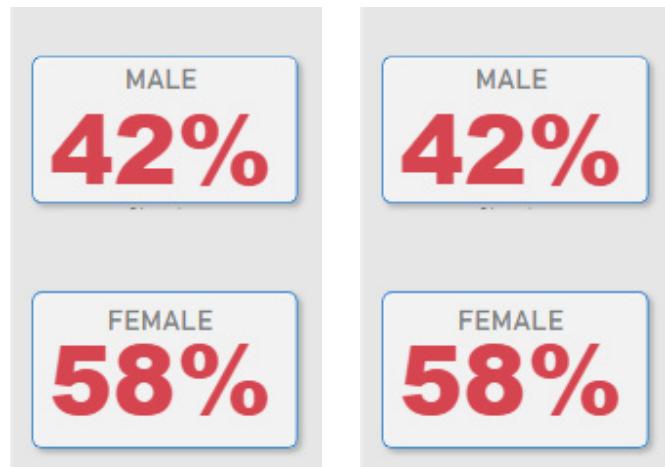


Figure 2: Participants Percentages

Figure 3: Percentage of Students that Want to flee and Had Already flee



Figure 4: Reasons Why Students Left Nigeria

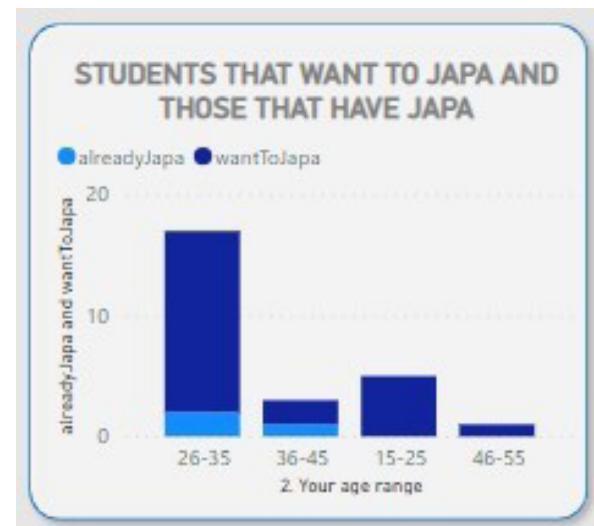


Figure 5: Cluster Column Chart for Students That Wants To Leave Nigeria and Those That Had Already Left

According to the respondents in figure 4 above, there are various reasons why students flee the country, and part of the reason is the 8-month strike embarked on by the Academic Staff Union of Universities (ASUU). During the period, some of the students gained admission to schools abroad and left as it can be seen in figure 5 above. Aside from the strike, other reasons why people want to flee Nigeria are stated in Figure 4 above.

From figure 5, comparing the analysis of the students that had the passion to leave Nigeria with those that had already left, people of the age range of 15 to 25 years are those that had the highest interest in leaving Nigeria and also those that had already left belong to that age range. It is amazing that those over 45 wanted to leave the country too.

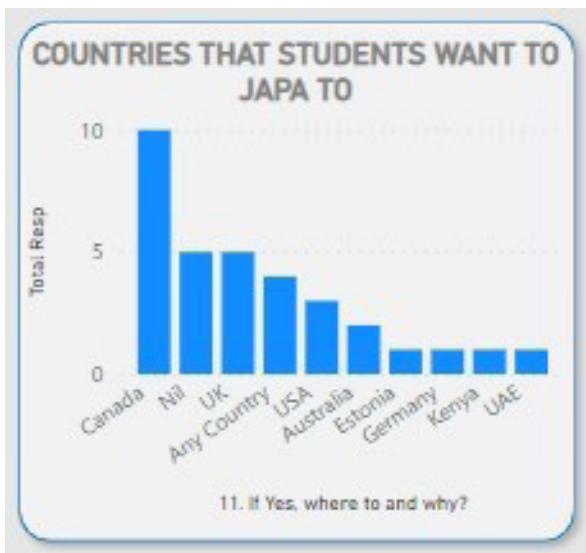


Figure 6: Countries of Interests That Students Want to Flee to

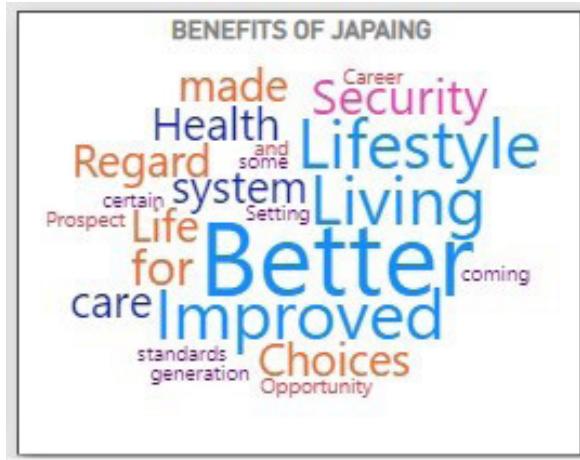


Figure 5: Benefits of Leaving Nigeria

From figure 6 above, Canada has the largest number of students interested in leaving the country, followed by United Kingdom and then United State. Estonia, Germany, Kenya, and the UAE have the same number of interested students going to those countries, but Australia has higher interests than those four countries. Some students do not mind going to any country. While some students are not interested in leaving Nigeria, majority of the final year students of Computer Science Master's program at University of Ibadan would like to flee from the country to be able to enjoy the good benefits as shown in Figure 7.

From figure 8, the participants stated the effects that students leaving Nigeria for other countries will have on Nigeria as it was established earlier in this research work. Some of the effects state are: the country will continue to be underdeveloped, drain brain/talent as professionals and the brilliant students would have all left the countries, there will be inflation and a prolong period of poverty and uncertainty in the living condition of those staying behind in Nigeria.

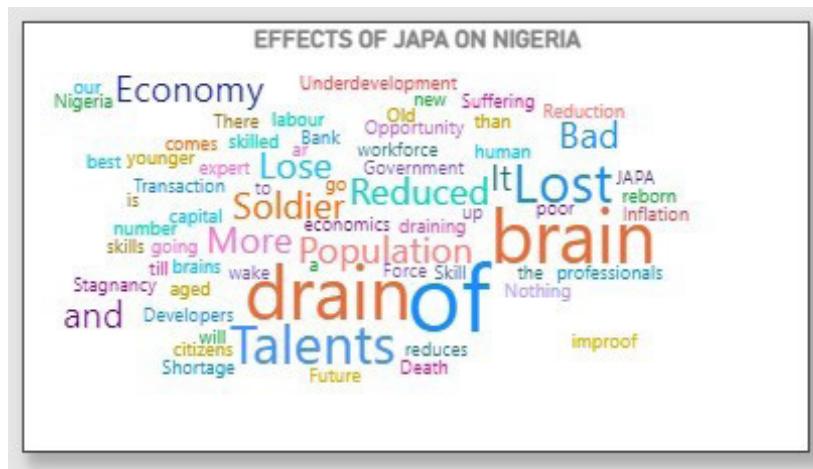


Figure 8: Effects of Leaving the Nigeria

CONCLUSION

There are so many reasons why presently students flee from Nigeria and more are becoming interested in fleeing from Nigeria on a daily basis. Some of the problems were highlighted by the students in this case study. This is not only limited to those stated but the fact that almost all the students wants to leave the country shows that Nigeria Government needs to work harder to improve the living standard of Nigerian students. This research is important to enable the Nigerian Government to be aware that aside a large number of Nigerian students from whom are professionals that had already left the country, the interest rate of those that are still within the country grow every day and very soon Nigerian higher institutions would have less students in their universities, hence there is need for the Nigerian Government to step in and help the Nigerian students. This research is relevant because it will help the Nigerian Government and other organizations in Nigeria to frequently get updated in what is in the mind of the students to better know how to support the Nigerian schools and students. This research is applicable in area of policy making by the Government and schools, welfare of the Nigerian schools, country development and so on. The University of Ibadan Nigeria needs to come up with strategy to motivate the current students since majority are focusing on how

to flee from Nigeria. In conclusion, students will like to have better life and secured future. If the situations in Nigeria are not improved, students will struggle just to manage to save enough funds for them to leave and secure better jobs and living outside the shores of this country. From the analysis from this research paper, it is better for students to flee from Nigeria.

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FACIAL EXPRESSION RECOGNITION USING EXTENDED CNN

Antika Saha¹, Rashed Mustafa^{1*} and Rezaul Karim¹

ABSTRACT

Facial expression recognition is an important problem in the field of computer vision. Computer vision is an interdisciplinary scientific field that deals with how computers gain high-level understanding from digital images. The facial expression recognition process formation of three stages they are face detection, feature extraction, and recognizing expression. The idea of expression recognition is helpful for people with physically disabled like hard of hearing and dumb to identify human facial expressions through the help of image processing and computer vision. The system can identify seven several facial expressions: anger, disgust, fear, neutral, happy, sad, and surprise. In the end, the design and implementation of the system are explained. The proposed method is a custom Deep Convolutional Neural Network (DCNN) model with more CNN layers and ten-fold cross-validation which is used to train and test various facial expression images with Google Colab. This paper worked on Kaggle facial expression dataset. The better accuracy of the model acquired is 85.0%, precision 0.83, recall 0.83, and f1-score 0.83 on the testing dataset.

Keywords: Convolutional neural network, Confusion Matrix, Cross-validation, deep learning, Facial expression, Feature extraction.

INTRODUCTION

The face is an essential part of an individual's human body, which plays a vital role in the extraction of an individual's emotional state. The face is responsible for communicating thoughts and ideas as well as emotions. A facial expression is one or more movements or areas of the muscles below the skin of the face. Through facial expressions, people can express their emotions. Identifying facial expression exploration has the capability to give computers to realize human emotions like anger, disgust, fear, happiness, neutral, sadness, and surprise. In nonverbal communication, the expression of faces plays an important role, which defining the interaction between humans and animals.

Deep learning is one kind of machine learning and artificial intelligence (AI) that follow the path of people getting particular categories of wisdom. It's a crucial material of data science that contains data and prognostic modeling. It's highly helpful to the scientists of data tasked with assembling, resolving, and understanding massive quantities of data; deep learning creates this method quicker and lighter. Sometimes deep learning is alluded to as deep neural learning or deep neural networking. Neural networks penetrate distinctly various forms, along with recurrent neural networks, convolutional neural networks, and artificial neural networks, and each has benefits for specific use cases. After 1960 this subject became more universal when a list of popular emotions was determined and several systems were recommended. There are seven basic popular emotions for humans. These are anger, disgust, fear, happiness, neutral, sad, and surprise.

As computers come gradually extensive and their connection with user's changes, they demand new materials to achieve a response from their correlations with those users and reciprocate appropriately. At present, there are various opportunities to derive responses from users like pulsation, articulation, gesture, visual communication, etc. But some of those opportunities are intrusive to users or do not allow sufficient or exact responses in order for a system to be dependable. After reviewing ten years of publications depending on Facial Expression Recognition build up a schematic diagram of the growth of research, where the x-axis denote the year and the y-axis denotes the total number of publications. The graphical representation of the growth of research is in figure 1.

One humble choice that provides a rational amount of facial expressions. The expressions of the face have been deliberated as a great source of knowledge to control the accurate emotions of a character (Ekman, et al, 1997). Even before Charles

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Darwin guided “studies on how people recognize emotion in faces” (Jabr, 2010), venerable minds like Aristotle, learned the significance of the expressions of faces (Russell, et al, 1997). Even so, it was before Paul Ekman managed cross-civilization analysis near the world that a set of common emotions like anger, disgust, fear, neutral, happiness, sad, and surprise; were finally approved (Bettadapura 1998, Siegman, 1978). Facial expressions can demonstrate individual emotions and show personal intentions in social situations. It can carry various emotional states and detect various physiological reactions. At present, facial expression recognition or facial expression based on the computer has motivated significant research work because of its capability to imitate human cipher skills. I have also been motivated to penetrate the advantages of the physically disabled like hard of hearing and dumb. But if the facial expression detection system can recognize their necessity by observing their facial expression then it becomes a lot lighter for them to make the associate facial expression detection system understand their demands. Currently, there is a vast amount of solutions available but still no consensus on what is the best solution when applied to images, and uncertainty.

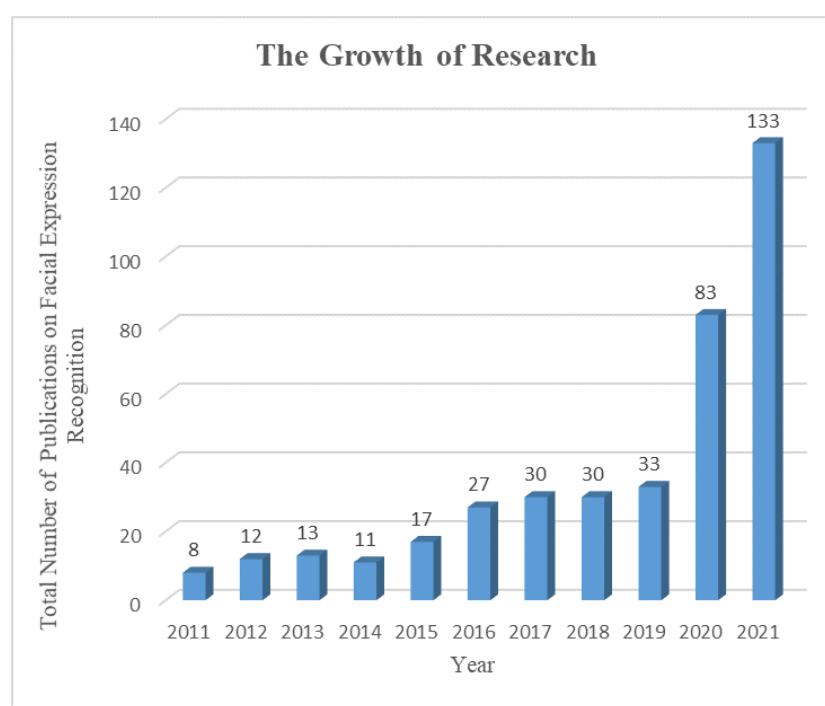


Figure 1: The graphical representation of the growth of research

LITERATURE REVIEW

This experiment has categorized six different facial emotions, which assemble into individual global expressions: anger, disgust, fear, happiness, sadness, and surprise. There is multiple research work that uses many techniques to identify facial expressions. Now is the time to discuss the research work on facial expression recognition and its limitations.

P. Liu, S. Han, Z. Meng, and Y. Tong (Liu. P, et al. 2014) proposed Boosted Deep Belief Network is operative for describing facial expression changes and appointed to form a boosted powerful classifier analytically by using a set of features. This framework can be learned from facial images’ extremely complicated features. Using that process they could classify the seven facial expressions and the classification rate is 41%.

Emad Barsoum, Cha Zhang, Cristian Canton Ferrer, and Zhengyou Zhang (Emad Barsoum, et al, 2016), the deep convolutional neural networks (DCNN) estimate the efficiency of four different patterns to train emotion identification on crowd-sourced labels. Here also handle turbulent levels by using a deep convolutional neural network (DCNN) for recognizing faces and crowdsourcing used to accumulate place of truth labels. Using that process they could classify the eight facial expressions and the highest accuracy is 85%.

Z. Meng, P. Liu, J. Cai, S. Han, and Y. Tong (Meng. Z, et al, 2017), proposed the identity-aware convolutional neural network (IACNN) process used training, expression, and recognition associated features are together assumed through a deep CNN scheme, which is collected from two duplicate CNN streams and prepared at the same time minimizing the categorization errors when maximizing the expression and recognize similarities. This method has been evaluated on two blatantly obtainable databases CK+ and MMI, the accuracies are 71.29% and 55.41%.

S. K. Lalitha and J. Aishwarya, (Lalitha. S. K, et al, 2021) proposed a raw convolutional neural network (CNN) classifier model. The output layer contains feature maps that reflect the knowledge of the image. Haar cascade classifier is used to identify the faces from the image. By using the proposed method and different types of datasets the average accuracy is 67%.

K. Chang, C. Chen and Y. Hung (Chang. K, et al, 2013) the diagram assesses the distinct predominance state of an expression depending on a single image. An effective descriptor spread covert, which is rendition invariant and can linearize deformation. For the ranking problem, this paper could not work on multiple image-based expression intensity estimations. Results describe

that the diagram with dispersed change omits. The accuracy is 71.29%.

According to the proposed work, the facial expression recognition problem has two main perspectives: validity and ability. Ability is counted in terms of time complexity, computational complexity, and space complexity. The targets of this research are to make a proposed method that has high accuracy and extreme computational complications. Here, ready an accurate set of data is a huge challenge. Another challenge is the process of creating an accurate extended CNN method for the emotional recognition of face purposes. Following the complications of the problem is necessary to be composed deep learning; the quantity of input is different.

METHODOLOGY

Deep learning current approaches are used for increasing the processing power of detailed datasets with results that overcome customary methods (Kaiming et al, 2015). The usual pipeline of deep learning for facial expression recognition systems comprises a preprocessing stage of the input image, which is organized by face alignment, data augmentation, and face normalization process. Those processed data are then passed to a neural network, mainly Convolutional Neural Network (CNN), Deep Belief Network (DBN), Recurrent Neural Network (RNN), Generative Adversarial Network (GAN), or Deep Autoencoder (DAE). After the features are turned out by the neural network those images are passed into a classifier that will classify the image. The overview of the facial expression recognition system is illustrated in figure 2. The facial expression recognition system includes the major stages such as image preprocessing, feature extraction, and expression classification.

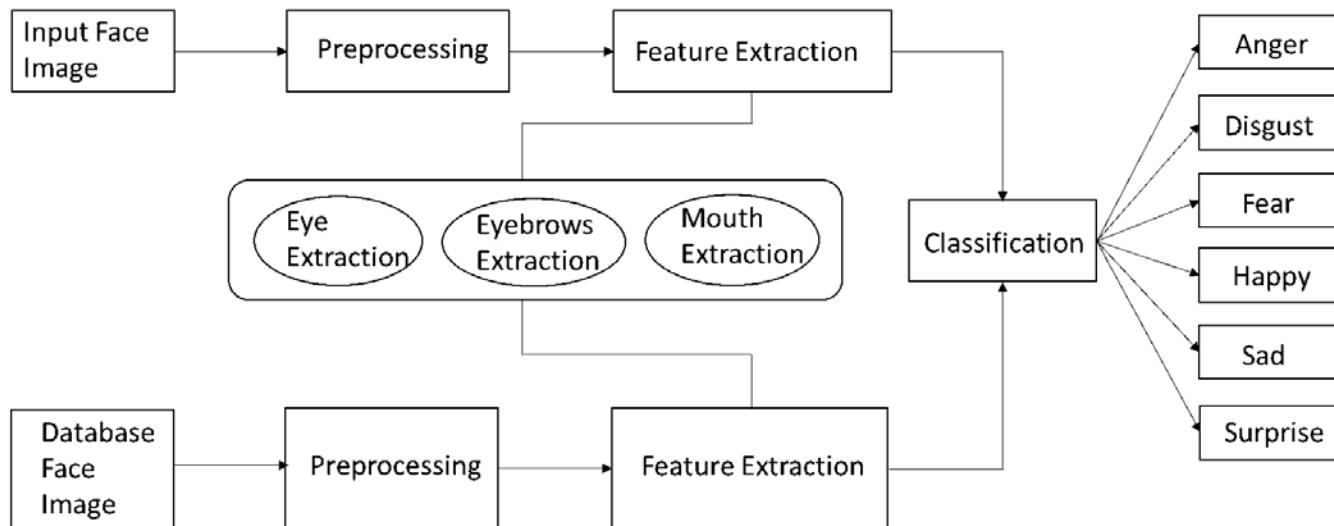


Figure 2: The architecture of the Facial Expression Recognition System

The progress and popularity of computer vision with deep learning are guided by the Convolutional Neural Network (CNN) algorithm. Convolutional Neural Network (CNN) can receive input images, train itself by learning filters to characterize distinct features between images and be capable to distinguish one image from another (Saha. S, 2018). The initial goal of Artificial Intelligence (AI) is to provide a set of algorithms used to resolve problems that humans can solve by instinct and spontaneously but this is a more challenging task for computers (Rosebrock. A, 2018). On the other hand, machine learning deals with the area of the lesson which delivers computers to know except being detailed programmed (Samuel A. L, 1959).

The proposed Deep Convolutional Neural Network model accepts an input image of 48×48 pixels and methods of various Convolution, Max-pooling, and Fully-connected layers giving the ultimate output of the other seven classes: anger, disgust, fear, happiness, sadness, surprise, and neutral. The flow diagram of DCNN is shown in figure 3.

In this system is a DCNN proposed method with 23 layers for training and testing facial images. It has 10 convolution layers, from those two convolution layers, have a 5×5 size of the filter and the other convolution layers have a 3×3 size of the filter, and the pooling layers have a size of the pool is 2×2 . After every convolution layer, an activation layer and batch normalization are included for gaining better accuracy. Every pooling layer is followed by the dropout layer for reducing the overfitting of the model. All the convolution layers are followed by two dense layers, every layer with 1024 hidden units, followed by a 60% dropout layer. The convolution-pooling batch is composed of convolution, activation, batch normalization, pooling, and

dropout gradually. Every fully-connected layer is composed of fully connected, activation, batch normalization, pooling, and dropout layers gradually. For classifying the image into the seven individual expressions of faces use a classifier that is softmax. Figure 4 shows the architecture of the implementation of a deep convolutional neural network. For the initial Convolution layer use the following equation:

$$B[i,j] = \sum_{k_1=0}^2 \sum_{k_2=0}^2 A[i+k_1, j+k_2] W_1[k_1, k_2] \quad \dots \quad 4.1$$

In equation 4.1, W_1 is the filter, and $k_1=0:2$, $k_2=0:2$. Every convolution layer used the same zero padding at $p = 1$ and stride $s = 1$. The same padding outline is used to develop the design of the architecture of networks more proficiently.

This process uses a K fold cross-validation technique to assess vertical models by separating the main dataset into a training dataset and a test set to assess it in occurrence. In this, the main dataset is modified and randomly delivered among k identically sizes sub-datasets. The outputs of this process can be built to give a distinct calculation. All surveys are applied for training and validation and each survey is applied for validation only one time. Here, using 10-fold cross-validation techniques.

As a batch normalization using an optimization algorithm that is Adam for finding better results. The results can be arranged in a Confusion Matrix that differs the predicted values from the true values. True positive

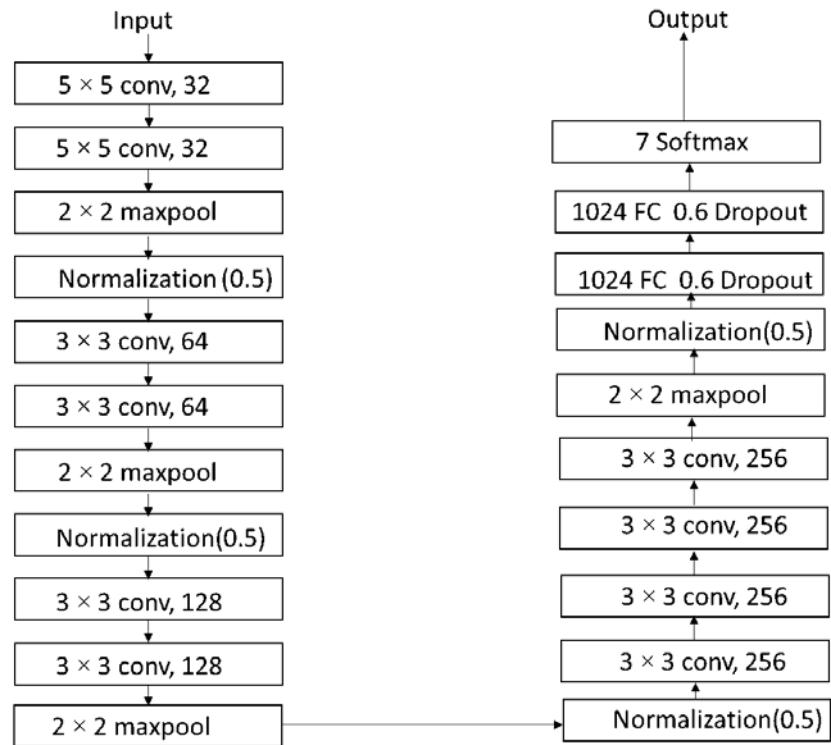


Figure 3: Flow Diagram of Deep Convolutional Neural Network

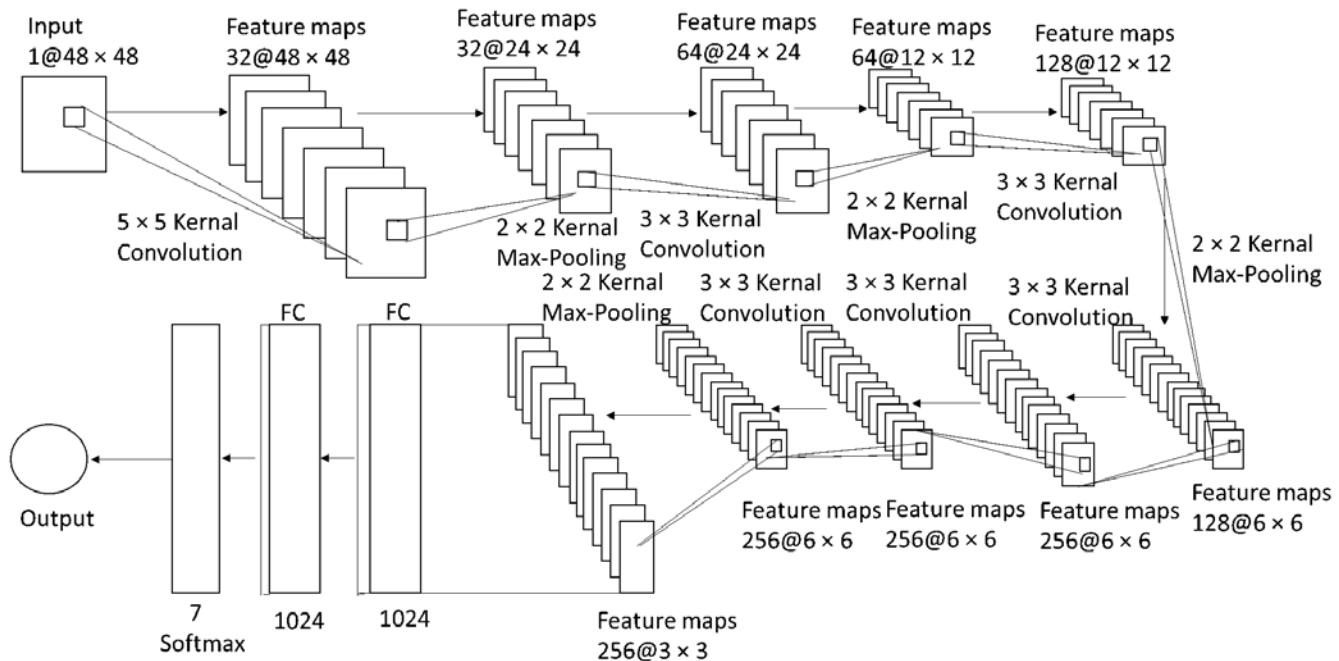


Figure 4: The architecture of the implementation of the Deep Convolutional Neural Network

meaning that data points are correct and false-negative meaning that they are incorrect. Accuracy is the fraction between the number of right predictions and the total number of predictions. It is applied for calculating the number of right predictions.

$$\text{Accuracy} = \frac{\text{TP} + \text{TN}}{\text{total}} \quad \dots \quad 4.2$$

Precision is the fraction between the number of right predictions and the total number of positive predictions. It is applied for calculating the ratio of right calculations between the positive ones.

$$\text{Precision} = \frac{\text{TP}}{\text{TP} + \text{FP}} \quad \dots \quad 4.3$$

The Recall is the fraction between the number of right predictions and the total number of all predictions. It is applied for calculating the cost of false positives in the model.

$$\text{Recall} = \frac{\text{TP}}{\text{TP} + \text{FN}} \quad \dots \quad 4.4$$

F1 score is the harmonic mean of precision and recall. It describes the number of correct predictions and the number of instances.

$$\text{F1} = \frac{2 \cdot \text{Precision} \cdot \text{recall}}{\text{Precision} + \text{recall}} \quad \dots \quad 4.5$$

DATASET EXPLORATION

The beginning of the experimental analysis of the proposed model uses different types of expression datasets. The size and color of human faces differ from each other. First, capturing the image using a camera or collecting the image from the internet. Then, the images are cropped in the face region and a face-alignment post-processing phase is directed. At last, the images are counted into similar communities of expressions. The dataset consists of a single .csv file bearing the column's emotion, pixels, and Usage. Every image illustrated in the 48×48 vectors in pixels is labeled with an encoded expression. The datasets are divided into training and validation datasets. The inconsistency is performed by using data augmentation processes or growing a cost-sensitive loss function during training.

Compared to other facial expression datasets, the FER dataset has more changes in the images, including facial repression, half faces, low-contrast images, and eyeglasses. The images were gathered using the Google image search API and resized as a result the face is high centered and holds about an equal amount of field in every image. The dataset is made of 28,709 training images, 3,589 validation images, and 3,589 test images with seven several emotions such as happiness, anger, fear, disgust, surprise, neutral, and sadness (Ian J, et al, 2013). The seven different expressions sample images from the FER dataset are shown in figure 5.



Figure 5: Seven different samples images from the FER2013 dataset

RESULTS

From the FER dataset, the expression of faces is available so the total number of samples of faces is 35887 and this dataset is used to appraise the proposed method. Compare to other evaluations, could not find any work which computes the cross-validation with the FER facial expression dataset. So now computes ten-fold cross-validation with the proposed model using StratifiedKFold tool on this FER dataset. The proposed model is built using the Sequential Keras backbone, which gives grouping a linear stack of layers into a Keras model. The proposed network is trained up to 200 epochs with 10-fold cross-validation where the datasets are divided into ten folds using StraightKFold machine learning tool by placing the exchanging parameter, optimizing the cross-entropy loss using Adam optimizer. Adam optimization is a stochastic gradient descent method that depends on the adaptive estimation of first-order and second-order moments. Using the Adam algorithm the method is computationally efficient, has little memory requirement, is invariant to diagonal rescaling of gradient, and is well suited for problems that are large in terms of data.

VGG19 (Simonyan, K, 2015) was one of the first architectures that used small kernel sizes and increased the depth of the

network with 19 layers, which led to a reduction in the number of parameters. The training results illustrated in figure 6 are the accuracy and loss learning curves. By using the ten-fold cross-validation on this VGG19 model the validation accuracy is 62.43%.

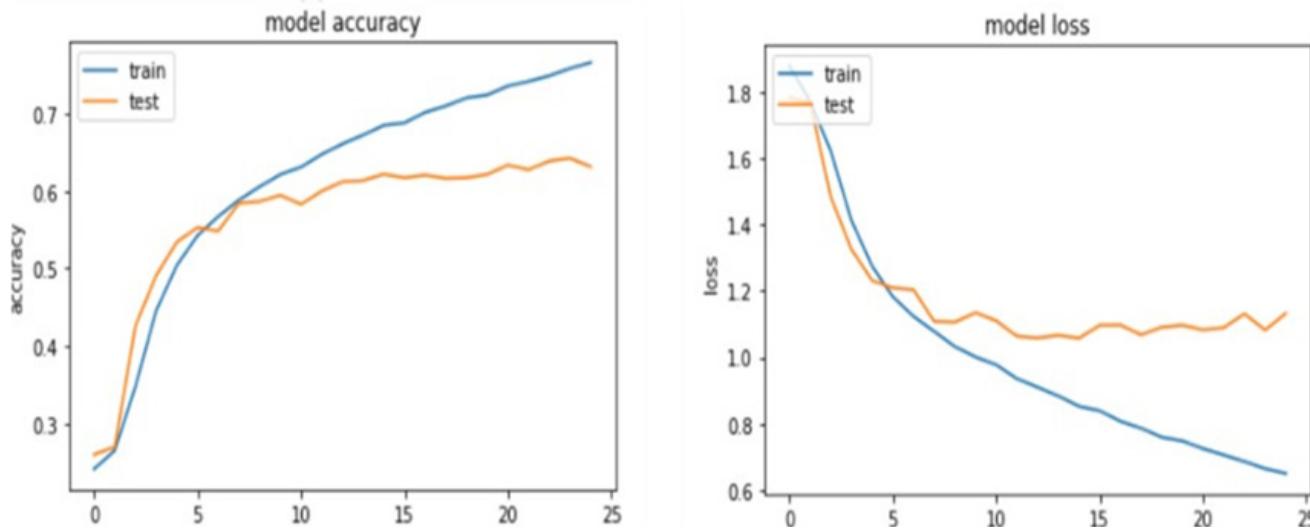


Figure 6: The VGG19 method accuracy, loss on the FER2013 dataset

By using the extended CNN proposed method and ten-fold cross-validation the validation accuracy is 85%. The facial expression recognition (FER) dataset given its size and all faces are aligned in the images. The accuracy and loss learning curves and the confusion matrix of the FER validation set are illustrated in figure 7.



Figure 7: The proposed method accuracy, loss, and confusion matrix on the FER2013 dataset

According to the extended CNN model calculated the accuracy with Adam optimizers those easy to find by the result of precision, recall and f1-score of the dataset FER2013 validation set using ten-fold cross-validation. The classification report containing precision, recall, and F1-score for each class is shown in table 1.

DISCUSSION & CONCLUSION

After that, this experiment computes by splitting the dataset using 10-fold cross-validation. For getting better accuracy split the datasets into 10% training and 90% testing, 20% training and 80% testing, 30% training and 70% testing, 40% training and 60% testing, 50% training and 50% testing, and so on. The best validation accuracy with ten-fold cross-validation is 85% compared with the

Table 1: Precision, Recall, and F1-score of the FER2013 validation set

	precision(%)	recall(%)	f1-score(%)
Angry	72	80	75
Disgust	79	66	72
Fear	88	92	90
Happy	84	87	85
Neutral	75	82	78
Sad	87	85	86
Surprise	94	91	92
Macro average	83	83	83
Accuracy			85

VGG19 model which gives the 62.43% accuracy value for recognizing the expression. Finally, the proposed extended CNN model with a ten-fold cross-validation process can classify facial expressions of humans i.e. happiness, anger, fear, disgust, neutral, sad, and surprise. Also, using the confusion matrix the model can be evaluated precision, recall, and f1-score. Using those different types of methods found the better accuracy of the model acquired is 85%, precision 0.83, recall 0.83, f1-score 0.83.

In this thesis, the target is to sketch an extension of the convolutional neural network to identify expression recognition of faces which helps the physically disabled like those hard of hearing and dumb. The direction of identifying the expression of faces is according to a sketch and improvement of a Convolutional Neural Network (CNN) able to forecast human emotions of faces. The CNN model form of ten convolutional layers arranged on the highest in all with the number of kernels redoubling in every obstacle. The extended CNN model learned on the FER2013 dataset, which makes with descriptions holding images of various lighting strikingly situations.

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SYNTHESIS, CHARACTERISATION AND COMPARATIVE STUDY OF HYDROGEL AND NANOGENELS OF PSYLLIUM

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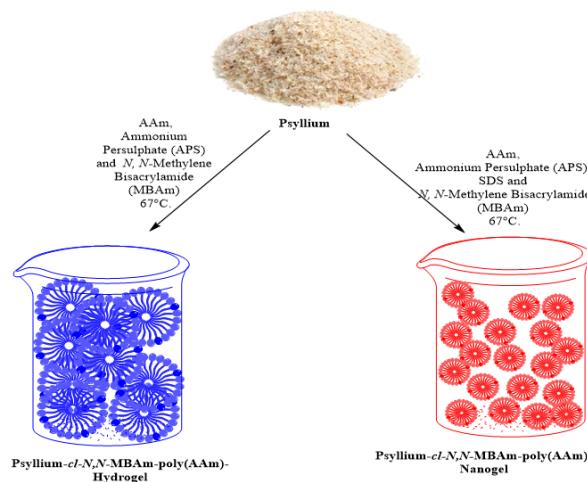
ABSTRACT

Natural polysaccharides are being explored as the matrices for attaining speciality materials for pharmaceutical, medicinal and environmental applications via chemical modification such as grafting. Psyllium polysaccharide-based hydrogels and nanogels have potential biomedical and water purification applications due to their advantageous properties such as stimulus responsiveness, biocompatibility, target drug delivery and stability. The present study aims to synthesise hydrogel and nanogels of psyllium and attain comparative data for the two to undermine their potential applications. Psyllium- cl-N,N-MBAm-poly(AAm) hydrogel (Psy-MBAm-AAm – hg) and Psyllium- cl-N,N-MBAm-poly(AAm) nanogel (Psy-MBAm-AAm – ng) were synthesised by grafting acrylamide (AAm) onto psyllium using ammonium persulphate (APS) as a free radical initiator in a redox system where N, N-methylene bisacrylamide acted as a crosslinker. The comparative study of synthesised gels was carried out by studying swelling characteristics at acidic and basic pH (7 and 4) and at varied temperatures for both matrices. The synthesised hydrogel and nanogels were subjected to characterisation by Fourier transform infrared spectroscopy (FTIR), Field Emission Scanning Electron Microscopy (FESEM), and Zeta Potential Analysis to get evidence for successful synthesis and nanogel formation.

Keywords: Hydrogel, Nanogel, Psyllium, Swelling

INTRODUCTION

Psyllium also known as Ispaghula and Isabgol is a natural polysaccharide obtained from *Plantago ovata* plant commonly known as desert Indian wheat (Mishra, S., Sinha, S., Dey, K. P., & Sen, G., 2014). More than 200 species of the *Plantago* genus (usually called psyllium) have been reported all over the world (Thakur, V. K., & Thakur, M. K., 2014) with an average height of 10 – 15 cm. The *Plantago* psyllium husk is the raw material for psyllium mucilage with arabinose (~22%), xylose (~75%), galactose (~1.5%), glucose (~0.7%), rhamnose and uranic acid as the main constituents (Cui, S. W., Wu, Y., & Ding, H. 2013). Psyllium husk is the main source of arabinoxylan (Li, Q., Wang, S., Jin, X., Huang, C., & Xiang, Z., 2020) and is of laxative nature so used for the treatment of constipation and diarrhoea due to its water-solubility and gel-forming capacity. Psyllium-based polymeric materials and hydrogels have been reported for various therapeutic and pharmaceutical applications like cholesterol reduction (Uehleke, B., Ortiz, M., & Stange, R., 2008), wound dressing (Ahmad, N., et. al. , 2021), drug delivery (Singh, B., Sharma, et. al., 2022, Kotta, S., et. al., 2022) tissue regeneration (Poddar, S., et. al., 2021), cancer treatment (Kumar, D., Gautam, A., & Kundu, P. P., 2022, Masood, R., & Mirafat, M., 2010) lower normal LDL level (Jovanovski, E., et. al.,



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2018, Everson, G. T., et. al., 1992) and Industrial applications like treating of coloured effluents from water (Druzian, S. P., 2021), wastewater treatment (Das, N., Ojha, N., & Mandal, S. K., 2021, Singh, J., Kumar, S., & Sharma, S. 2022) such as removal of Hg(II) from the solution (Kumar, D., Pandey, J., Khan, N., Kumar, P., & Kundu, P. P. 2019) to mention a few.

The hydrophilic nature of the psyllium husk imparting its excellent swell ability is one of the defining properties for its varied applications (Singh, B., Chauhan, G. S., Bhatt, S. S., & Kumar, K. (2006), Singh, B., Sharma, N., & Chauhan, N. (2007), Singh, B., & Chauhan, N., 2009). The psyllium hydrogel is explored for wide applications in various fields and little work is done with the psyllium-based nanogels. Hence, in order to get a better picture of the broad application area of Psyllium-based materials we synthesized hydrogels and nanogels of the same and tried to understand what effect the particle size has on the characteristic properties of the gels. Psyllium- cl -N, N-MBAm-poly(AAm) hydrogel (Psy-MBAm-AAm – hg) and Psyllium- cl -N, N-MBAm-poly(AAm) Nanogel (Psy-MBAm-AAm – ng) were synthesized by crosslinking Acrylamide (AAm) onto psyllium using ammonium persulphate (APS) as a free radical initiator. The synthesis of hydrogel and nanogel was characterized by swelling studies at different pH, and temperature and by subjecting them to FT-IR, FESEM, and Zeta Potential to get evidence for the successful synthesis of hydrogels and nanogels.

MATERIALS AND METHODS

Material

Psyllium, N, N-Methylene Bis-acrylamide (Loba Chemie Pvt. Ltd.), Ammonium Persulphate (Loba Chemie Pvt. Ltd.), Sodium Lauryl Sulphate (Loba Chemie Pvt. Ltd.) and Acrylamide (Loba Chemie Pvt. Ltd.), Acetone (Qualikems Fine Chem Pvt. Ltd.), were used as received. Electronic Balance BL-220H of reliability 0.001 g, water bath with thermostat, all other reagents were analytically pure and all solutions were prepared with distilled water.

Synthesis of Psyllium-cl-N, N-MBAm-poly(AAm) hydrogel (Psy-MBAm-AAm – hg)

Psyllium commonly known as Ishab Ghul obtained in the form of small needles. The raw Psyllium was warmed at 35°C to remove all the moisture content present in it and the dried Psyllium needles were crushed to a fine powder. 0.500g of AAm, 0.015g of Ammonium Persulphate (APS) and (0.015g) of N, N-Methylene Bisacrylamide (MBAm) were dissolved in 10 ml distilled water. To the homogeneous mixture 1.0g, powdered Psyllium was added slowly with continuous stirring on a magnetic stoller. The homogeneous mixture thus obtained was sonicated for 60 min at 67°C. The Pool thus obtained was cooled to room temperature (25°C) and the hydrogel formed was extracted with acetone. The homopolymer thus formed was extracted in a 60:40 ratio solution of acetone and ethyl alcohol. The extracted hydrogel was left undisturbed at 40°C in a hot air oven for 24 h. The dried hydrogel was crushed to fine powdered with pestle and mortar and labelled as Psyllium- cl -N, N-MBAm-poly(AAm) hydrogel (Psy-MBAm-AAm – hg) shown in Figure 1.

Synthesis of Psyllium-cl-N, N-MBAm-poly(AAm) nano gel

For the synthesis of Psyllium-cl-N, N-MBAm-poly(AAm) nano gel (Psy-MBAm-AAm – ng), 0.500g of AAm, 0.015g of ammonium persulphate (APS), 0.015g of N, N-methylene bisacrylamide (MBAm) and 0.01g of sodium dodecyl sulphate (SDS) were mixed in 10 ml of distilled water. To the homogeneous mixture 1.0g, powdered Psyllium was added slowly with continuous stirring on the magnetic stirrer. The homogeneous mixture thus obtained was sonicated for 60 min at 67°C. The Pool thus obtained was cooled to room temperature (25°C) for 2h and the gel formed was extracted with acetone. Extract with acetone was again homogenized in a



Figure 1: Psy-MBAm-AAm – hg hydrogel



Figure 2: Psy-MBAm-AAm – ng Nanogel

sonicator at 30°C for 20 min. The homopolymer formed was removed by extracting the pool in a solution of 60:40 ratio of acetone and ethyl alcohol. The extracted nanogel was left undisturbed for cooling at 40°C in a hot air oven for 24h. The dried gel was labelled as Psyllium– cl –N, N-MBAm–poly(AAm) Nanogel (Psy-MBAm-AAm – ng) shown in figure 2.

Separation of Hydrogels and Nanogels

The hydrogel and Nanogel synthesized with a cross-linker were treated repeatedly with ethyl alcohol to remove the homopolymer formed during the reaction. The unreacted and insoluble product was removed by simple filtration, without drying and treated with ethyl alcohol and water to get the constant weight. The dried gel was again treated with acetone.

Swelling Study

Psy-MBAm-AAm – hg hydrogel was dried at room temperature till constant weight is obtained and 0.050 g was measured with the help of electronic balance and immersed in a solution of pH 4.0 at room temperature (25°C). The swelling study was carried out using the following formula (Singh, B., Chauhan, G. S., Kumar, S., & Chauhan, N., 2007):

$$\text{Percent Swelling} = (\text{Weight of the Hydrogel} - \text{Weight of the Xerogel}) / \text{Weight of the Xerogel} \times 100$$

The pH 4.0 solution was prepared in distilled water with the help of a buffer tablet and was tested with a Digital pH meter. The palate of Psy-MBAm-AAm – hg was immersed in 10ml pH 4.0 solution and taken out after 5 min for weight measurement. The experiment was performed in duplicate and repeated for 10, 20, 40, 80, 160 and 320 minutes time intervals. The swelling study was also carried out in a solution of pH 7.4 at the same time interval. The Swelling behaviour of Psy-MBAm-AAm – hg was also studied on the same pH and after the same time interval at a temperature of 37°C.

For the swelling study of nanogel, Psy-MBAm-AAm – ng nanogel was dried at room temperature to a constant weight and grinded to a fine powder. 0.010g of Nano gel fine power is taken in a centrifuge tube filled with 10 ml solution of pH 4 and 7.4 at room temperature (25°C).

The tube was left undisturbed for 4 minutes and centrifuged for 1 minute at 2000 rpm in a centrifuging machine. The water content was decanted off and blotted dry then the weight of swelled nanogel was calculated. Each experiment was performed in duplicate at the different time spans of 10, 20, 40, 80, 160 and 320 min at the same temperature. The Swelling study of nanogel was also carried out under the same conditions in a solution of pH 4.0, 7.4 at 37°C.

RESULTS AND DISCUSSION

Psyllium cross-linked hydrogel and nanogel were characterized by FTIR, FESEM, Zeta potential and Swelling Study

FT-IR

FT-IR spectra of Psy-MBAm-AAm – hg and Psy-MBAm-AAm – ng were recorded using F.T. Infra-Red Spectrophotometer Model RZX(Perkin Elmer) 8010 between 400 to 4000 cm⁻¹ to study the modification. The FT-IR of hydrogel and nanogels of

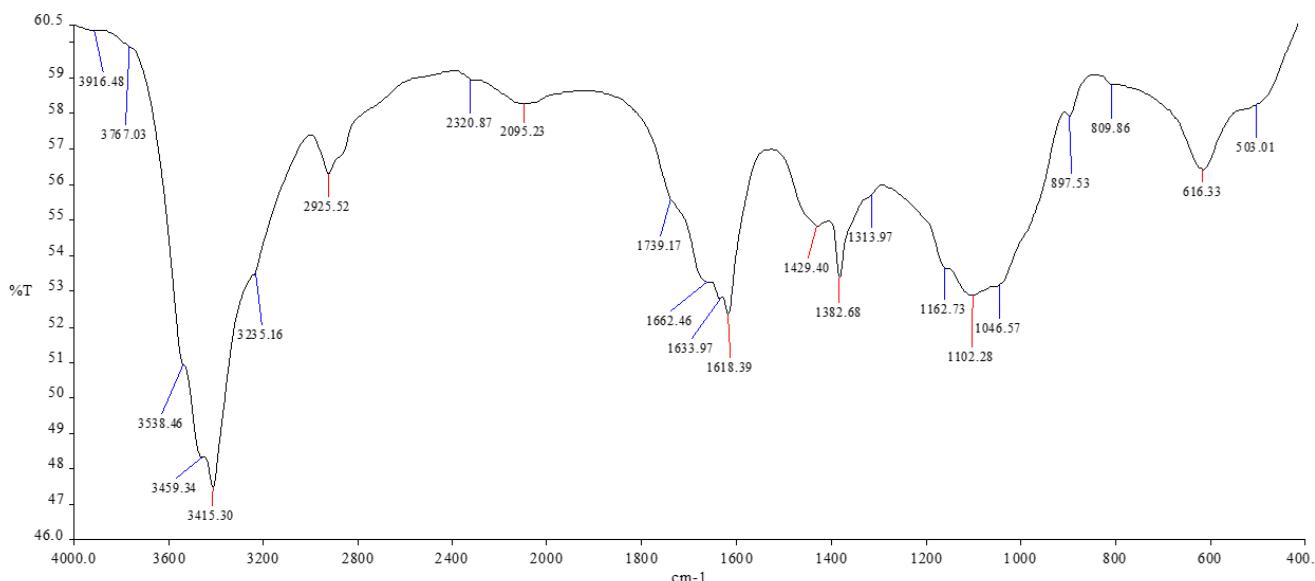


Figure 3: FT-IR of Psy-MBAm-AAm – hg hydrogel

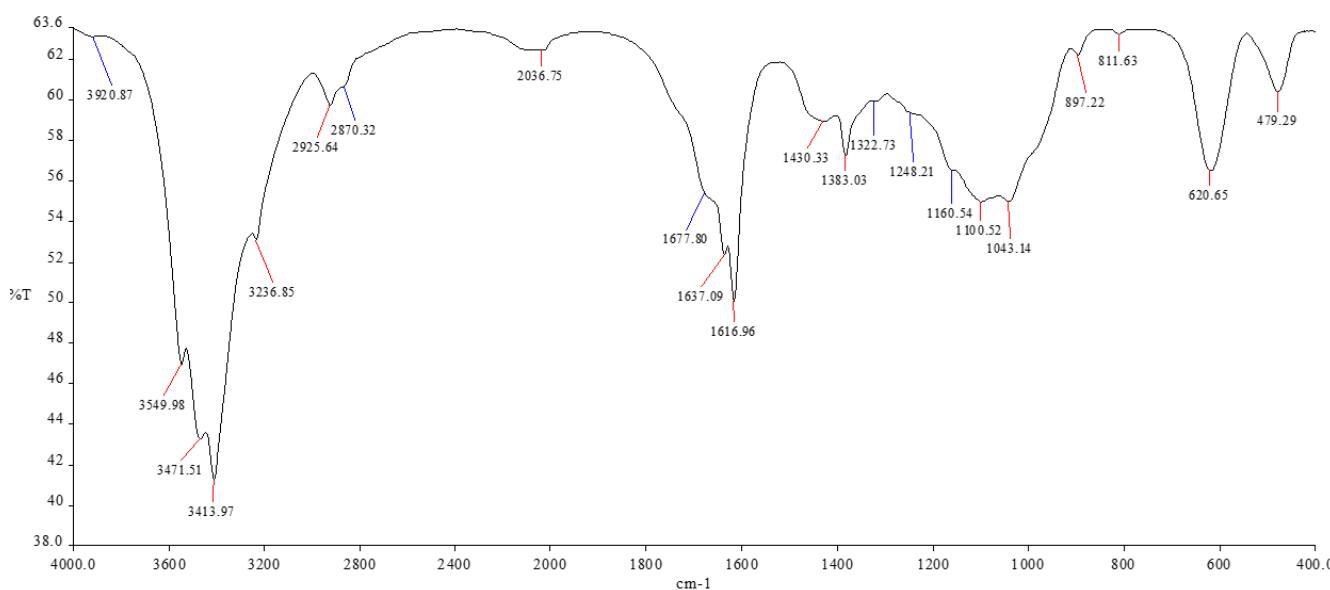


Figure 4: FT-IR of Psy-MBA-AAm – ng Nanogel

psyllium is presented in Figure 3 and Figure 4 respectively. The broad absorption band due to -OH starching is observed at 3415.30 cm^{-1} for the hydrogel and nano gel. At 2925.52 cm^{-1} the band is observed due to asymmetric starching vibration – OH and – CH. The band at 1466.27 cm^{-1} witnessed the C-H deformation vibration.

The characteristic peaks are observed at 811.63 cm^{-1} , 620.65 cm^{-1} for the grafted Psyllium hydrogel backbone. The absorption band at 1618.39 cm^{-1} and 1616.96 cm^{-1} in the FT-IR of Psyllium hydrogel and nanogels respectively are witnessed due to the stretching of the –C=O of the –COOH group and C-O-C starching vibrations.

The out of plane bending of -NH and -CN groups in gel results in a peak at 1382.68 cm^{-1} . -NH and -CH bending, generate a band at 897.22 cm^{-1} , while the band at 1034.14 cm^{-1} is due to C – O starching. In the case of Psy-MBAm-AAm – ng an additional peak at 479.29 Cm^{-1} is noticed due to C–C ring deformation in psyllium and -CH₂ wagging.

FESEM

The surface morphology of Psy-MBAm-AAm – hg hydrogel and Psy-MBAm-AAm – ng nano gel was studied using Hitachi SU 8010 Series and images at different resolutions are presented in Figure 5 (a and b). FESEM data indicated the somewhat rough surface of the hydrogels with small grooves and ridges. FESEM images of nanogel indicate structural heterogeneity in its surface, where nanoparticles of size 37.4 – 97.2 nm in diameter get accumulated together to form an aggregate molecule of diameter 500 nm . The agglomeration rate is observed to increase over time due to the hydrophilic and H-bonding nature of the nanogels.

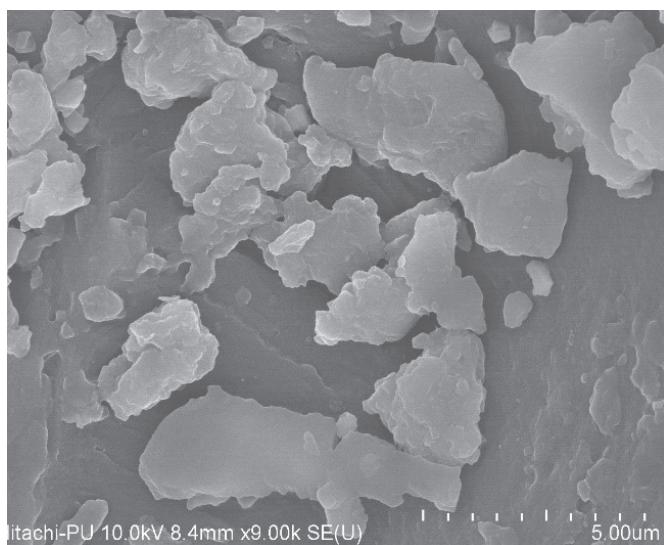


Figure 5(a) FESEM of Psy-MBAm-AAm – hg

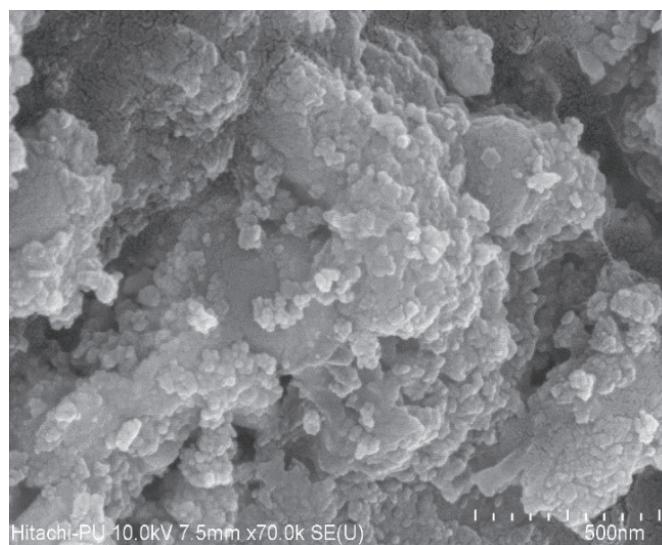


Figure 5 (b) FESEM of Psy-MBAm-AAm – ng

FESEM indicated successful synthesis of nanogels of Psyllium acrylamide nanogels crosslinked with MBAm.

PARTICLE SIZE ANALYSIS

The successful synthesis of Psy-MBAm-AAm – ng nanogel is also verified by Zeta Size particle analysis. 1g/L of aqueous solution at 25°C is used to study the size distribution of nanogel using Malvern Zetasizer Nano ZS. Zeta potential is the electrokinetic potential of the colloidal system and is the measure of phase difference in the boundaries of Solid and liquid phases in a colloidal solution.

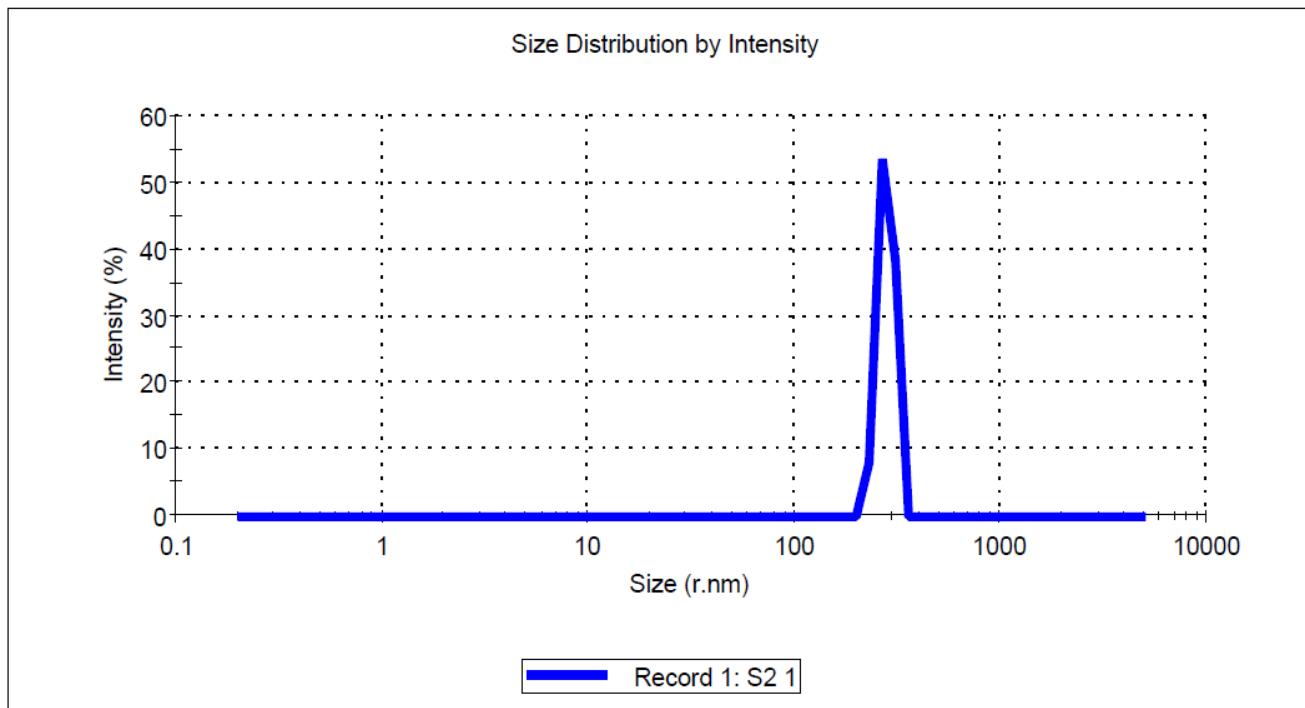


Figure 6: Zeta Sizer Analysis of Psy-MBAm-AAm – ng Nanogel

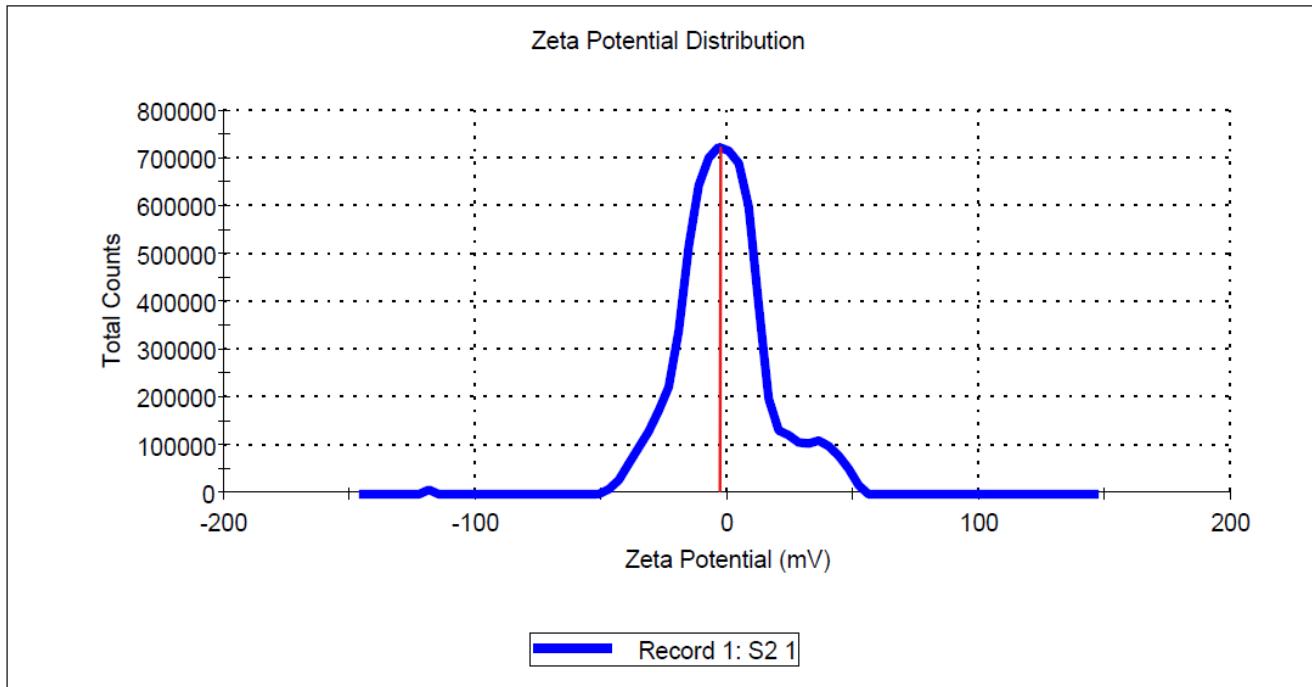


Figure 7: Zeta Potential Analysis of Psy-MBAm-AAm – ng Nanogel

The Polydispersity Index (PDI) value of Zeta Sizer (Figure 6) is 0.558, verifying that nanoparticles are of varied size with little variation at 25°C. The average radius Z (Z- average) of synthesized nanoparticles in an aqueous dispersion medium was 278.7 nm at 100% intensity.

Since the Zeta potential is the measure of the surface charge of the nanoparticles in the solution and hence is used to measure the stability of nanoparticles in the dispersion medium. The higher the positive or negative value of the Zeta potential (ξ Potential) more will be the stability of nanoparticles in the dispersion medium.

In the case of Psy-MBAm-AAm – ng nanogel the Zeta potential value of 93.3 % particles is -3.52mV, indicating that these nanoparticles were negatively charged and have a rapid coagulation tendency in aqueous solution. The 6.5 % of nanoparticles have a higher positive zeta potential of 39.3 and are moderately stable with a positive charge, while only 0.1 % of nanoparticles with -119 mV zeta potential show excellent stability with Negative charge in aqueous solution. In nutshell, average particles are negatively charged with a zeta potential of -1.37 mV and get rapidly coagulated. Due to hydrophobic interactions, these nanoparticles have a higher affinity for water molecules in an aqueous solution and are hence responsible for swelling behaviour. The ξ Potential curve for Psy-MBAm-AAm – ng nanogel is shown in Figure 7.

SWELLING BEHAVIOUR

The swelling characteristics for the synthesized hydrogel and nanogel observed at 4.0 and 7.0 pH (25°C and 37° C) are presented in Table 1 and Figure 8. Figure 9 shows the swelling behaviour of Psy-MBAm-AAm – ng nanogel at different reaction conditions. Table 2 gives the details of the variables used.

Table 1: % Swelling of Psy-MBAm-AAm – hg and Psy-MBAm-AAm – ng Nanogels

Duration	Psy-MBAm-AAm – hg				Psy-MBAm-AAm – ng			
	T1 = 24°C		T2 = 37°C		T1 = 24°C		T2 = 37°C	
	pH = 4	pH = 7	pH = 4	pH = 7	pH = 4	pH = 7	pH = 4	pH = 7
% Swelling	S _{w1}	S _{w2}	S _{w3}	S _{w4}	S' _{w1}	S' _{w2}	S' _{w3}	S' _{w4}
5 Min	176	200	228	276	1434	898	2012	1710
10 Min	294	314	300	372	1554	990	2178	2118
20 Min	398	420	386	460	1872	1016	2286	2204
40 Min	532	580	596	648	2068	1054	2746	2374
80 Min	666	762	750	888	2290	878	2620	2362
160 Min	866	1074	1006	1190	2556	778	2450	2352
320 Min	1232	1398	1256	1500	2804	776	2138	2288
640 Min	1624	2406	1288	1716	3212	908	2114	2266

Table 2: Variable used with their description

Sr. No.	Variable	Description
1	Psy-MBAm-AAm – hg	Psyllium- cl –N, N–MBAm–poly(AAm) hydrogel
2	Psy-MBAm-AAm – ng	Psyllium- cl –N, N–MBAm–poly(AAm) nanogel
3	AAm	Acrylamide
4	APS	Ammonium persulphate
5	SDS	Sodium dodecyl sulphate
6	MBAm	N, N-Methylene Bisacrylamide
7	%Sw	Percent Swelling

Swelling Behaviour of Psyllium – cl – poly(AAm) Hydrogel and Nanogel

An aqueous medium was used to carry out the swelling study of the polymeric network of psyllium hydrogel and nanogel. The swelling was carried out till equilibrium swelling is obtained at a fixed pH maintained at a particular temperature.

The Swelling Percent (%S_w) of the gel was calculated as using the following formula (Singh, B., Chauhan, G. S., Kumar, S., & Chauhan, N., 2007):

$$\% S_w = (W_{sw} - W_d) / W_d \times 100$$

Where W_{sw} = Weight of swollen Gel
 W_d = Weights of swollen gel

The swelling behaviour of the hydrogel and nanogel networks was studied as a function of time, temperature and pH. The data thus obtained indicated that the increase in surface area on the psyllium matrix on conversion to nanogel resulted in a prominent increase in the percent swelling at all studied pH and temperatures.

Effect of temperature and pH on the Swelling of Psy-MBAm-AAm – hg Hydrogel

The swelling studies carried out at the two temperatures indicated the percent swelling of Psy-MBAm-AAm – hg hydrogel increases initially at all pH and temperature due to the penetration of solvent molecules into the crosslinked matrix. A steady increase was noticed up to 320 min at all reaction conditions. However, at 37°C and pH 4, the % Swelling becomes constant. At pH 7 (24°C) a sharp increase in swelling behaviour was noticed and hydrogel behaves as a super absorbent. Whereas under other conditions the %swelling of hydrogel was observed to increase at a constant rate. The nanogel exhibited almost double %swelling than the corresponding hydrogel at pH 4 (24°C and 37°C).

Effect of temperature and pH on the Swelling of Psy-MBAm-AAm – ng Nanogel

In Psy-MBAm-AAm – ng, the variation of % Swelling is not as regular as noticed in the case of a hydrogel. The significant effect of temperature was noticed at pH = 4, nanogels act as super observant at normal room temperature (24°C) but at 37°C the maximum swelling is noticed at 40 min and thereafter a steady decrease in swelling up to 320 min was observed till it becomes constant. This change in swelling behaviour is due to rupturing of the polymeric backbone at high temperatures. At high temperature, the binding forces are mainly hydrogen bonding which binds the different polymer chains together and creates a cavity for holding the solvent molecule becomes inoperative and release the excess water molecules back.

This results in the deswelling of the nanogels and retains only those solvent molecules which help in maintaining the equilibrium in different forces. The further increase in the time period results in the dissolution of nanogels. The Porosity and particle size of gels play a crucial role in maintaining the balance between different cohesive forces. From Table 1, it is observed that at T_1 and pH = 4, initially, the rate of adsorption of water molecules by Psy-MBAm-AAm – hg hydrogel is eight folds than that of Psy-MBAm-AAm – ng and starts decreasing with the increase in time and finally reach to double. The high rate of adsorption by nanogel is attributed due to the high porosity and particle size and

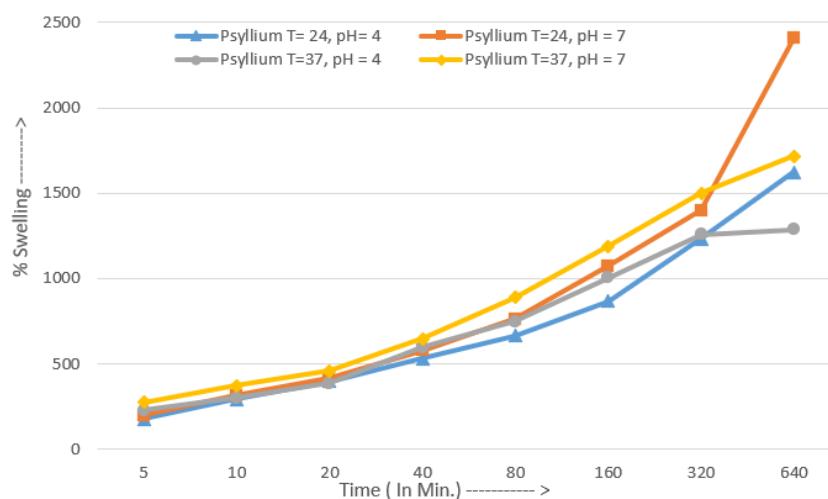


Figure 8: Swelling behaviour of (Psy-MBAm-AAm – hg) Hydrogel

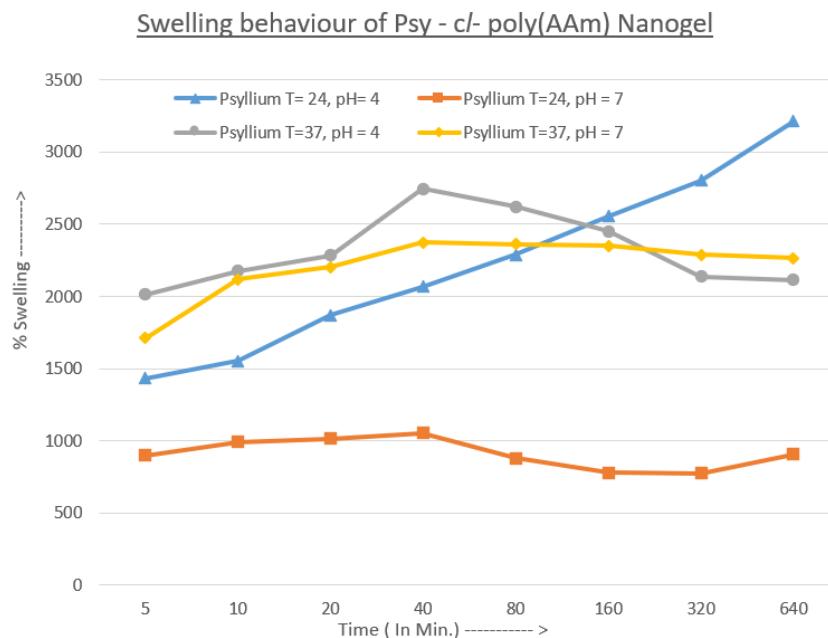


Figure 9: Swelling behaviour of Psy-MBAm-AAm – ng Nanogel

the decrease in adsorption is because of the occupancy of the empty void by solvent molecules in the polymeric chain. The same adsorption behaviour with a higher adsorption rate is observed at pH = 7 and the same temperature. The lower swelling rate at pH = 4 is due to the breaking of the polymer network chain in an acidic medium. At high-temperature T₂, and low pH the Psy-MBAm-AAm – hg swelled continuously but for Psy-MBAm-AAm – ng, adsorption of the solvent molecule increases initially, and reaches the maximum at 40 min and then starts decreasing. The swelling of Psy-MBAm-AAm – ng nanogel at T₂ and pH = 7 is much higher than that of Psy-MBAm-AAm – hg hydrogel.

CONCLUSIONS AND FUTURE PERSPECTIVE

The paper aims to draw a comparative between the psyllium-based acrylamide grafted hydrogel and nanogels. Psy-MBAm-AAm – hg and ng were successfully synthesized and characterized. The FESEM and particle size analysis data indicated that the nanogels of size below 250nm that exhibited negative zeta potential value were obtained. The swelling characteristics studied at varied pH and temperatures indicated that the nanogels exhibited greater swelling than the corresponding hydrogels due to an increase in the surface area.

The results pointed towards the potential applications in site-specific drug loading and release studies as at body temperature high % swelling was observed at acidic pH. The nanogel exhibiting a continuous increase in water absorption at room temperature and in mildly acidic pH, opens the application of the same for sewage or industrial wastewater management. There are a number of applications already reported for hydrogel but the method reported in this paper is fast and simple and also suitable for the synthesis of Psyllium – Acrylamide-based nanogels.

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NOVEL METHOD FOR EXTRACTION OF LIGNIN CELLULOSE & HEMICELLULOSE FROM *Pinus roxburghii* NEEDLES

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ABSTRACT

Lignocelluloses are becoming a major area of attraction for the researchers for their sustainability and cost effectiveness. The ease of functionalization of these matrices along with remarkable physical and chemical properties and tunable functional sites, make them incredible materials for tissue engineering and drug delivery among other applications. The present study focuses on the extraction of biomaterials lignin, cellulose and hemicellulose from the *Pinus roxburghii* (PR) needles following a single source procedure. A green chemistry approach is followed ensuring minimum wastage with maximum output from the raw material. The *Pinus roxburghii* (PR) needles were collected from the local area, washed thoroughly, dried in the oven at 45°C for few days and grounded to powder. The PR powder was subjected to treatment with acetic acid: formic acid in the ratio of 1gm/10ml mixture at different concentration to study the yields, for 1hr at low temperature followed by 3hrs treatment at 110°C. The concentrated mother liquor thus obtained was filtered, diluted and left undisturbed for 48h. Lignin precipitates obtained were separated and dried in oven at 30°C. Subsequently, the residue from the extracted lignin was washed to neutralise the pH and dried in oven. By using 5% NaOH reflux treatment at 90-110°C for 3h, hemicellulose was recovered from the pre-treated pine needles mother liquor from which the lignin had been extracted. The mother liquor was treated with ethanol and acetic acid to precipitate hemicellulose in the freezer. The residue obtained after the removal of hemicellulose was washed again to and subsequently bleached to get cellulose. An excellent yield of the desired products was obtained. The extracted products were subjected to characterization studies namely FTIR and HRMS etc to get evidence for successful extraction. The analysis revealed that the PR leaves contained about 1.5gm% to 3.0gm% Lignin, 15-30wt% Hemicellulose and rest is Cellulose. Further, aim is to use the extracted material to prepare the nano-hydrogels for the drug delivery and other applications.

Keywords: Cellulose, Characterization, Extraction, Hemicellulose, Lignin

INTRODUCTION

In the recent times, the utilization of the bio-waste for value added products has received solemn attention of the researcher's world over. With the eminent threat of the natural resources being depleted at an alarming rate various studies have been preformed to replace the petroleum based products by agro-based polymers products such as cellulose, hemicellulose, chitosan and lignin (Dhumal, Ahmed et al., 2019). These biomaterials have huge potential for varied applications and pose an efficient method for utilization of bio wastes. The organic wastes such as shed leaves, bark of trees etc. have a considerable potential for the extraction of lignocellulose material as renewable feed stocks for the production of a spectrum of biomedical materials, chemical and other sustainable materials (Nuruddin M.,et al., 2011).

Lignocellulosic materials (lignin+cellulose+hemicellulose) are one of the most important natural sources for the production of high value added materials for biomedical industries or biopolymers for agro based industries and are very attractive research candidates due to their biodegradability, low density, easy availability, excellent mechanical properties, eco-friendly and socio-economic nature. Lignocelluloses being a low cost and renewable feedstock for producing bio fuels, bio-based chemicals, biomedical materials and bio-degradable materials have been receiving considerable attention these days. The three dimensional network of lignin with cellulose and hemicellulose in these entities is responsible for providing strength and stiffness to the

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plants.

In nature, lignin is the most abundant aromatic material with methoxyl, hydroxyl, phenolic, carbonyl and carboxylic groups on aromatic rings as phenylpropane polymer 3-D structure. The aromatic and hydroxyl group play a crucially important role in the anti-microbial (Raj T. et al., 2022) and anti-oxidant activities. Lignin has become a smart choice for use in the paper and pulp industries due to its easy availability, low cost, eco-friendly nature, high aromatic content and good pest resistant nature (García, A. et al., 2017). Along with that lignin is also being explored as a suitable candidate for applications such as for sensor making (Kumari S., et al., 2014) antimicrobial agent (Yang, W.; et al, 2016), food packaging material (S. Domenek, et a., 2013), drug delivery (Alqahtani M. S.,et al. 2019), gene delivery, tissue engineering (Kumar R., et al., 2021) , as a binder (Farhat, W., et al., 2017) several laboratory aromatic reagents (Wang, H.; et al., 2019 & Rinaldi, R et al., 2016), as Scaffold materials (Salami M. A., et al, 2017), matrix for drug loading and release and many health care products (Gordobil, O.; et al., 2018 & Dominguez-Robles et al 2020). The lignocelluloses material is organized as micro-fibrils linked together to form cellulose fibres. These fibres once extracted have been reportedly used for the production of ethanol (Bassem B. et al., 2011, Hallac, B. B., Ragauskas, A. J., (2011) , raw material for paper industries, manufacturing flexible electronic film, coating for packaging, receptacles for drug delivery and optical digital storage media (Abitbol, T et al., 2016). The properties of cellulose fibers are affected by many factors such as source, climate, harvest, maturity, retting degree, decortications, disintegration (mechanical, steam explosion treatment), fiber modification, textile, and technical processes (spinning and carding).(Van de Velde K, 2001). Cellulose and hemicellulose get associated with lignin through covalent bonds to form lignin-carbohydrate complexes in the primary and secondary cell-wall of wood. The hemicellulose has been used to produce alcohol by fermentation and sorbitol and also have important applications in cosmetics, papermaking, explosive, antimicrobial and drug delivery (Girio et al., 2010, Zhao et al., 2014). Hemicelluloses can be used in value-added industrial applications including hydrogels (Hu, L.S et al., 2018), thermoplastics, blends composites and nano-composites. (Kirsi S. Mikkonen 2013, Fredon et al., 2002, Farhat, W., et al., 2017, Azhar, S et al., 2015). Hence these materials are of utmost importance and we need to look for novel methods for the extraction of these materials.

Till date many successful methods such as alkali extraction (Sun, B., et al., 2014), steam explosion (Biermann et al., 1984), hot water (Leppänen et al., 2011), enzymatic hydrolysis (Nhuan et al., 2011), bioconversion method (Himmel et al., 2007), and the hydrothermal method (Feria et al., 2012) have been employed for the extraction and isolation of lignocelluloses material. In its native area, *Pinus roxburghii* generates a bulk a quantity of needles waste after the shedding. A significant quantity of these waste remains un-utilized and catch fire, generating a large quantity of smoke that severely impact the human health and the environment. Due to current demands and wide range of applications the researcher have focused more on the isolation of lignin, cellulose and hemicellulose from different resources such as pines Needles (Raj et al., 2022), pines cones (Rambabu, N et al., 2016) rice Husk (Johar, N et al., 2012), straw of wheat, jute, banana stem, hemp and flex straws, sun flower stalk (Fortunati, E., et al., 2016) pine oil residue, pine apple leaf fibers and sisal fibers (Siqueira, G. et al. 2010) etc. Cellulose and hemicellulose interactions in the cell wall play an important role in the excellent flexibility of wood-based materials. At present, the methods of extracting hemicellulose from plants can be divided into physical pre-treatment and chemical pre-treatment. The former includes steam explosion, hot water extraction, ultrasound, and microwave-assisted methods.

In the present study we report another method for the extraction of these three plant backbone forming materials from the *Pinus roxburghii* needles. We aim to develop a method that ensures have maximum extraction of the desired products with minimum waste and apply and hence extracted and characterized materials for speckled applications. The *Pinus roxburghii* (PR) needles were employed for the extraction of lignin, cellulose and hemicellulose following previously reported methods and formulating a novel eco-friendly procedure. The lignin, cellulose and hemicellulose yield was checked and found with highest yield of cellulose 60-70gm%, Hemicellulose 15-30wt% followed by Lignin (1.5gm% to 3.5gm%) and subjected to FT-IR and HRMS characterisations to get evidence for successful isolation.

EXPERIMENTAL

Raw Material

Pinus roxburghii (PR) needles were collected from forest near Palampur India. The collected needles wash properly and cut into 10mm pieces. Sample was air/oven dried and stored in the room temperature.

Chemical used

All chemicals and reagents utilized are of standard quality. The formic acid (assay 85%) (Sisco Research Laboratory Pvt Ltd),

NaOH flakes (central Drug House (P) Ltd Daryaganj New Delhi), Ethanol (Changshu Yangyuan Chemical, China, Acetic Acid (RDC Limited Delhi), Double distilled (DD) water (Lab prepared) and Sterilising Water (Sun Rise Pharma) were used as received.

Extraction of Lignin, Cellulose and Hemicellulose

Step-I

Extraction of lignin from PR needles was carried out following an amalgamated approach with steps from a standard method (Raj et al., 2022) along with formulation of novel steps. PR needles were collected, separated from the branch, washed thoroughly with distilled water and dried under sunlight followed by grinding them to powder stage using a mixer grinder. Acetic acid (AcA), Formic acid (FA) and water were used in the ratio of 5.5:3.5:1 by volume for extraction of lignin. One part of the powdered pines needle was combined with the 20 parts of solvent (AcA: FA: Water in 5.5:3.5:1) in a round bottom flask. The resultant mixture was heated to 70-80°C for one hour, and subsequently next 3hrs at 110°C. The reaction mixture was allowed to cool and filtered. Thus obtained solid residue was washed with 1:1.5 ratio of dilute acetic acid (3.0wt%) solution at 60 °C keeping 20-25min incubation time for three to four washes and the decanted solutions were mixed with mother liquor to left for evaporation under constant stirring to reduce the volume to 10ml at 30-40°C and diluted to 300ml to precipitate the lignin. Precipitated lignin was filtered by using Whatman filter paper number 42 and dried. The work has been reported by Raj T. et al., 2022. The yield of the extracted lignin was determined using the following formula:

$$\%Wt = \frac{Wi - Wr}{Wi} \times 100$$

Where, W_i = initial weight of Pinus needles. W_r = Weight of residue left after the Lignin precipitation. $\%W_t$ = Weight %age of component.

The left over pine waste post lignin extraction was employed for separation of cellulose and hemicelluloses bagasse and mother liquor respectively. This paper focuses on the same.

Step-II

The residue obtained after the removal of Lignin was neutralised by washing with DD water and dried in oven followed by treating with an alkali solution (5% wt) of NaOH (Kumari S., et al., 2014). The mixture was transferred to round bottom flask and heated to 60°C for 2 hrs followed by reflux for 4 h at reflux temperature. The solid was then filtered and washed thrice with DD water to get cellulose fibrils. These cellulose fibrils were bleached by aqueous chlorite thrice at 100-130°C for 4 h. Thus obtained sample was of Cellulose. The yield of the extracted cellulose was determined using the following formula:

$$\%Wt = \frac{Wr - Wp}{Wr} \times 100$$

Where, W_r = Residue weight that left after the removal of Lignin. W_p = Weight of precipitate formed.

$\%W_t$ = Weight %age of Cellulose.

Step-III

The filtrate obtained in the First step was treated with 25% acetic acid and transferred to 96% ethyl alcohol to maintain a pH of 4.5 at 4°C. The mixture was left for two days to allow the hemicellulose to precipitate and settle to the bottom. The clear layer above the precipitate was removed by vacuum suction. The precipitates obtained were washed with 60% ethanol and frizzed-dried to get the hemicellulose powder. Scheme 1 gives the graphical representation of the extraction process. The yield of the extracted hemicellulose was determined using the following formula:

$$\%Wt = \frac{Wp}{Wr} \times 100$$

Where, W_r = Residue weight that left after the removal of Lignin. W_p = Weight of precipitate formed.

$\%W_t$ = Weight %age of Hemicellulose.

On applying the above mentioned approach the obtained yield for extracted lignin, cellulose and hemicelluloses indicated that PR leaves contained about 1.5gm% to 3.0gm% of lignin, 15-30wt% of hemicellulose and remaining is Cellulose. Scheme 2 represents the images of the as extracted samples of lignin, cellulose and hemicelluloses from *Pinus roxburghii* needles.

Characterization

The extracted lignocelluloses material was characterised by various techniques such as FT-IR (spectrum 100 Perkin Elmer with HATR & DRIFT) for vibrational analysis of samples, and HRMS (Xevo XS QToF mass spectrometer, Waters ACQUITY

UHPLC, Mass Range 20-4000amu, Resolution 40000 FWHM Mass Accuracy: Typically <1ppm, Sensitivity: Full Scan Sensitivity up to 500fg on column with S/N> 100:1 in ESI MS mode for reserpine, Ionisation Method: API ESI positive & negative, APCI positive & negative, Direct infusion for Mass Analysis (MS, MS/MS), UHPLC with PDA detector to get the evidence of successful extraction.

The FTIR and HRMS data indicated that the extracted lignin, cellulose and hemicellulose are of excellent purity.

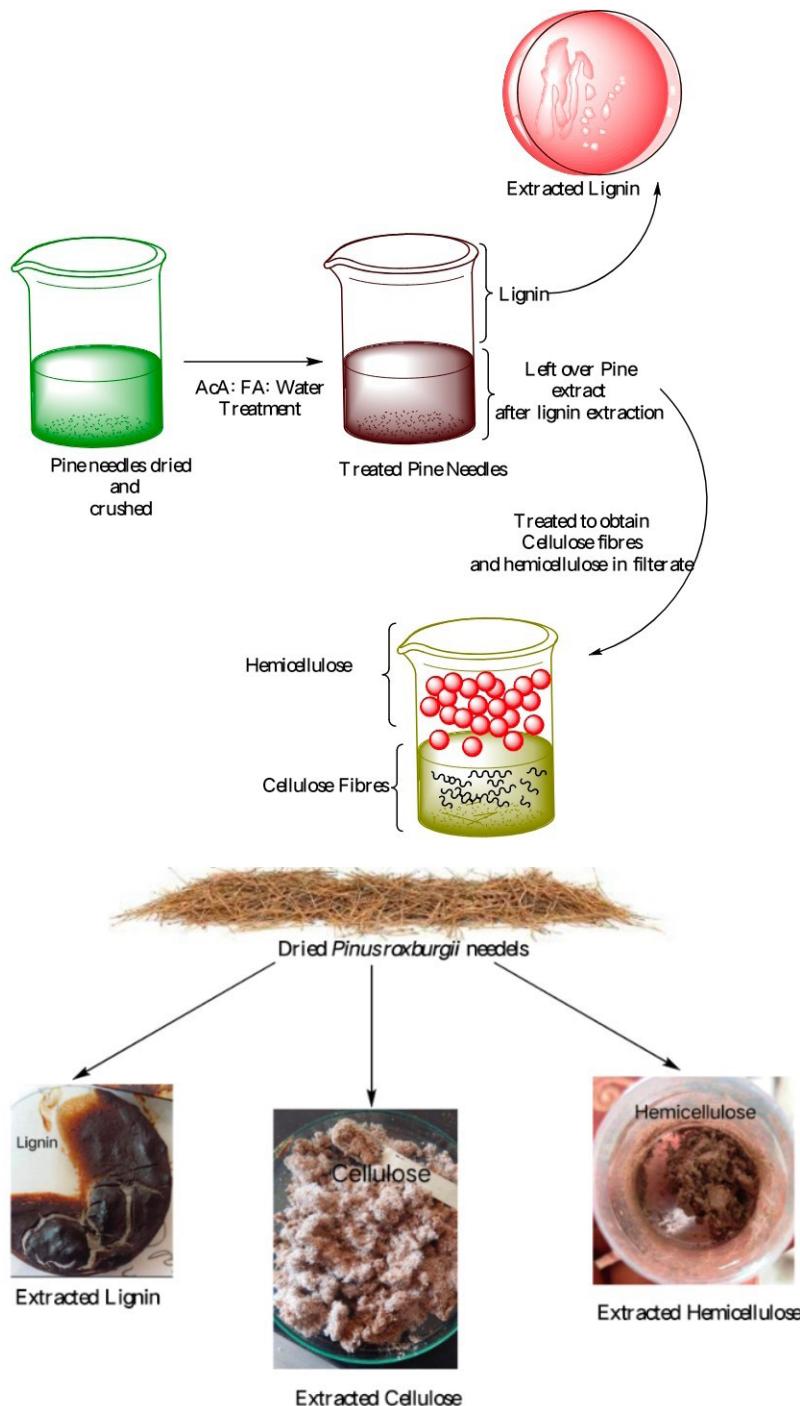
RESULTS AND DISCUSSION

The FTIR results indicate the successful separation of all lignin, cellulose and hemicellulose from the PR needles (Sun et al., 2000). The stretching observed at 1510cm^{-1} represents the $-\text{C}=\text{C}-$ groups of the aromatic ring of lignin compounds. The absence of this characteristic peak in the spectrum of cellulose and hemicellulose points towards the successful separation of lignin from the other two components. The prominent peak at 1734cm^{-1} of the residue is attributed to either acetyl or an uronic ester group of hemicelluloses indicating the successful isolation of the three components.

FTIR of Lignin, Cellulose & Hemicellulose

FTIR of extracted lignin sample:

The FTIR data obtained for the extracted samples indicated successful extraction of the same from the PR needles. The characteristic functional groups with their values are mentioned in the Tables-1, 2& 3 for lignin, cellulose and hemicelluloses respectively. The band for $-\text{OH}$ was observed at 3414.00cm^{-1} ($3500\text{--}3100\text{cm}^{-1}$), $-\text{C}=\text{O}$ group conjugation appeared at 1716.65 cm^{-1} and 1631.78cm^{-1} , $-\text{C}-\text{O}$ stretching for S-type of lignin (Figure-1 & Table-1) was also observed. The $-\text{C}-\text{O}-\text{H}$ group was indicated by the stretching at 1029.99 cm^{-1} (Figure-2 & table-2) and $-\text{C}-\text{O}-\text{C}$ bridge between sugar units was justified by stretching at 1045.41cm^{-1} (Figure-3 & Table-3). The spectra obtained for lignin, cellulose and hemicellulose, exhibited major differences in the region of $1800\text{--}600\text{ cm}^{-1}$. The stretching peak at 1122.57 cm^{-1} is designated to aromatic C-H stretching characteristic of S-type unit of lignin, whereas at 1273.08 cm^{-1} the characteristic stretch bands is comparable to ether & G-type unit in lignin in the pines needles(Figure-1 & Table-1). The stretching observed in the range of $1850\text{--}1450\text{ cm}^{-1}$ represents the functional groups generally originating from lignin and at 650 cm^{-1} for cellulose and hemicelluloses components. (Owolabi A. L et al., 2018, Brinchi L, et al., 2013). The characteristic peak of lignin's aromatic ring vibration presents at wave number $1200\text{--}1400\text{ cm}^{-1}$ almost disappeared after chemical and mechanical treatment.



Scheme 2: Representation of the images of the extracted samples of lignin, cellulose and hemicelluloses from *Pinus roxburghi* needles.

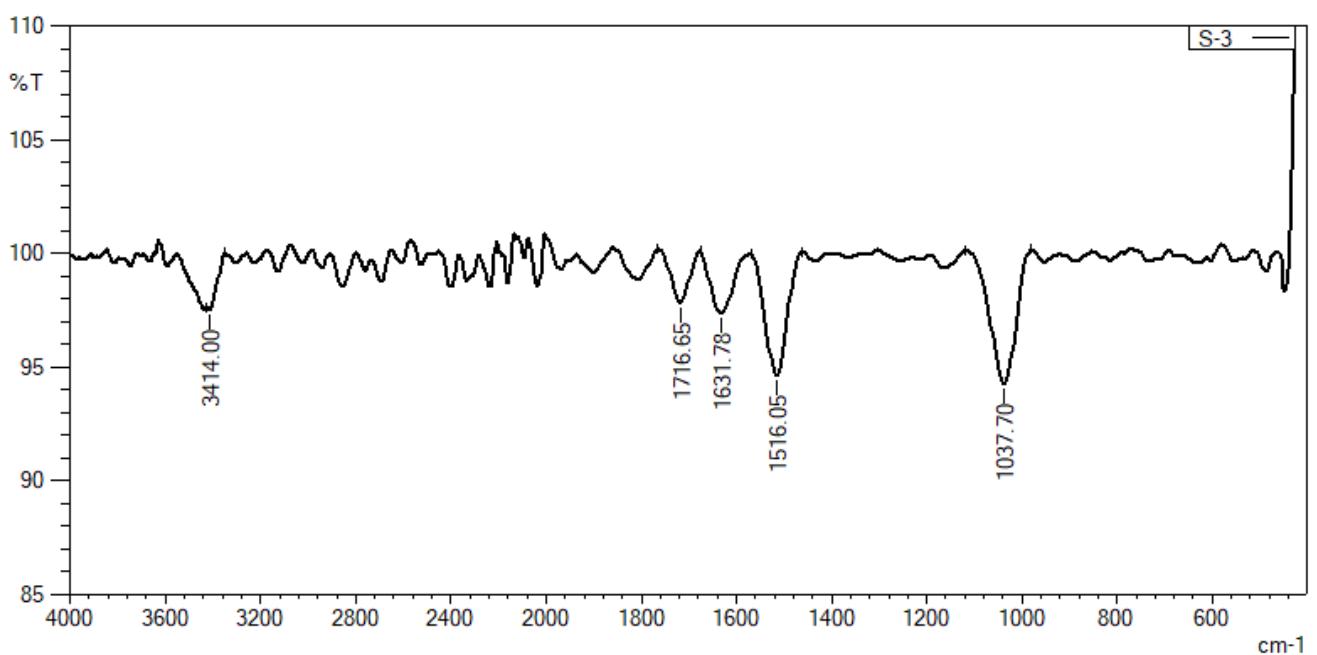


Figure 1: The FTIR spectra of the extracted lignin

Table 1: The FTIR spectral peaks of lignin extracted from *Pinus roxburghii* needles.

S. NO	Reference Frequency Cm ⁻¹	Peaks appears in the present study (cm ⁻¹)	Functional groups	Compounds
1	3450-3300, 3500-3100	3414.00	-OH polyphenols -OH str Ar. Aliph.	phenolic group
2	3500-3100	3502.72, 3174.83	-OH str	Ar. Aliphatic grp
3	2960-2925, 2950-2850	2958.80, 2924.08	Str -C-H, -CH ₃ ,-CH ₂	Alkyl, aliphatic grp
4	2500-2100	2503.60, 2102.40	C=C conj.	Benzene ring
5	2500-2100	2499.74	C=C conj C=C Str.	Benzene ring
6	2500-2100, 2156	2156.41	C=C conj. C=C str.	Benzene ring
7	1720-1715	1718.85	Str. C=O unconj.	Carbonyl group
8	1650-1600, 1647	1631.78	Str. C=O conjugation.	Carbonyl group
9	1600-1500, 1515	1516.05	Ar. rings of S -type	S-type ring
10	1450-1600,	1450.46	Ar. Stretching of Lignin, OH Str	Aromatic, aliphatic alcohol
11	1450, 1460	1458.18	-OH Str Ar,	Aromatic grp
12	1272-1220, 1200-1215	1273.08	-C-O stre,phenol,	Ether & G-type grp
13	1115-1125, 1121	1122.57	Ar. C-H stretching S-type unit	S-type unit
14	1030, 1041, 1040	1037.70	C-O bond in aromatic S-type	S-type unit
15	825, 835	825.53	S-type aromatic C-H bonding	S-type unit
16	800-785	786.93	C-H out of plane defor S-type	S-type unit
17	627-500	628.79	C-H Str	Aromatic Hydrogen

FTIR of extracted cellulose sample:

The FTIR spectra for extracted cellulose exhibited various characteristic peaks such as ones at 3525 cm⁻¹ and 1650 cm⁻¹ indicative of the non-aromatic moieties. A weak peak around 2900 cm⁻¹ was observed (Figure 2) for alkali treated extracts indicating complete removal of hemicellulose moiety from the extract. The stretching frequency for untreated cellulose fibre was observed around 1423.47 cm⁻¹ for -C=O group (Owolabi A. L et al., 2018). The detailed analysis of the data obtained is presented in the table 2 which strongly indicates successful extraction of cellulose form the pine needles.

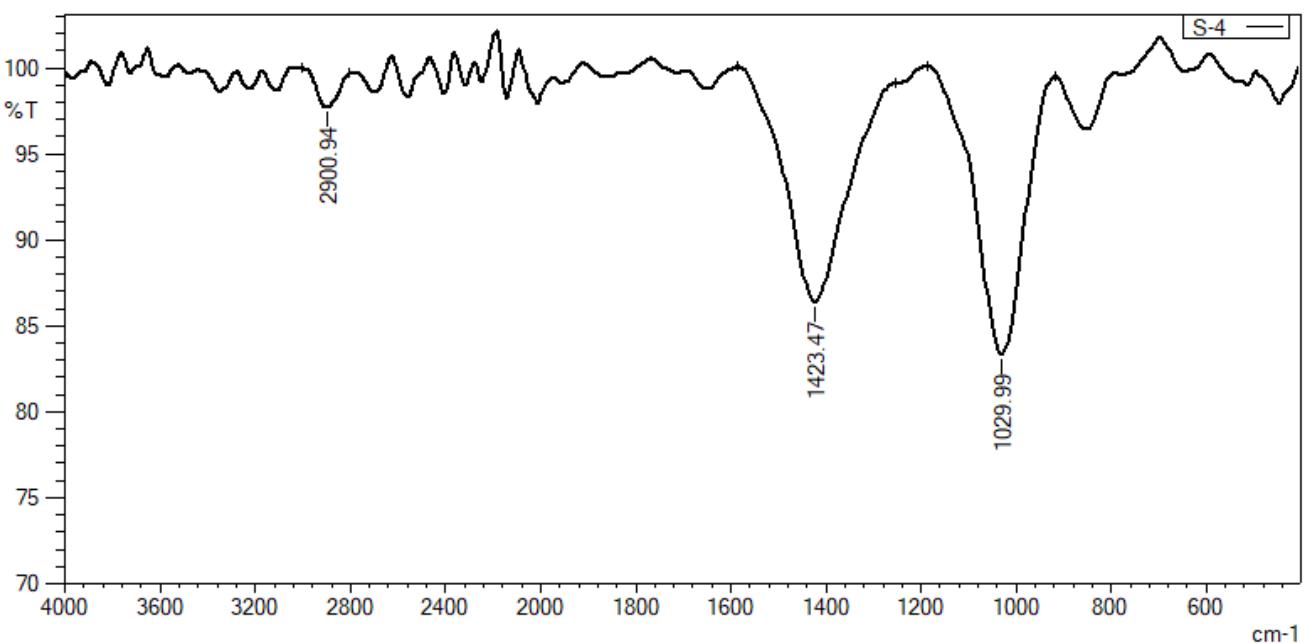


Figure 2: The FTIR spectra of the extracted Cellulose.

Table 2: The FTIR spectral peaks of Cellulose extracted from *Pinus roxburghii* needles.

S. NO	Reference Frequency Cm^{-1}	Peaks appears in the present study (cm^{-1})	Functional groups	Compounds
1	4,000-2,995	3996.50	-OH Acid, methanol	Alcoholic group
2	2950.85	2900.94	asymmetric and the symmetric stretching of methylene (-CH ₂ -) groups	-CH ₂ group
3	2,890	2889.36	H-C-H Alkyl, aliphatic	Aliphatic group
4	1440-1400	1423.47	-OH bending	Acid
5	1,640	1612.49	Fiber-OH Adsorbed water	
6	1,270-1,232	1546.05	C-O-C	Aryl-alkyl ether
7	1,170-1,082	1029.99	C-O stretching	Pyranose ring Skeletal
8	1,108	1107.14	-OH, C-OH	Alcoholic groups

FTIR of the extracted hemicellulose sample:

In the FTIR of the extracted hemicellulose it was observed that the ring vibrations overlapped with stretching vibrations of side group -C-OH bonds and -C-O-C glycosidic bond vibrations. Asymmetric and symmetric stretching of the carboxylate ions was seen at 1600 cm^{-1} and 1380 cm^{-1} . The stretching frequencies at 1060.84 cm^{-1} , 1037.70 cm^{-1} and 898.82 cm^{-1} were indicative of the mannose and glucose units (Gupta et al., 1987). The detailed analysis and spectrum is represented in figure 3 and table 3.

High resonance mass spectrometry (HRMS) of extracted Lignin, cellulose and Hemicellulose.

The HRMS data pointed towards the cross linked nature of the lignin exhibiting various characteristic chemical linkages and consequently, unique functional groups appearing at predictable regular position relative to the others in the mass spectra (Raj T., et al, 2022, Jens Prothmann et al., 2018). The corresponding values at 105.0694 to 130.0 indicate the presence of the methoxy groups. Mass to charge ratio (m/z) at 157.1003, 179.0844 and m/z 209.1316 with standard error are ascribed the presence of 4-methoxybenzaldehyde, 4-methoxycinnamic acid and 3, 4-dimethoxycinnamic acid respectively, that constituted major peaks in the spectra of lignin (Figure 4). Other signal at m/z 271.0956 corresponding to the signal obtained in the (Raj T., et al., 2022) (Figure 4). These are very close to the values reported for the groups present in lignin (Ruochun Zhang et al., 2021). The m/z values obtained in the HRMS of cellulose at m/z 158.9638 (Figure 5) and m/z at 158.9638 (Figure 6) correspond to the value of single monomer unit of cellulose and hemicellulose respectively (Lunsford K. A. et al., 2011). Large number of

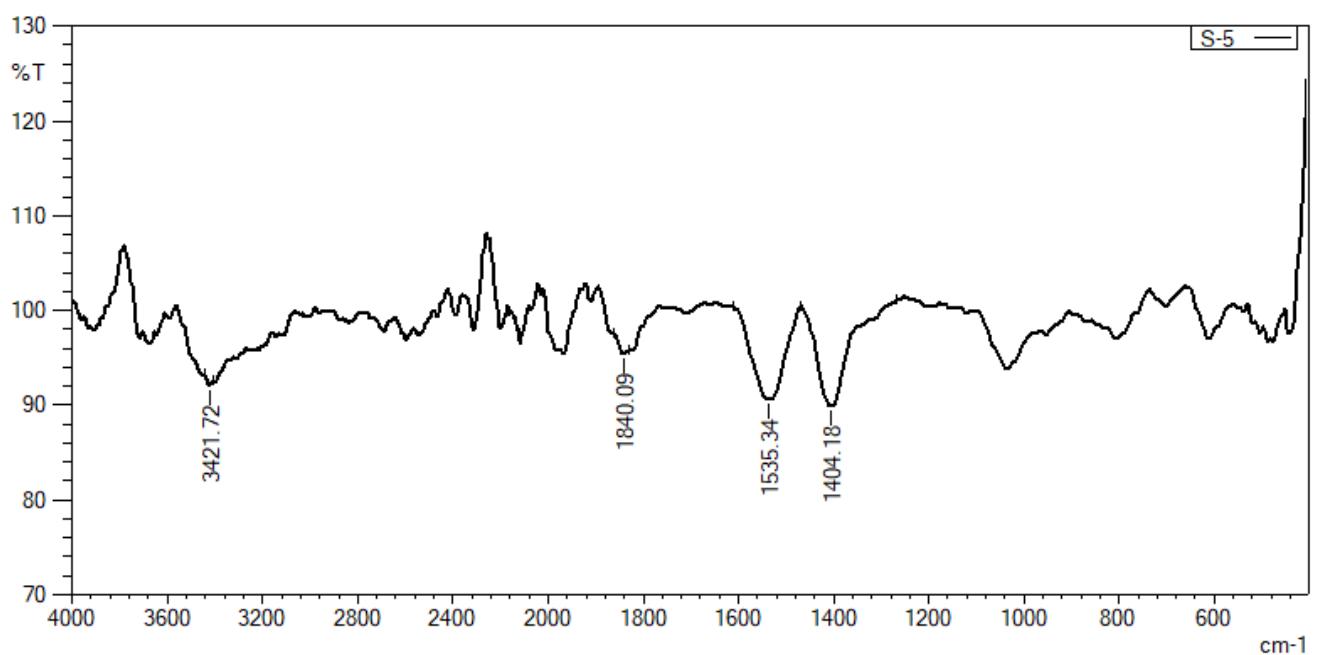


Figure 3: The FTIR spectra of the extracted Hemicellulose.

Table 3: The FTIR spectral peaks of Hemicellulose extracted from *Pinus roxburghii* needles.

S. NO	Reference Frequency Cm ⁻¹	Peaks appears in the present study (cm ⁻¹)	Functional groups	Compounds
1	4,000-2,995		-OH, Acid, Methanol	Acidic group
2	3000-3640 Farhat W, 2017	3421.72- 3001.23	Stretching vibrations of -OH	Alcoholic group
3	2889, 2890	2889.36	Symmetric and Asymmetric vib. Of C-H	Alkyl group
4	1765-1715	1770.05	C=O ketone and Carbonyl	Carbonyl group
5	1505	1535.34	C=O streaching	Carbonyl group
6	1483	1404.18	CH ₂ bending	
7	1108	1107.00	-OH, C-OH Group	
8	1045	1045.41	Asymmetric stretching of C-O-C bridge between the sugar units.	Pyranose ring structure
9	985	983.69	C-O stretching characteristics of sugar st.	Sugar units
10	898	898.82	Characteristics of β-glucosidic linkage between the sugar units	Glucose & Mannose units

glucose units attached through glycosidic bonds to form linear chain of cellulose with high degree of crystallinity. The hydroxyl groups attached with carbon atoms of cellulose monomer units makes a internal hydrogen bonding network. The calculated mass value for each of glucosyl units ($C_6H_{10}O_5$) is 162m/z. After the comparison, the peaks m/z values with calculated mass are as: m/z 974.8090 (6 glucosyl units) 1178.7690 (7 glucosyl units) 1240.7610(8 glucosyl units) 1450.7228(9 glucosyl units) 1586.6989(10 glucosyl units) (Figure 5).. The difference with the calculated value is almost equivalent to the number of water molecules removed during the bond formation. (Jung, S., et al., 2010) These value shows the successful extraction of cellulose from the needles. (Figure 5). The major constituent of hemicellulose in soft wood is O-acetyl-(4-O-methylglucurona)-xylan to gather with small amount of glucomannan in alkaline condition. From the lower mass peaks of the spectra indicate that the cellulose and Hemicellulose obtained is depolymerised completely. The main subunits of Hemicellulose obtained after the

extraction are xyloglucan, Xylan, β -glucose & Glucomannans. Which are shown in peaks as as: m/z 873.6373 indicates the five units of Hexose, m/z 895.5160 indicates hexose-5units & 1 acetyl group and m/z 917.6600 indicates hexose-5 units & two acetyl groups attached (Figure 6).

The low mass spectral ranges predominately contain peaks of monomeric glucose, xylan, phenols and aromatic acids, the most important of which are the representative of C6-C3 units (phenyl propane type) of hydroxycinnamic acids and m/z 158.9638 representative of cellulose. These values provide strong evidence for the presence of G-unit coniferyl alcohol and S-unit Synapyl alcohols in the extracted lignin and the high m/z 1110.7838 to m/z 1654.6804 represent the presence of cellulose polymeric structure of glucose units (Wei, L. S., et al., 2018) (Figure 5). These units are the key components in the 3D structure of the coniferyl lignin, cellulose and Hemicellulose. Similarly, the m/z values obtained for hemicellulose namely at 289.1737, 377.2256, 425.1477, 513.2003, 585.4064, 713.4513 and 873.6373 m/z indicates the partially de-polymerization of hemicellulose (Figure 6) & support the successful extraction of hemicellulose from the *Pinus roxburghii* needles.

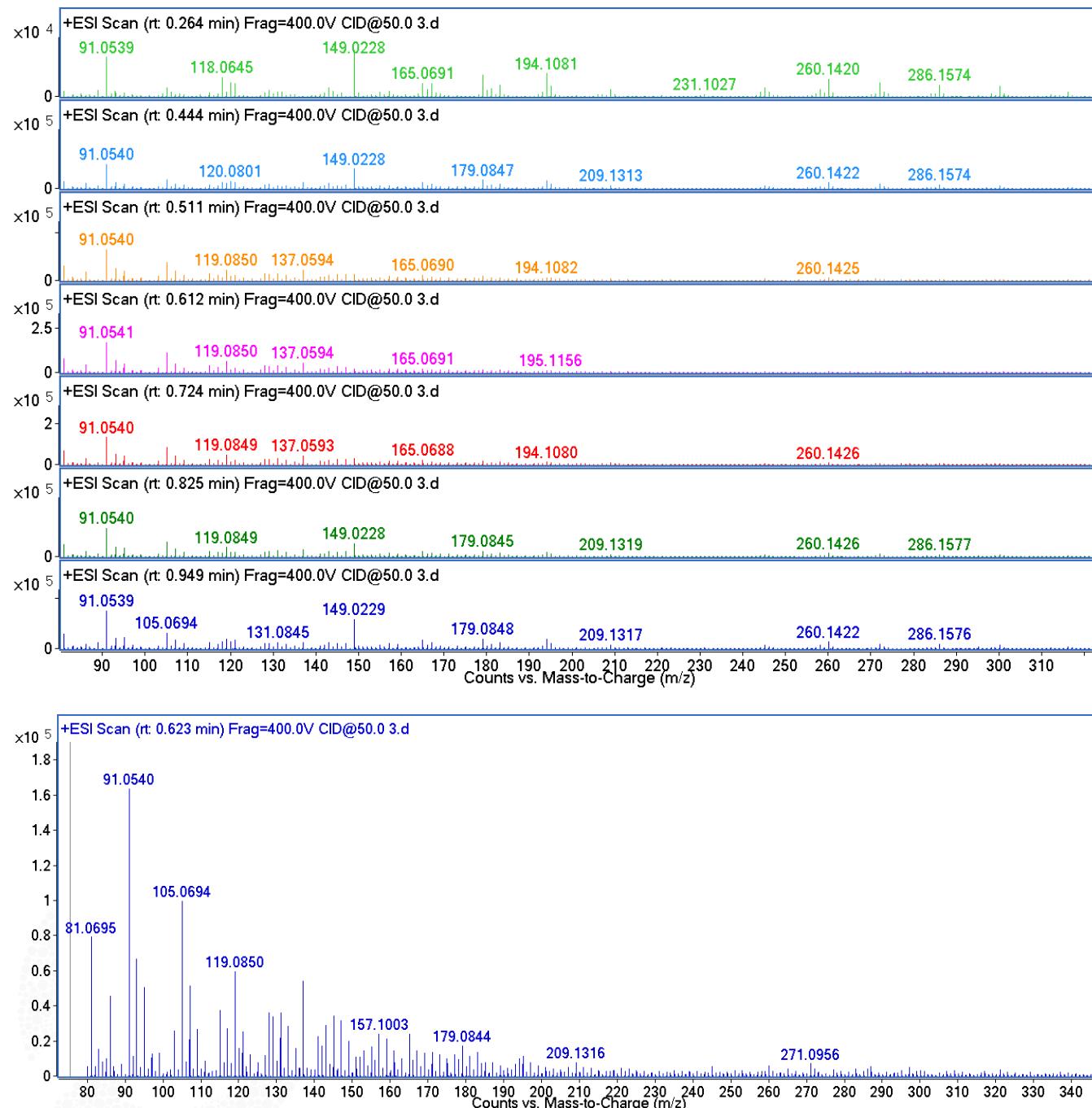
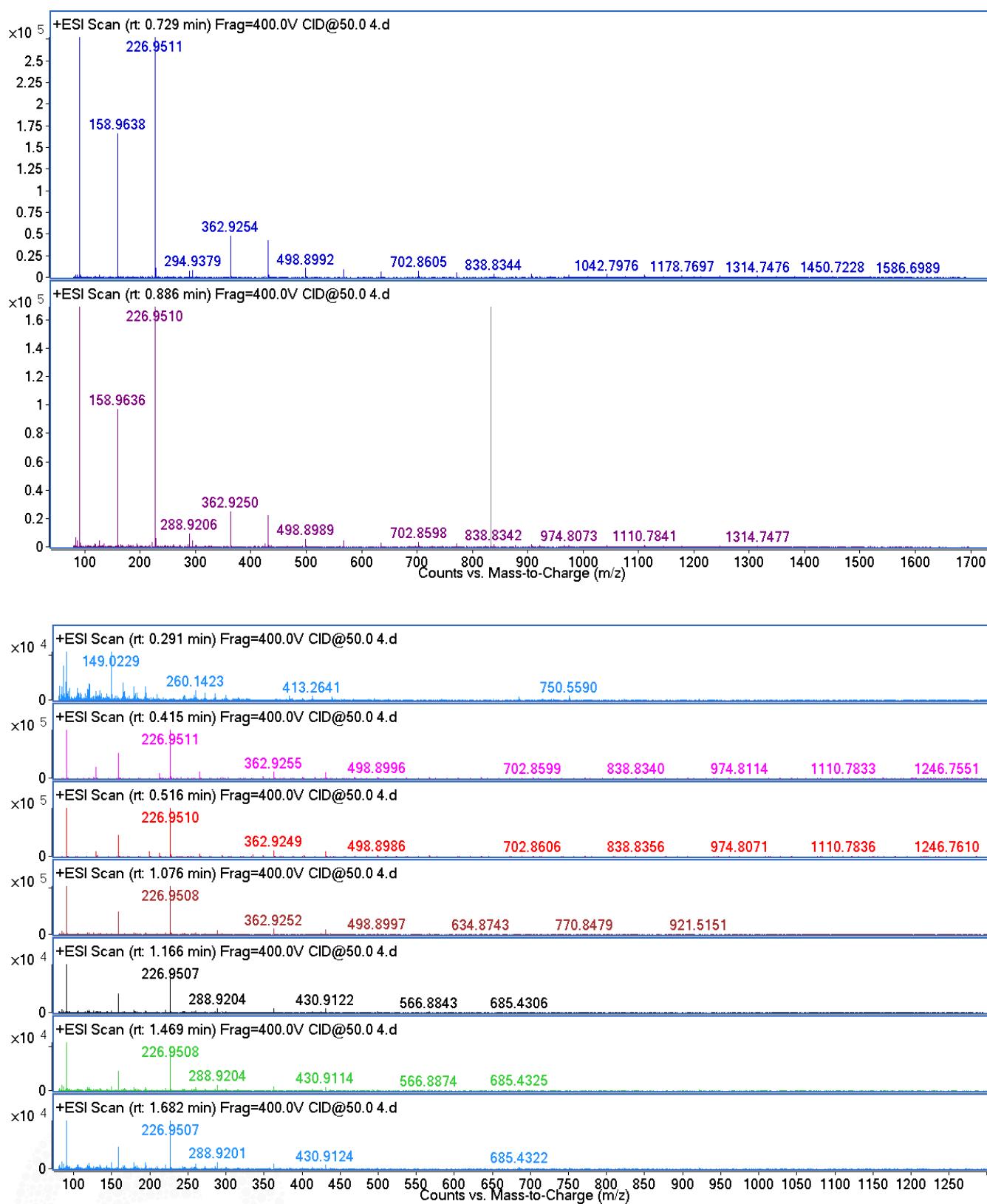


Figure 4: The HRMS spectra of the extracted lignin.



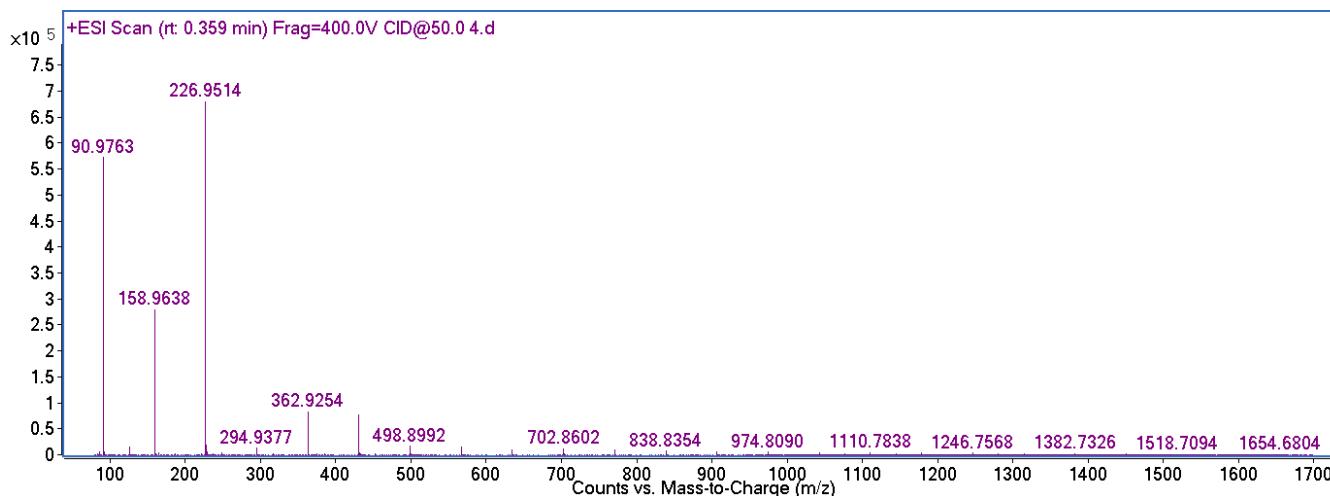


Figure 5: The HRMS spectra of the extracted Cellulose.

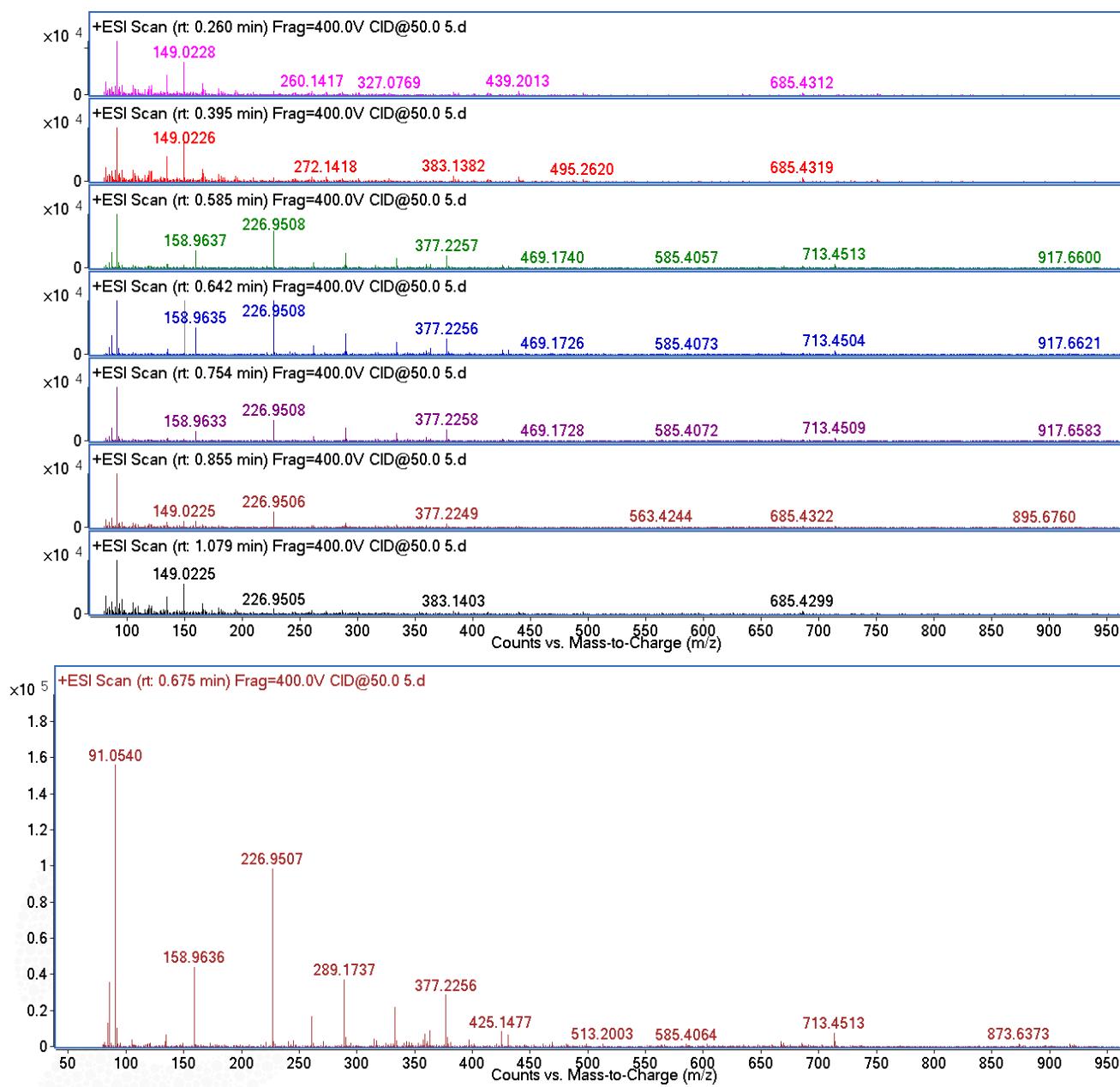


Figure 6: The HRMS spectra of the extracted Hemicellulose.

CONCLUSION

The aim of the study was to depict the versatility of the PR needles to act as a raw material for extraction of various natural products namely lignin, cellulose and hemicellulose. More specified and comprehensive approach was introduced to widen and simplify the existing synthesis methods to attain a good yield of the desired derivatives. The lignocelluloses products have great potential in preparation of matrices for sensor, antimicrobial and drug delivery applications. The present work is the second phase of the previously reported work of Raj T. et al., 2022 wherein they reported the extracted of lignin from *Pinus roxburghii* needles and prepared its silver incorporated composites guar-gum and agar-agar that exhibited excellent antimicrobial properties. The pre-treated *Pinus roxburghii* needles from which the extraction of lignin had been carried out were employed for extraction of cellulose and hemicellulose subsequently. The characterization results indicated a good yield of the extracted products with excellent purity. This method follows a green chemistry approach with minimum wastage of the raw material. This method helps counter the scientific challenges such as waste, fire pollution, to prepare more and more economic and eco-friendly materials in the near future with sustainable development.

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ANALYZING INTERACTIVE ARCHITECTURE AS AN APPLICATION TO A POST-PANDEMIC SITUATION IN THE CONTEXT OF DHAKA

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ABSTRACT

The COVID-19 pandemic in 2020 has resulted in an increase in domestic violence. People must spend the majority of their time indoors, which forces them to use technologies like smartphones. Many individuals have gone through periods of unemployment, economic hardship, and faith-damaging family ties. In these circumstances, people had no choice but to engage with the virtual environment. In Dhaka, the rooftop was used only as a playground and a location for outdoor recreation during this particular incident. Interactive architectural installations modeled after residential neighborhoods may play a significant role in encouraging a sense of community belonging, particularly for young and elderly people. Residents may use their rooftops and connect with collaborative architecture that uses all of their senses to communicate with people. This kind of interactivity could be used to combat the pandemic melancholy by mounting interactive architecture on the roof. This article discusses a potential interactive architecture that may be built on the rooftop and foster community spirit in order to aid the impoverished community during or after a pandemic. This article reviews many types of research and offers some recommendations for an interactive design that might be employed in the future to promote neighborhood activities, giving the depressed population a chance to participate in a community within the home.

Keywords: Interactive Architecture, Pandemic (COVID-19), Community, Rooftop, Community Interaction

INTRODUCTION

In December 2019, a coronavirus disease (COVID-19) emerged in Wuhan, China, inducing widespread terror and dread. It has spurred a worldwide concern. It is turned into a global health issue (Chen et al., 2020). At the beginning of 2020, the World Health Organization (WHO) labeled COVID-19 a global pandemic. Bangladesh got infected with the virus in March 2020. Bangladesh has been named the second most devastated country in South Asia (Hossain et al., 2021). Self-quarantine practices, minatory quarantines, travel limitations inside or outside the city, restrictions on overseas flights, and finally, curfews were all used to start social distancing in various countries. Flexible and home office practices have begun at companies, while physical education has been halted and online education has begun (GÜZEL et al., 2020). Bangladesh's government, enforced social separation by putting the country under lockdowns like other countries such as China, Italy, Spain, and India (Rahman and Islam, 2020). People in Bangladesh face economic, psychological, medical, and sociological problems, as well as domestic violence. It entails the use of violence and other forms of abuse to establish dominance and terror in a relationship, making it vulnerable (Hossain et al., 2021). The occurrence of brutality is random or continual in pattern (Kaur and Garg, 2008). During the Pandemic period, familial violence has skyrocketed (Campbell, 2020). Boredom and depression are common psychological phenomena for people. There is no recorded report on how to treat family violence survivors' physicians in literature (Sharma and Borah, 2020). As a result of the pandemic, people have been compelled to confine their lives to their homes, which has resulted in increasing community activities rather than public space. People's lifestyles, interactions, jobs, and social, and emotional status have all changed because of the pandemic (Rahman and Islam, 2020). Physical activity and proximity to natural features and greenery are prominent for preserving contentment and physical and mental health, according to studies on health and wellness (Mitra et al 2020, Jacob et al 2020, Alessandro et al 2020, Amerio et al 2020, Sinha et al 2020, Hanzl 2020). The impact of COVID-19 on the shared environment like rooftops or ground floors or front streets, as well as changes in cities and neighborhoods, make changes at an urban scale (Rahman and Islam, 2020).

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During a pandemic, residents' mobility has been restricted, forcing them to spend more time at home, raising the challenge of how they meet their demand for open space. The use of roofs and verandas has increased in this period, as has neighborly interaction on the rooftop and ground level (Rahman and Islam, 2020). People of all ages suffered in numerous ways because of their inability to engage in even the most basic physical and social activities. Life was trapped in a virtual environment that was increasingly private. A playground or social interaction was difficult for children. Individuals are forced to spend their time at home in this situation, and they chose their rooftop as an interaction zone. Many occupants have utilized their roof as an outdoor playground, flying kites, for example. They have been stranded in their home for over a year. The rooftop is used for a variety of purposes in Bangladesh, but during this horrific period, people have found it as the sole place where they could interact with others and nature. It would be a great development for the community if the rooftop is utilized as a communal interaction space for a certain neighborhood.

In this condition, the use of technology has achieved its pinnacle, but it is restricted to the virtual realm and more personal areas. Using interactive technology on rooftops may be able to shift the contact between residents into a new realm, allowing Bangladesh to move in new and beneficial directions. "Interactive architecture can be defined as the total integration of the disciplines of interaction design and architecture" (Fox, 2010). Physical structures are static and do not respond to others. Instead, interactive architecture responds to its users as if they are already living entities. When interactive technology is used in architecture, an environment will be able to rearrange itself and automate physical reactions inability to answer, interact, adapt, and be responsive. Interactive technology is created with the intention of creating settings and objects that can adapt to changing interpersonal, social, and environmental needs (Fox, 2010).

The purpose of this study is to contribute to the resolution of this horrific social condition and that inhabitants in the context of Bangladesh are connected through communal participation using interactive technology in their communities. Searching through the case studies on how interactive technology can affect the physical setting of a community positively in many cases and finding the futuristic notion of using an interactive architecture that could change the emotional health of the neighborhood positively and collectively.

LITERATURE REVIEW

Defining Interactive architecture

Interactive architecture did not emerge to gain traction until the mid-nineties. A lot of efforts beyond academia have now been completed, enabling prospective customers to get some faith in this form of experimental architecture. The comprehensive merger of such fields of communication art and construction is known as interactive architecture. This is based on the merging of embedded computation (intelligence) as well as a practical analog (kinetics) which can be adaptive. Whenever these two departments get combined, an environment will also be able to restructure on its own and regulate physical transformation to react, interact, adapt, and communicate. By identification, reactive architecture is a two-way path. Individuals should be viewed as "participants" rather than "users" when interacting with architecture. The future of architecture will rely on innovation and fully uncharted techniques and applications. Modernization from other industries with found comparable results and responsive advances will continue to affect and advance interactive architecture. Humankind is benefited from technology in many ways such as electrically powered actuators and human-machine interfaces transformed conventional mechanical and hydraulic control mechanisms in the auto sector with "drive-by-wire" technology. The introduction of formerly inconceivable tools for capturing knowledge/analysis has been signified by a surge in sensor invention and manufacture (Fox, 2010). Interactive architecture is preprogrammed with a sense of responsiveness that creates a relationship between humans and machines. We can say interaction is a 'mutual reaction' between artificial intelligence and humans (Hoberman, 1999). Using interactive technologies to aid in the planning and enhancement of the rooftop's use could result in a location for proper relaxation and activity. Building space can be used for more than just physical interaction owing to communicative embedded technologies. Changing from a well-communicating space neighborhood to a new, unknown collaboration space is a possibility. To improve people's interactions, several interaction technologies were deployed. Emotionally, mentally, and physically, embedded interactive devices can connect with individuals. Sensor systems based on technology can read human emotions and, by doing so, they may be able to change their character, which will benefit people.

Benefits of interactive architecture

Humans can get benefit from Interactive Architecture in a variety of ways. It can also give safety and security in methods that no fixed design could do. Buildings account for half of all CO₂ emissions; thus, green building has a big advantage. To achieve

easy implementation, connection, and production, making the place adaptable are interactive system. Interactive Architecture investigates how elements in the physical environment may present just when they have been required and then vanish or change once they are no longer required. They are well-suited to adaptation and react to emerging requirements (Fox, 2010).

MATERIALS AND METHODS

In this study, a mixed-methods technique is used. Review of the legal framework for acquiring information in the event of a pandemic through literature review. Using methods to collect information and examine related information from books, websites, journals, and other sources. Words, photos, and objects are used to create information output. Some case studies are considered to better understand the concept of interactive technology in architecture and the development of design concepts. The rooftop will communicate with the entire neighborhood through some futuristic technologically advanced installation that will give a communal feeling of togetherness to residents.

LEARNING THROUGH INTERACTIVE ARCHITECTURE

Study 01 (D-tower):

According to Spuybroek, "the tall premade epoxy construction in a bulbous, vegetal form catches interest day and night in its various incarnations, which is related to a Gothic dome with the legs and facade connecting with the same continuity, and it is a seamless combination of multimedia," says Spuybroek, "where architecture is part of a larger interactive system of relationships." Every evening, the D-tower light goes on from the street illumination, so observers can monitor the tower's color through a webcam at www.d-toren.nl/webcam. It consists of three components which are a physical construction (the tower), a poll of the questionnaire, and a website. Each of the three components communicates with the inhabitants. Its written website (www.d-toren.nl) records People who have higher emotions in response to a questionnaire created by artist QS Serafijn, assessing the degree of their love, hatred, happiness, and terror experiences. 50 residents will complete the questionnaire every six months after that the responses are then translated into various 'landscapes' that are shown on the internet after the questions are exacted. Based on the response from the residents, the technique reveals the emotional life. according to Spuybroek, every one of the ins and outs of individual feelings, involves ongoing discussions about different connotations. Each month, the questions get increasingly specific, and the replies are plotted in distinct "landscapes" on the website. based on these responses to the questions The tower abstracts the sensations using color, displaying "the State of the Town" in the color of the heartfelt feelings that are output as the results of a vote regarding individuals' emotions on the D-website, showing blue for happiness, red for love, green for hate, or yellow for fear. However, according to Lars Spuybroek of NOX, it still has not been yellowed (for fear). The landscape will portray the highs and lows of moods in each of the town's zip codes. Someone could identify which emotions would be the strongest that day after walking through Doetinchem in the evening.



Figure 1: D-Tower, Doetinchem, 2003 (source: <https://www.bollinger-grohmann.com/en.projects.d-tower.html>)

The project shows intense (sensations, characteristics) and the comprehensive (space, amounts) start to play separate roles, wherein individual interaction, coloring, wealth, values, and emotions become linked realities,' says the artist. Conducting romantic life or expressing the deepest ideas in an architectural vehicle, which looks to be replaced by the classic country greens

in which people who chat and share togetherness, is an advanced sociological idea. It also allows locals to leave their personalized messages underneath the tower, which are displayed on the website's emotive landscapes, and post a picture and a short statement to the portal, which will be connected to the environment through a little interactive digital flag. To connect the inhabitants strongly, the tower will send handwritten love notes and flowers from "love addresses" to "hate addresses." Community is surprised at the end of each year, and the tower will give a \$10,000 award towards the location to the most powerful emotions. It is a wider engaging connection system with the residents using a communication architecture system in a collective manner. Community engagement is high in this installation.

Study 2 (Bubbles):

Another exploration project that adapts well to the urban context named Bubbles which is a flexible spatial inflatable performance artwork. The installation is made up of enormous pneumatic volumes that expand and compress in response to the arrival of viewers and in the absence of visitors, the spatially distributed bags occupy the entire site with the formation of a transparent bubble with transparency filling. A technologically advanced sensor system detects occupants' movement, and increasing activity enables the space to open and become accessible (Lin, 2019).

Study 03 (Bloom):

There are numerous examples throughout the world in which architects have discovered how interactive technology and architecture have positively impacted the environment and human feelings. "Bloom," an architectural research project by DOSU Studio Architecture, is on display at the Materials and Application Gallery in Los Angeles. The project is intended to monitor instrument indexing time and temperature using a materialistic experiment, structural modernization, computational system, and pattern building into an environmentally conscious model. It is covered with a smart thermobimetal which turns into curls when heated by the sun or ambient temperature changes. The research claimed to have developed a passive, sustainable technique for minimizing dependency on artificial climate-control technologies.

It demonstrated a unique structural strategy that prioritized distributed structural stresses while reducing infrastructure requirements. It showed how computer technologies may be used to design, analyze, and fabricate complex surfaces. The highly interactive skin interacts with the temperature and response to provide shades and ventilation in a certain area of the shell without power or controls. With the use of progressive digital software, the skin is made up of around 14,000 laser-cut parts, which necessitate the function. The structure is self-supporting with 414 hyperbolic paraboloid-shaped fixed panels which examine the materials' ability to act as a shell. The ultimate prolog shape is lightweight and flexible, grounded on the complete geometry and material combination to ensure inclusive stability. One portion of the surface faces the sun directly, while the other side is shaded, causing no reaction, or curling in a single panel. As a result, each panel has a wide range of tile shapes and functions. In "Bloom", the total structure is completely associated with the structural capability of each hypar panel. Sung is working on bris-Soleil systems and curtain-wall panels that include responsive thermobimetal and glass in passive shading systems (Furuto, 2012).



Figure 2: Bubbles, 2019 (source: https://foxlin.com/portfolio_item/bubbles/)



Figure 3: Bloom, Los Angeles, 2012 (source: <https://www.archdaily.com/215280/bloom-dosu-studio-architecture>)

Study 04 (DUNE):

Investigating the interactions between people, technology, and the environment is the fundamental objective of Daan Roosegaarde (1979), with the concern of a sociological visionary who collaborates on future landscapes with his team in Studio Roosegaarde. The project DUNE is his light-based interactive landscape installation that reacts with human action. Hundreds of fibers in this alloy of nature and technology glow in response to the sounds and actions of visitors. Visitors become active participants in the project, strengthening social interactions with others as well as community participation in the landscape, with the creation of future relationships between urban space and people, where hundreds of interactive LED (Light Emitting Diode) lights and noises are included in DUNE (Roosegaarde, 2006-2018, [studioroosegaarde, 2010](https://www.studioroosegaarde.net/project/dune)).



Figure 4: DUNE, Rotterdam, 2007 (source: <https://www.studioroosegaarde.net/project/dune>)

Study 05 (Noosphere):

Noosphere is a spherical artificially intelligent structure created by architect Philip Beesley, who was inspired by the fast-evolving technology and culture of responsive and interactive systems. His major idea is that future architecture may incorporate living functions and that structures may start thinking and caring for themselves. According to human Individual sounds that swell to strong melodies and soften to faint murmurs are generated by networks of high-fidelity, panoramic speakers. Hundreds of microscopic processors respond to viewers' movements and gestures with unique illumination, audio, and movement. "SmartWrap," a customizable nylon membrane base, and polymeric cover that could modify its accountability level, light, or coloring, and show photos. "Kinect Motion Sensors" are used to learn a visitor's body posture, profundity, and proximity. Over time, through Physical Data, Vocal Detection, Systems "Shape memory alloys" and mode-developing materials that can change their molecular, electromagnetic, tensile, electrical, or thermodynamic conductivity dependent on their connection (Beesley, 2019) (Futurium, 2020).

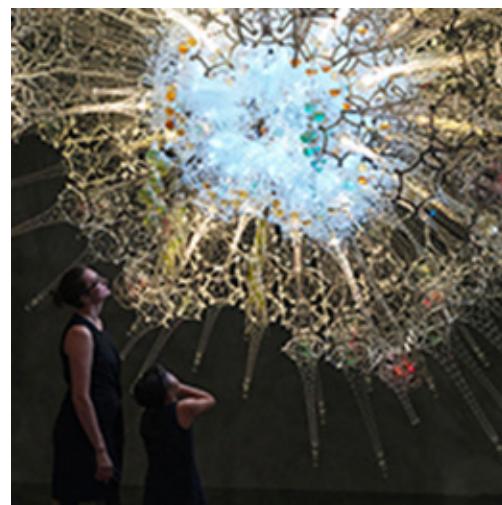


Figure 5: Futurium Noosphere, Berlin, 2019 (source: <https://livingarchitecturesystems.com/project/futurium-noosphere/>)

Study 06 (Burble)

The Burble has taken on a variety of embodiment and has performed at events and gatherings all over the globe. It was inspired from the tale of 'Jack and the Beanstalk.' considering the distinct situation, each version is composed and constructed to the requirements of the site. The Burble was originally constructed from 1 km of carbon composite poles and 1 km of Mega D12 high-performing cruising cord, making it one of the largest global interactive architecture installations. The project's goal was to see how regular citizens can create, develop, and influence the local civic environment on a large scale, even if it is just for a single evening. The color of the pattern varies in reaction to how it is modified instantaneously by individuals clinging to the railing below. Visitors' activities have an impact on either the run-time reaction (color changes) or how the structure reacts to individuals since they designed the architecture in the first instance. It is the 'multi-mediated' interactive design that is revolutionizing and redefining our workplace, recreation, and home environments in every sphere of professional and personal life as a visual platform.



Figure 6: Open Burble, Singapore Biennale 2006 (source: <https://www.haque.co.uk/work/burble/>)

Study 07 (Luminous Motion)

The Light Art Project is an interactive luminous sculpture erected in Winchester Cathedral. The installation is a futuristic illumination appearance of stainless-steel optical fibers that is motivated by the medieval Christian concept of the 'axis Mundi.'

Through the vocabulary of illumination, the designer intended to investigate notions of paths and communication networks in the modern metropolis. During the day, the powerful fiber optic light points are very visible, and at night, they are magnificent. Everyone with a cell device can manipulate and adjust the colors of the illumination, allowing for virtual conversation across the realm. A user can modify the color coding of a telecommunication tower by using a smart telephone. As a result, a vast number of individuals would be able to see it (Freeman 2008). The focus of this work is on state-of-the-art routes, both actual and virtual (Freeman, 2002).

Study 08 (Sky Ear)

Now ' Electromagnetic fields (EMF) are common everywhere in our environment. It is a very responsive installation on an urban scale that floats in the sky with 'LED-embedded balloons' and listens for 'electromagnetic waves. Now ' Electromagnetic fields (EMF) are common everywhere in our environment. The sky ear creates an electromagnetic environment just outside our natural sensibilities with a luminous cloud of tens of thousands of helium balloons and sensors device the cloud demonstrates natural electromagnetic surroundings to modernize the environment, as well as how personal cell phone calls and texts influence existing and future electromagnetic fields. Devices with a connection to the internet, all start contributing to a vibrant and dynamic strain culture in metropolitan centers. This project aims to research spatially in the urban context for the communal neighboring connections. The cloud analyzes the electromagnetic environment caused by events, cell phones, police, and paramedics frequencies, and TV stations because it lifts into the sky. The balloons vary in color depending on the amount of electromagnetic energy they receive and connect with one another to form broader rhythms. Folks call to cellphones attached in the cloud to hear environmental microwave "whistlers" and "spherics." These calls have changed the colors and patterns that show all over its cover. It floats with the wind and different electromagnetism then the cloud shines and varies. The "spherics" and "whistlers" those individuals hear on smartphones are the noises in the environment. Sky Ear is a one-night festival wherein visitors can call into a shimmering "cloud" of cell devices and helium balloons and hear the sounds of the sky (Haque, 2004).

RESULTS AND DISCUSSION

In addition to the case study, embedded or interactive architecture or installation procedures can be performed in the subcontinent. Interactive architecture is a way to connect people to the most advanced technological system. In some project, people are connecting their emotions to the project and get feedback from it which give a collective notion for the city. Citizens of the city are able to know about others' feelings through the project.

D-Tower, for example, used a questionnaire poll to collect data from individuals based on their emotional state, which was subsequently saved on a website. The d-tower has changed color because of this information, indicating the collective emotional state of most citizens (NOX,2005). Some influential strategies are employed in this project to engage citizens in the feedback collection process. This initiative has an impact not just on the neighborhood but also on the city. Through the collective concept, individuals may observe and grasp the emotional state of all citizens (Study 01).

In Bubbles several simple systems interact in a way that causes more complex behaviors to emerge as a collective and occur as emergent behavior. In Bubbles several simple systems interact in a way that causes more complex behaviors to emerge as a collective and occur as emergent behavior. It creates a robotic environment that is built using mechanical assemblies. These projects have prompted visitors to command as emergent and bottom-up (Fox, 2010). It makes visitors believe that it can respond or react to its unexpected behavior. It is an urban scale project which allows visitors to interact with (Study 02).

Bloom, another project, raises awareness about solar energy and its influence on tourists. It is an environment-conscious design that provides shade to visitors while also providing a city-scale space beneath it (Study 03).

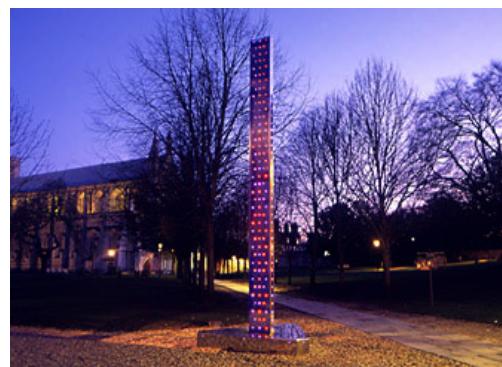


Figure 7: Luminous motion, Winchester, 2002 (source: <http://www.peterfreeman.co.uk/winpg.htm>)



Figure 8: Sky Ear floating above Greenwich Park, 2004 (source: <https://haque.co.uk/work/sky-ear/>)

DUNE is an interactive landscape built on motion and sound sensors. This form of the interactive landscape is appropriate for youngsters to interact with (Roosegaarde, 2007). This proposal introduces a tremendous new concept of the technologically interactive urban environment that can be installed anywhere, even on a tiny balcony. This project is installed in numerous locations as an example of the tunnel that secures the environment for passersby with its sensor system that interacts with the passers and offers them a smile by glowing and whispering. This project is much appreciated in many aspects, including the enhancement of social interaction between people, children, and the provision of a sense of security based on on-site demand (Study 04).

Another artificially intelligent installation that reacts to visitors by glowing, changing shape, and producing sound. Its reaction has changed based on its memory, which is why visitors find it completely different on each visit. This intelligent structure provides visitors with a sense of interaction with the robot, which has the memory to recognize specific movements and gestures, as well as a sense of communal interaction (Study 05).

The Burble experience is a revolutionary idea that has been implemented in a variety of events around the world. It is large enough to modify the city's skyline for one night. It is animated as it sails on a massive scale with the wind. Individuals can use a smart device and their fingers to create patterns, lines, and circles that change color and pattern depending on the surface of the burble. This is a collaborative and collective interaction with the installation. It has a significant impact on community activity and improves the cohesiveness of events (Study 06).

Luminous Motion is a construction that connects people with their smartphones and provides illumination based on the actions of visitors. The idea of the project links the physical and spiritual worlds. This sculpture facilitates virtual connections between people and responds to them. People's collective action allows the light to shine promisingly at night and promotes communal interaction (Study 07).

Finally, an outstanding project Sky Ear is an interactive installation that floats in the sky and communicates with users via smart devices. It alters the environment by changing the colors and sounds. When it floats and whispers at night, the entire environment changes magnificently, which connects people. Collaboration on a one-of-a-kind action improves the community environment in a collective manner (Study 08).

CONCLUSIONS

A variety of anomalies in socioeconomic, behavioral, educational, and cultural contexts conclude the post-pandemic situation. We are unable to move static architecture such as buildings, but we can propose some interactive, adaptable, and embedded architectural interventions that could be constructed in any location in the neighborhood while keeping the Bangladeshi context in mind. This type of installation can benefit both the neighborhood and the people. Interaction design in architecture has become increasingly prevalent. The interaction will be truly seamless. However, while we will interactive systems, we must also recognize that it is the responsibility of useful design to express interaction from a design standpoint (Fox, 2010). An interactive system that connects the entire neighborhood could be effective for residents. Keeping in mind that some interactive installations are considered as installation projects on the top of the roof in this study to achieve collaboration, interaction, and collectiveness within the neighborhood. These proposed futuristic installations are experimental installations in the context of Bangladesh.

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TIME DOMAIN APPROACH FOR ROLLING ELEMENT BEARING FAULT DETECTION AND DIAGNOSIS IN VIBRATION MONITORING

Chahinez Beldjaatit^{1*}, T. Sebbagh¹, and H.Guentri²

ABSTRACT

One of the most crucial techniques for equipment maintenance is vibration condition monitoring. This is done to identify failing components and improve the machine's safety and reliability. The health of the bearing has an extensive impact on the life of the rotating machine. This is because bearings are the most vital and critical part of rotary equipment. The vibration signal analysis can give us a better understanding of various defects occurring in mechanical systems. The current work focuses on the fault detection of ball bearings using time domain analysis in vibration monitoring. Several statistical features are calculated in this type of analysis for three bearing conditions essentially: healthy, inner race fault, and ball fault condition. When the results from both healthy and defective conditions are compared, it becomes clear that default conditions result in an increase in the majority of the statistical parameter values. The effect of changing the fault diameter and the load on the bearing state is observed through a variation of statistical parameter values. The results show that time features can clearly distinguish between all bearing situations.

Keywords: Condition monitoring, Fault analysis, Rolling element bearing, Time domain analysis.

INTRODUCTION

Considering the rapid development in science and technology, the numerous applications in industries where rotational mechanical equipment is used results in the effective operation of systems such as compressors, automobiles, industrial fans, and steam turbines (Li, et al., 2018). The technology for defect identification has taken center stage in prognostics and health management (PHM), it is essential for maintaining mechanically intelligent equipment (Wang, et al., 2018).

Early fault detection in rotating machinery reduces damage hazards and hence decreases the need for costly emergency repairs. Condition monitoring via vibration analysis is useful tool and effective technique for determining the state of the rotary machine. Most rotating equipment failures are caused by rolling element bearings (Zhang, at al., 2011).

The analysis techniques applied to process the raw vibration signals for rolling element bearing condition monitoring split into the following categories: Time-domain, frequency domain and time-frequency analysis methods. In time domain technique, parameters like kurtosis, skewness, crest factor, and RMS are monitored for bearing fault detection (Shiravastava & Wadhwani, 2014). For frequency domain analysis Fast Fourier Transform (FFT) is used to convert time domain vibration signals into frequency components are used for fault detection and location. The most valuable method of all is time-frequency analysis since it provides both time and frequency information. Typical techniques for time-frequency analysis include Short Time Fourier Transform (STFT) (Thomazilla, et al., 2019), Wigner-Vill Distribution (WVD) (Baydar & Ball, 2001) and wavelet transform (Shukla, at al., 2021). Time domain analysis is the simplest and most straightforward of the three methodologies, time domain statistical features are widely used to detect and classify different types of bearing faults using various architectures of deep learning (Magar, at al.,2021).

In this paper, time domain analysis of fault bearing has been presented. Several time features parameters are calculated and extracted from vibration signals of different states of bearing, comparing the statistical features allows for the identification of bearing faults. This is due to the presence of effective informations in these parameters about energy content in vibration signals.

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The paper is structured as follows: The bearing dataset is described in Section II. Section III describes the fault diagnosis technique. The time Domain features computation is presented in Section IV. In Section V, the monitoring results and discussions are presented. The last Section concludes the paper.

Dataset Description

The dataset used in this study is obtained from the Case Western Reserve University Bearing Data Center (CWRU Bearing Data Center) is shown in figure 1. It allows access to ball bearing test information for both healthy and damaged bearings. Electro-discharge machining (EDM) was used to collect the vibration signal data under the following four experimental conditions: (a) normal state, (b) inner race fault, (c) outer race fault, and (d) ball fault with fault depths from 0.18 mm to 0.71 mm (0.007 inches to 0.021 inches). The vibration data was recorded using accelerometer with a sampling rate of 12 KHz and 48 KHz for drive end bearing faults. the vibration signals were collected for motor loads of 0 to 3 hp at motor speeds of 1720 to 1797 rpm. In our study, the sampling frequency chosen is 12 KHz for three cases: normal condition, inner race fault, and ball fault. Each fault type bearing includes four kinds of fault degrees (0.18 mm, 0.36 mm, 0.53 mm, and 0.71 mm). Figures (2a), (2b) and (2c) represent respectively the vibration signals of normal state, inner race fault and ball fault.

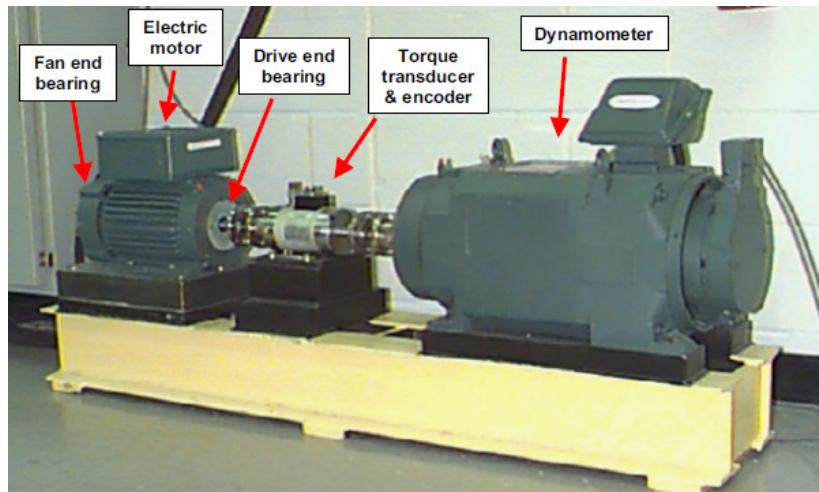


Figure 1: CWRU test rig (Smith & Randall, 2015)

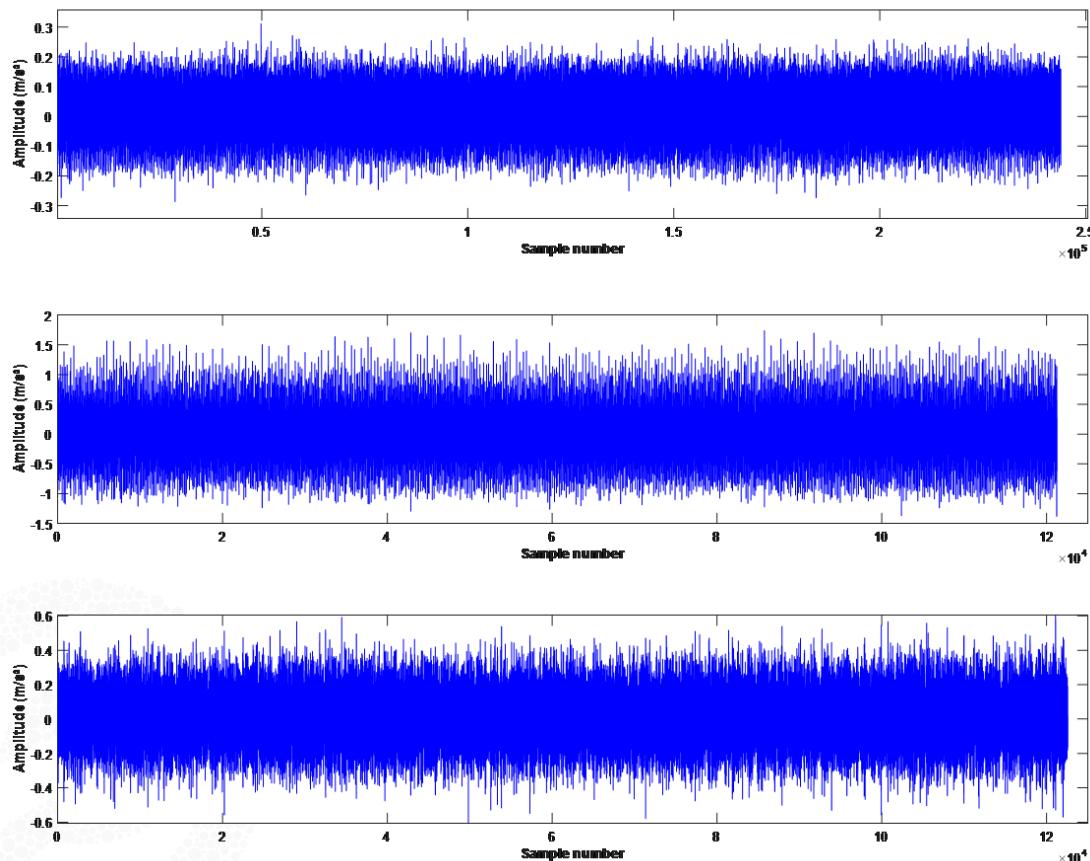


Figure 2: Vibration Signals (0 hp, 0.18 mm): (a) normal state, (b) inner race fault and (c) ball fault.

Time Domain Analysis

The process which some characteristics features as well as statistical parameters are computed from vibration data is known as time domain analysis. For effective fault diagnosis, feature extraction turns as an important step. In the current work, the statistical features used for time domain analysis are: root mean square (RMS), kurtosis (Kur), peak value and standard deviation (SD). In this section the mathematical description of proposed features is presented.

Root Mean Square (RMS)

The root mean square (RMS) value indicates the energy content in the vibration profile, it is a Gaussian random process with amplitude modulation (Nayana & Geethanjali, 2017). RMS is presented as :

$$RMS = \sqrt{1/N \sum_{i=1}^N (X_i)^2} \quad (1)$$

Where X_i is the raw vibration signal, i is the sample number and N is the number of sampling points.

Kurtosis (Kur)

Kurtosis is the normalized version of the fourth moment of the signal, it is a statistical measure of the random variable distribution. The approximate value of kurtosis for normal bearing is 3 (Casarendra & Tjahjowidodo, 2017). When the value is more than or equal to 4, it denotes that there is some degree of damage. Kurtosis is given by:

$$Kur = \frac{\frac{1}{N} \sum_{i=1}^N (X_i - \bar{X})^4}{RMS^4} \quad (2)$$

\bar{X} is the mean value of the time series X_i

Peak Value

Peak Value is maximum amplitude at some point. A breakdown with immediate impact is always detected using the peak value (Fu, et al., 2015), it is obtained as:

$$\text{Peak Value} = \max (X_i) \quad (3)$$

Standard Deviation(SD)

Standard Deviation is the degree of depression of the data points relative to its mean. It can alternatively be defined as the RMS of signal. SD is represented as:

$$SD = \sqrt{1/N \sum_{i=1}^N (X_i - \bar{X})^2} \quad (4)$$

Time Domain Features Computation

The time domain analysis features are extracted from vibration signal of both healthy and defective bearings. The results of statistical parameters are given in table 1.

Table 1: Statistical parameters in time domain of vibration data.

Fault Diameter (mm)	Load (hp)	Bearing state	RMS	Kurtosis	SD	Peak value
0	0	Healthy bearing	0.0738	2.7642	0.0727	0.3113
	1		0.0664	2.9306	0.0652	0.3459
	2		0.0643	2.9251	0.0631	0.3592
	3		0.0659	2.9572	0.0647	0.3065
0.18	0	Inner race fault	0.2915	5.3959	0.2912	1.7390
	1		0.2929	5.5423	0.2928	1.5808
	2		0.2995	5.5638	0.2995	1.6396
	3		0.3136	5.2911	0.3136	1.6715
0.18	0	Ball fault	0.1392	2.9847	0.1387	0.6070
	1		0.1391	2.9638	0.1390	0.6596
	2		0.1473	2.8314	0.1472	0.6046
	3		0.1536	2.8897	0.1536	0.7206

0.36	0	Inner race fault	0.1978	21.9574	0.1948	2.0108
	1		0.1655	22.0843	0.1655	2.0301
	2		0.1631	21.8662	0.1630	1.8805
	3		0.1808	18.1640	0.1808	2.1269
0.36	0	Ball fault	0.1527	17.7692	0.1526	2.2785
	1		0.1409	8.8371	0.1408	1.3398
	2		0.1435	9.7522	0.1434	1.8391
	3		0.1337	14.8587	0.1336	1.9892
0.53	0	Inner race fault	0.5254	7.4450	0.5252	3.7880
	1		0.4418	7.6667	0.4418	3.6861
	2		0.4889	8.0580	0.4889	3.6231
	3		0.4487	8.3451	0.4487	3.6150
0.53	0	Ball fault	0.5254	7.4450	0.5252	3.7880
	1		0.4418	7.6667	0.4418	3.6861
	2		0.4889	8.0580	0.4889	3.6231
	3		0.4487	8.3451	0.4487	3.6150
0.71	0	Inner race fault	0.8384	3.3967	0.8384	4.7851
	1		0.8376	3.1956	0.8375	3.9307
	2		0.8413	3.2863	0.8413	4.5410
	3		0.8231	3.3169	0.8231	4.3469
0.71	0	Ball fault	2.0771	3.8715	2.0771	10.9310
	1		2.0299	3.9103	2.0298	11.6740
	2		2.1457	3.7723	2.1457	10.4419
	3		2.1450	3.8991	2.1449	11.3639

RESULTS AND DISCUSSION

Feature extraction technique is applied on bearing vibration signals under different conditions of motor loads (0 to 3 Hp). The statistical parameters such as RMS, Kur, SD, and Peak Value are shown graphically in figures 3- 13.

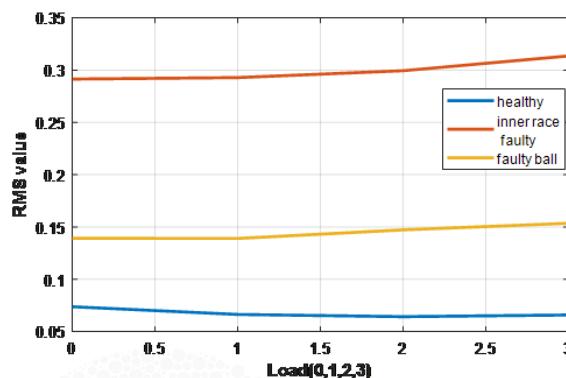


Figure 3: Variation of RMS Value for three bearing states.

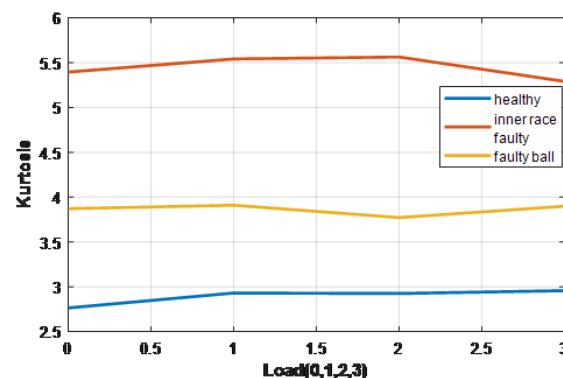


Figure 4: Variation of Kurtosis Value for three bearing states.

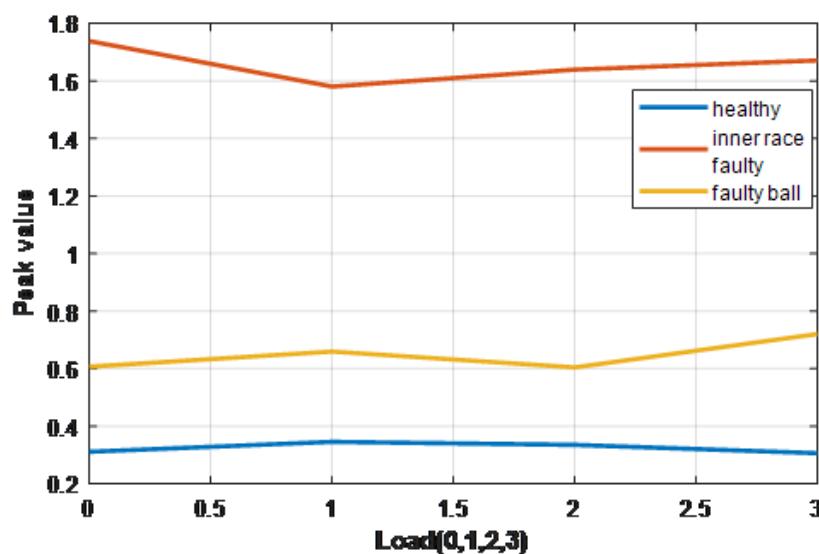


Figure 5: Variation of Peak Value for three bearing states

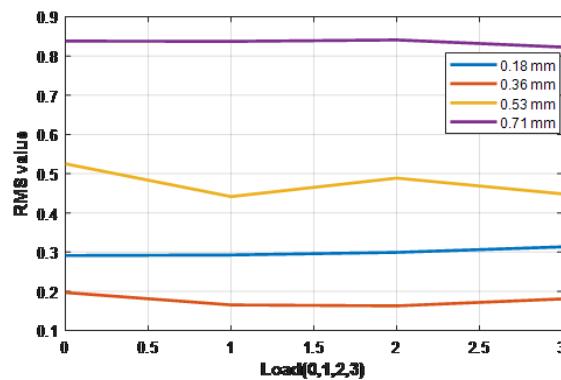


Figure 6: Variation of RMS Value for inner race fault with different diameters

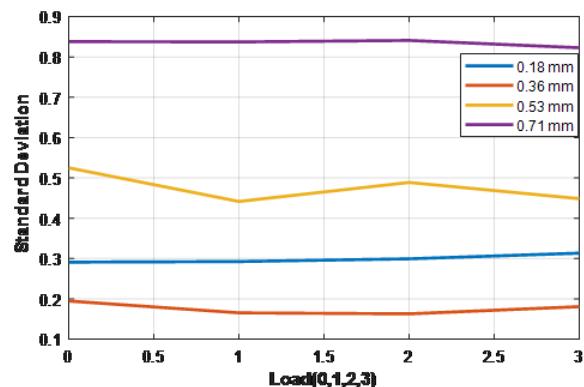


Figure 7: Variation of Standard Deviation for inner race fault with different diameters

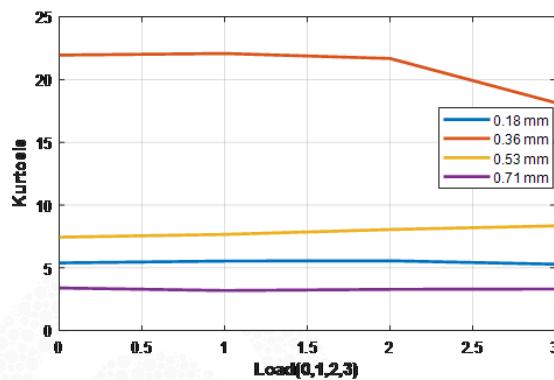


Figure 8: Variation of kurtosis for inner race fault with different diameters

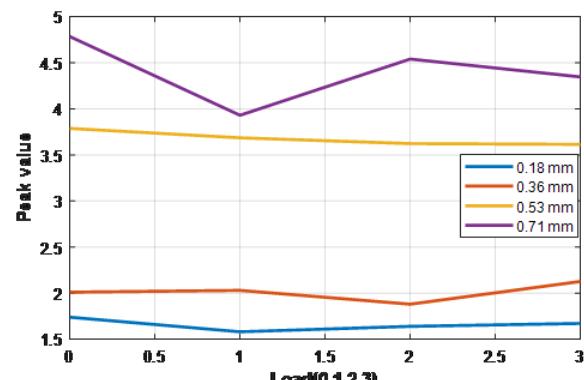


Figure 9: Variation of Peak Value for inner race fault with different diameters

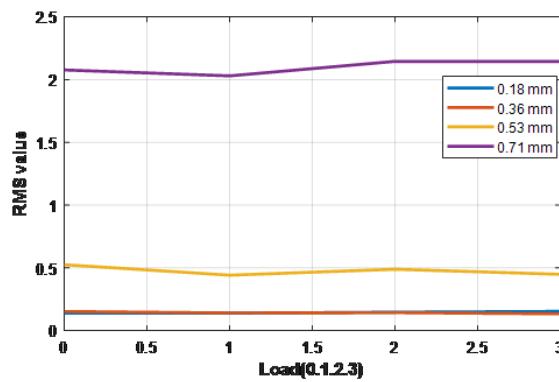


Figure 10: Variation of RMS Value for ball fault with different diameters.

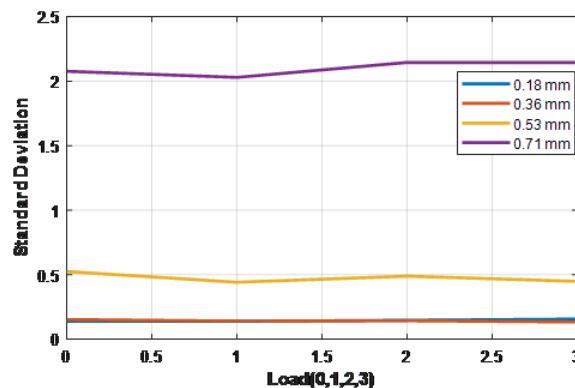


Figure 11: Variation of Standard Deviation for ball fault with different diameters.

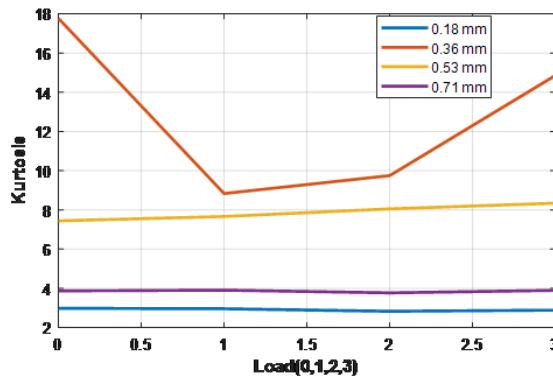


Figure 12: Variation of kurtosis for ball fault with different diameters.

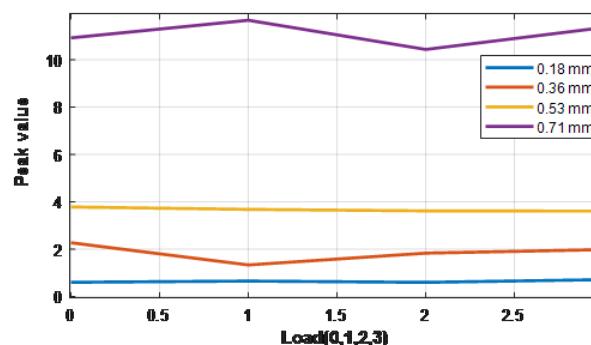


Figure 13: Variation of Peak Value for ball fault with different diameters.

From table 1 and the previous curves of time statistics indicators depending different loads (0,1,2,3), the first important observation we made was that for defective bearings, when rolling element pass over the defective zone of the bearing, the time features amplitude abruptly changes and increases. It can serve as a crucial clue in detecting faulty bearing. Figures 3-5 show that the separation between normal and defective bearing are very clear. We also note that the change in the load does not have a significant effect on the change in statistical parameters values.

The RMS , Kurtosis, and Peak values for each of the three bearing states taken into consideration in this work are shown in figures 3, 4, and 5. It can be observed that the RMS ,Kurtosis and Peak values of healthy bearings are lesser compared to the faulty bearings. The values of these indicators are greatly increased for inner race fault. This demonstrates that inner race defect produced higher energy and asymmetry in vibration data.

Figures 6, 7, 9,10, 11 and 13 represent the variation of RMS, Kur, SD and Peak Value for inner race and ball faults respectively with different diameters of defect. It can be easily noticed that the root mean square, standard deviation and peak values increase as the diameter of the damage increases. This indicates that the diameter of the fault has a big impact on the degree of damage development which leads to an increase in the dispersal and the number of peaks in vibration signals. Another note regarding the curves of RMS and SD respectively shown in Figures 6, 7, 10 and 11, they are identical. This is due to their almost equal values, which supports what is stated in the definition of standard deviation above.

In figures 8 and 12, it can be seen that kurtosis value for bearing with inner race fault and with ball fault is maximum in diameter of 0.36 mm. However, for defect case, a rise in peaks causes a flattening of the data distribution which results in a low value kurtosis.

CONCLUSION

The present paper includes a study on the time domain analysis technique for the monitoring of bearing's condition. This type of analysis displays the energy content of the vibration signal with time. It was implemented on the bearing data under different conditions and statistical features of the vibration signal were extracted to detect the bearings defect. The main time domain parameters that were used in this work are: RMS, Kurtosis, Standard Deviation and Peak value. Due to the wide variation in these values, thus showing clear identification between healthy and rolling element fault conditions of bearing. Hence, these results evince that time domain approach are capable of clearly differentiating between all bearing states, including normal, defective race and defective ball.

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TREND ANALYSIS OF RESEARCHES IN ETHIOPIAN CONSTRUCTION INDUSTRY

Elias NEZIF^{1*}

ABSTRACT

This study identifies the research trends of the Ethiopian construction industry in the last twenty years. The paper aims to search, categorize, and analyze the research trend of partnering related studies in construction using a desktop search method from several construction-related journals. A series of related articles from partnering journals are viewed based on searching and categorizing published journal articles from 2003 to 2022. The number of publications in the field with a good reputation was used to determine the most influential journals. A critical examination of the conference revealed that categorizing and evaluating, as well as the use of partnering, were common in construction industry research, while quantitative research techniques were used to analyze research trends in the industry. From numerous article reviews, future research areas were forecasted.

Keywords: Ethiopian construction industry: publications: research trend: survey analysis.

INTRODUCTION

Ethiopia's construction industry is a large-scale sector in the country. So far, several studies have been made to solve existing problems, enhance existing work types, and so on. Several universities in the country and almost all over the world are conducting MSc theses and PHD dissertations on the industry under different themes. Some research is being conducted despite the country's limited technological capacity and other constraints. This conference aims to address the trend of construction industry research in the country. Because the construction industry is so vast, several researchers have proposed various classifications for trend types.

There has been no study conducted in the last 20 years to examine research trends in the Ethiopian construction industry. A failure to gain a broader view of the state of research may result in overlooking core aspects or duplicating efforts (Yalcinkaya and Singh 2015). However, no systematic effort has been made to date to prevent the reputational damage caused by research issues. Reviewing research periodically helps to easily identify research gaps in the construction industry.

To ensure continual improvement in the construction industry's performance, its challenges must be identified so that integrated solutions that suit the context can be provided. The Ethiopian construction industry, like that in most developing countries, faces challenges that impede its development (Mengistu & Mahesh, 2019). The challenges that are being faced by the industry can be reduced by conducting a large amount of research and forecasting future construction target areas.

Several scholars categorize construction industry research in different ways, but Binghamton University introduced the most suitable categorization of topics. These topics were then reviewed and approved by a committee of construction management faculty from Brigham Young University, with feedback from additional construction professionals. Each theme was then defined so that it was relevant to the industry, allowing participants in the survey instrument to form an accurate opinion (Graham, 2010). Categories of construction industry research topics are: training/human resources, management/risks, technology/innovation, economics/cost control, globalization, estimation/bidding, scheduling, design/BIM, performance, safety, industry overview, legal/contracts, sustainability, computer systems/expert systems, project delivery, productivity/optimization, constructability, materials/equipment, project/quality management, facilities management, procurement, and heavy civil construction.

Now a days, the construction industry has seen rapid growth in projects of increasing size and complexity. These projects are usually very complicated in nature. Project complexity increases as a result of rapid changes in the environment, increased product complexity, and increased time pressure. Megaprojects are usually beset with poor performance outcomes, such as cost overruns and schedule delays, partly because of their increasing complexity and underestimation of this complexity (Luo et al.,

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2017). In Ethiopia, the trend for research in complex construction industries is very low compared to other themes. The study aims to find out the trend of construction research in the country. This provides a solid foundation for trend research in the construction industry. Many studies have shown that project success is dependent on the complexity of a project and that traditional project management methods are not enough to properly address this complexity (Remington and Pollack 2007). Proper understanding of project complexity is essential to ensuring effective management; therefore, much research has been undertaken on this subject.

MATERIAL AND METHODS

In this conference, local and foreign journals were taken as the primary method for quantitative analysis of published papers. This method was selected over qualitative manual methods of review in view of the considerations discussed below.

In order to analyze the trend of research in the construction industry, it is necessary to fix a benchmark for how to classify research topics. The systematic approach is mainly characterized by the selective inclusion of studies and biased interpretations in order to promote one's view. After several reviews of systematic approaches from several papers, it was found that the topic categorization approach of a committee of construction management faculty from Brigham Young University was followed (Graham, 2010).

These themes were then reviewed and approved by a committee of construction management faculty from Brigham Young University, with feedback from additional construction professionals. (Graham, 2010). As indicated on the study, research in the construction sector can be categorized into 22 themes, and those themes are used to categorize research trends in the sector. Ethiopian journals and foreign journals are searched using network technology. In other words, this is a quantitative method conducted by computational tools based on network analysis principles, in which the explored footprints from previous studies are investigated to trace the development of knowledge in a domain over time (Hosseini et al. 2018).

The current study searches for general Ethiopian construction industry research works from all universities and all over the world. Therefore, the conference work mainly has four steps. (1) searching for research papers, (2) categorizing research by topic, (3) analyzing research topics and identifying trends, and (4) forecasting future research areas

While analyzing the topics of the construction industry, a trend is computed with a significant P value that becomes more significant with extra data (Rowlinson, 2017), where the values above the p value are considered the research trend areas, while the topics with lower P values are not considered.

RESULTS AND DISCUSSION

Influential Journal on Ethiopian Construction Industry Research

A total of 129 publications were found by searching all universities in the country and all over the world. Six Ethiopian journals were found where construction research is published. Addis Ababa University (Zede Journal of Engineers and Architects), Adama Science and Technology University (Ethiopian Journal of Science and Sustainable Development), Bahr Dar University (Abyssinia Journal of Engineering and Computing (AJEC)), Wollega University (Science, Technology, and Arts Research Journal), Wollo University (Ethiopian Journal of Science and Technology), Ethiopian Association of Civil Engineers Journal, and Ethiopian Association of Civil Engineers Journal were reviewed. Table 1 shows the journals for repeated publications from the international journals searched: International Journal of Engineering Research and Technology (IJERT), Journal of Engineering Design and Technology (JEDT), and International Journal of Construction Management (IJCM).

Zede Journal of Engineers and Architects (ZJEA) ranked first among 129 publications searched, publishing 33% of journals published on Ethiopian Construction Industry, while Ethiopian Association of Civil Engineers Journal (EACE) ranked second, publishing 21% of the research. Furthermore, the International Journal of Engineering Research and Technology (IJERT) and the Journal of Engineering Design and Technology (JEDT) ranked third and fourth, publishing 12% and 10% of the research, respectively. (Table 1) shows the percentage of Ethiopian Construction Industry Journal publications.

Table 1: Journals with Number of publications

No	Journals	No of Publications	Rank
1	Zede Journal of Engineers and Architects(ZJEA)	42	1

2	Ethiopian Association of Civil Engineers Journal(EACE)	27	2
15	International Journal of Engineering Research and Technology(IJERT)	15	3
12	Journal of Engineering Design and Technology(JEDT)	13	4
7	International Journal of Construction Management(IJCM)	6	5
5	Ethiopian Journal of Science and Sustainable Development(EJSSD)	6	5
8	African Journal of Science, Technology, Innovation and Development(AJSTID)	3	7
13	Journal of Materials in Civil Engineering(JMIC)	3	7
17	Journal of Management in Engineering(JME)	2	9
10	Water International(WI)	2	9
4	Abyssinia Journal of Engineering and Computing (AJEC)	2	9
3	Ethiopian Journal of Science and Technology(EJST)	1	12
6	Science, Technology and Arts Research Journal(START)	1	12
9	International Journal of River Basin Management(IJRBM)	1	12
11	International Journal of Architectural Heritage(IJAH)	1	12
14	International Journal of Business and Economics Research(IJBER)	1	12
16	Journal of Construction Project Management and Innovation(JCPMI)	1	12
18	Advances in Civil Engineering(ACE)	1	12
19	Journal of Water Recourse Planning and management(JWRP)	1	12
Total		129	

Ethiopian Construction Industry Research Trend

The key target of this conference is to find and sort out construction industry research trends, and so far the research publications have been categorized into 22 topics (trends), some of which have reputations while others don't have research publications made in relation to the themes. A statistical tool is now used to distinguish between trend and non-trend research. P-value, also known as probability value, is used to understand the statistical significance of a finding.

The trend of the research paper is taken as 95% of the searched research topic's reputation. The data analysis' mean is 6.79, and the standard deviation is 2.94. The reputations greater than the mean minus two times the standard deviation are used in 95% of the research that identifies trends in the Ethiopian construction industry. For construction industry research, an observation greater than 0.91 is considered a trend. Aside from constructability, facility management, and civil construction, the remaining 19 themes are trends in published research on Ethiopia's construction industry. Material/equipment and technology/innovation ranked first and second, with 26% and 12%, respectively, of the published research made, whereas design/BIM and performance both ranked third, based on the published construction research made in the last two decades. The Ethiopian construction industry research trend is summarized in Table 2 below.

Table 2: Rank of Research Trend in Ethiopian Construction Industry

No	Theme	Theme	No of Publications	Rank
1	Materials/ Equipment	33	26%	1
2	Technology/ Innovation	15	12%	2
3	Design/ BIM	14	11%	3
4	Performance	14	11%	3
5	Computer systems/ Expert systems	8	6%	5
6	Productivity/ Optimization	8	6%	5
7	Economics/ Cost Control	7	5%	7
8	Project Delivery	5	4%	8
9	Project/ Quality Management	5	4%	8

10	Management/ Risks	4	3%	10
11	Training/ Human Resources	3	2%	11
12	Safety	3	2%	11
13	Scheduling	2	2%	11
14	Legal/Contracts	2	2%	11
15	Sustainability	2	2%	11
16	Globalization	1	1%	16
17	Estimating/ Bidding	1	1%	16
18	Industry Overview	1	1%	16
19	Procurement	1	1%	16
Total		129	100%	

CONCLUSION AND RECOMMENDATION

As per the study, research on the Ethiopian construction industry in the last two decades has mainly focused on materials, innovation, design, and performance, whereas computer systems and expert systems, productivity and optimization, economy and cost control, project delivery, project and quality management, and management and risks are secondarily focused areas. The third research trend in the construction industry is Training/Human Resources, Safety, Scheduling, Legal/Contracts, Sustainability, Globalization, Estimating/Bidding, Procurement, and Industry Overview. In comparison to the sector's economic contribution and numerous other possible research areas, research on Ethiopia's construction industry has been extremely limited over the last two decades. In this conference, almost all publications were searched, but only 129 papers were found, which indicates the construction research industry in the country is lagging behind. For example, lots of Ethiopian university journals have not published a single paper on this industry; only Addis Ababa University is addressing the construction research needs of the industry. It is recommended that professionals, academicians, and universities get involved in research. It is also suggested that complex project research, heavy civil engineering constructions, building information modeling, and 3D printing be considered.

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LANDMARK AND TOURIST SPOTS USING AUGMENTED REALITY

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ABSTRACT

It takes a lot of time to build a system design like this, but why not use the rising technology to build an Augmented Reality design? Using the appropriate technology can help minimize the time needed to make one. It can also show other people that society is catching up in terms of new applications and technologies. One example is Augmented Reality (AR), a technology that produces a mixed experience by superimposing digital data on a real-time image of the physical environment. Augmented Reality has now progressed to the point where real-time applications are considered and needed. Consequently, this study uses Augmented Reality to show the 3D model of the selected LandmarkLandmark and the Municipality of Dumangas, Iloilo, by pointing the Android mobile device to the provided Landmark marker. However, synthetic elements must be rendered and aligned in the scene accurately and visually acceptable way. To address these issues, real-time, robust, and efficient model-based tracking was proposed for a mobile camera - a virtual map for the Municipality of Dumangas, Iloilo. This would help the Municipality of Dumangas to promote their tourist attraction and the people who want to locate different tourist spots in Dumangas, Iloilo. This application would also provide the user with information about the specified Landmarks. The Landmark Tourist Spots Using Augmented Reality would test the user to know the Landmark in Dumangas, Iloilo.

Keywords: Augmented Reality, Tourism, Augmented Reality Benefits, Mobile Applications, Location Awareness.

INTRODUCTION

Tourism is one of the economic areas with the most substantial growth rates worldwide. There is undoubtedly a certain allure to travel. People's expectations for the caliber of games have been rising simultaneously. It has been observed that many Filipinos need more knowledge about their history and other facts about the country, such as heroes, national symbols, heritage sites, and how the Philippines gained freedom from the colonizers. As a Filipino, the developer incorporated information about Philippine history, specifically on Panay Island, in designing the game.

In connection with this, a study entitled "Landmark and Tourist Spots using Augmented Reality" was developed. This study has aimed to create a game that will help ease the difficulty of navigating a location, specifically Dumangas, while at the same time incorporating enjoyment and, at the same time, developing one's strategic capabilities. Landmark and Tourist Spots using Augmented Reality is a 3D platform game developed using Game Maker Studio as the main engine. This is aimed at creating a difference from one another through the integration of Augmented Reality. There is a level progression in the game where the difficulty increases in parallel to its level.

The game was developed to aid in learning Philippine history, primarily Dumangas, Iloilo, on the island of Panay. This system would help the users understand and gain more knowledge about different tourist spots and landmarks in the Municipality of Dumangas, Iloilo.

The game's contents would be based on the different histories of the municipality of Dumangas in Iloilo. For the municipality of Dumangas, the game would be based on <http://dumangas.gov.ph/about-us/history-of-dumangas/>. It is the Dumangas Municipality's local blog about the rich history of Dumangas. For Iloilo City, the game would be based on <http://www.ilovalocity.gov.ph/history>; it is the City's local blog about its history. And in other municipalities, it will be based on their history blog. And the result of the evaluation of IT Experts using McCall's Software Evaluation Criteria for Software Quality Model and the result of the evaluation of the respondents using the ISO/IEC 9126-1:2010 Software Quality Model Characteristics.

The term "augmented reality" refers to a live or direct view of an actual, physical environment whose elements have computer-

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generated sensory input, such as sound, video, graphics, or GPS data, added to or enhanced them. It is connected to a broader idea known as "Mediated Reality," in which a computer or other device alters one's perception of Reality. Therefore, technology works by improving how one now perceives Reality. Contrarily, virtual Reality simulates the actual environment in place of it. Traditionally, augmentation is done in real-time, inside a semantic framework, and with ambient components like sports scores on TV while a game is in progress. The information about the user's immediate environment in the actual world becomes interactive and digitally manipulable with the aid of cutting-edge AR technology.

A software development kit (SDK) for augmented reality applications called Vuforia is available for mobile devices. It employs computer vision technologies to instantly identify and follow simple 3D objects, such as boxes and planar images. When real-world photographs are viewed through a mobile device's camera, this image registration functionality allows developers to position and orient virtual items, such as 3D models and other media, in relation to those real-world images.

REVIEW OF RELATED LITERATURE

One of the most significant technological breakthroughs in recent years has been the creation of augmented Reality, which has improved accessibility, education, and entertainment inside the confines of a smartphone. Since its first release, numerous studies and applications have been produced using this as a starting point.

The main goal of augmented Reality was to provide a platform that would enable users to gain a deeper understanding of a specific place or activity by displaying the intended environment inside a virtual 3D representation. However, innovators have made augmented Reality more advantageous to a world that is gradually beginning to rely entirely on its necessary technology.

Nowadays, most people will turn to their smartphone or another mobile device as soon as the term "AR" is spoken. A sufficiently strong processor, a practical display or monitor, a variety of sensors, and input choices are all necessary for AR to function correctly.

"Typically a smartphone contains a processor, a display, accelerometers, GPS (global positioning system), camera, microphone, etc., and contains all the hardware required to be in an AR device," the Interaction Design Foundation says in "Augmented Reality – The Past, The Present, And The Future" on its organizational blog.

A few foresighted marketing teams have already started incorporating augmented Reality (AR) technology into campaigns targeted at tech-savvy consumers. The Lego app displays an AR version of a full Lego set when users look at the box through their smartphone camera. Accessory manufacturers use AR to let customers "try on" different styles of watches or glasses to see how the product looks. Comic book films have paved the way for their upcoming theatrical releases with AR Snapchat filters. Numerous ground-breaking smartphone apps and games that inspire effect also use augmented Reality. The "10 Best Augmented Reality Apps And AR Apps For Android" article by Android platform expert Joe Hindy on Android Authority.com discusses AR apps.

Hindy claims that Google Translate now uses augmented Reality (AR) to instantly translate languages by pointing a smartphone camera onto a sign, page, or screen printed in a foreign alphabet. Quiver uses augmented Reality to turn colored photos into three-dimensional figures, taking the current enthusiasm for coloring books to new heights. Additionally, AR can be found in online directories, star maps, instant messaging apps, games, and QR code scanners.

Why are AR and VR technologies being introduced into the market now? Virtual reality technology is not a new concept. The concept of virtual Reality was well-known by the start of the 1990s. The first home VR system, called Virtual Boy, was unveiled by Nintendo. The consoles didn't do well since they had a limited selection of games, no color (just red and black), and were awkward to use. A couple of years went by with no significant news. Then, in 2014, Oculus Rift brought up the subject of virtual Reality once more, and businesses worldwide started to develop their own VR headsets. Since the spring of 2016, some intriguing goods have been introduced and are currently being produced in large quantities.

Even better news is on the way. According to the United Nations World Tourism Organization, tourism has demonstrated incredible power and resilience over the past few years and has expanded rapidly. As a result, tourism is one of the global economic sectors with the greatest growth. The number of foreign tourists arriving worldwide reached 1'235 million in total, up 46 million (overnight visitors) or 3.9% over 2015, according to the most recent UNWTO World Tourism Barometer.

With a video system, augmented reality's initial method overlays digital images of Reality with AR. This involves taking away the marker or substituting virtual items for it. Another option is combining transparent mirrors and lenses with an optical vision system with real-world perception. Projectors, handheld displays, and head-mounted displays can all use it. They also benefit

from not having parallax because they maintain the resolution of the real world. These methods are safer since they can be utilized without power, making them perfect for use in the military and medicine. For interaction and registration, additional input devices are needed, like a camera. Projective systems are created using the third method, which involves projecting the AR onto real things. These screens have the benefit of providing a wide field of vision by covering huge surfaces. However, the interaction needs additional input devices. Every time the surroundings or the distance from the projection surface changes, projectors also need to be calibrated.

Since they have been in use for almost a decade, customers have not embraced smart glasses. 2021 appears to be a good year for AR smart glasses to become more widely available because many new devices are anticipated to be released this year.

Although augmented Reality (AR) has a lot to offer society, it can totally transform the training sector. This technology has many applications, ranging from corporate training in soft skills to hands-on learning in industries like manufacturing and healthcare.

The VR/AR Association's 2019 Training Industry Sector Report includes a list of more than 100 businesses willing to assist enterprises in transforming their training capabilities using AR. But despite this level of interest, the adoption of AR in training is still nascent. While there are several disruptive startups and promising investments, the use of this technology training is yet to go mainstream.

The year is 2022, and the future appears before anyone anticipates it. Technology that humans formerly could only imagine is now available to everyone. The list of novel technologies includes artificial intelligence, machine learning, blockchain, autonomous vehicles, and CRISPR. In 2022, augmented Reality will be one of the technologies that will significantly impact retail. AR empowers customers to perceive and customize goods in 3D while buying.

Keeping customers interested during the purchasing process is getting harder and harder to do. Retailers may create a new way to compete with an altogether new consumer experience that results in more sales and increased customer satisfaction with augmented Reality.

"Augmented vs. virtual Reality have one big thing in common. They both have the remarkable ability to alter our perception of the world. Where they differ, is the perception of our presence."

Table 3: Displays the contrasts between physical, augmented, and virtual reality while attempting to explain the differences

<ul style="list-style-type: none"> • User is immersed in a mix of the real world and a virtual world • Interact with both worlds and clearly distinguish between them • Uses a smartphone, tablet, or other mobile device 	<ul style="list-style-type: none"> • User is immersed in an entirely virtual world • Hard to differentiate between reality and virtual reality • Uses head-mounted display or glasses



between AR and VR.

Dynamics and interactivity are crucial needs for augmented reality application entertainment ability and the power relationship between the user's context and the content that is delivered (delivered content should be current, real-time, local, and generally context-sensible), as well as a user interface that is clear and functional avoiding cognitive fatigue, easy application navigation for efficient usage of the application. Most of the functionalities are added and extrapolated, plus additional ones that the analytic procedure uncovered to generate tours for those investigating areas of interest automatically. Communication enables direct channels to service providers, agents, and other users.

METHODOLOGY

In this research study, the researcher also visited various sites relevant to the current study and used different applications to study how the system works.

For the application, some prototypes were made to run tests to see the improvement of the project. Versioning control was done to minimize application redundancy and improve application development's fluency.

Research Questions

In the area of AR, several research topics might be posed. The following inquiries are the most suitable for analyzing this thesis' aim attainment.

Which AR technologies are appropriate to improve the user experience for tourists?

- Where do AR technologies have gaps? (Correlation of sound and image, interactivity, area of vision, resolution.)
- Security considerations (using the AR device while strolling around, theft prevention)
- Comfort features (fitting of the devices, weight, easy handling)

Which case study should be used to improve tourism through AR/?

- Which issues need to be addressed?
- Which product is it?
- The mission and vision statements.
- What marketing opportunities are there?

Which business model fits this case study the best?

- Who are the clients?
- Software Design and Development Component

Rationale

This research is without development or experiments. There are several reasons for this decision:

Scope: Before developing a service or product on the wrong technologies, ascertain the need and appropriate technology.

Time: Time constraints for conducting the research

Costs: The cost of the powerful PC/Laptop and mobile phone needed to power the devices is PHP 80,000.00.

System Architectural Design

Project Development

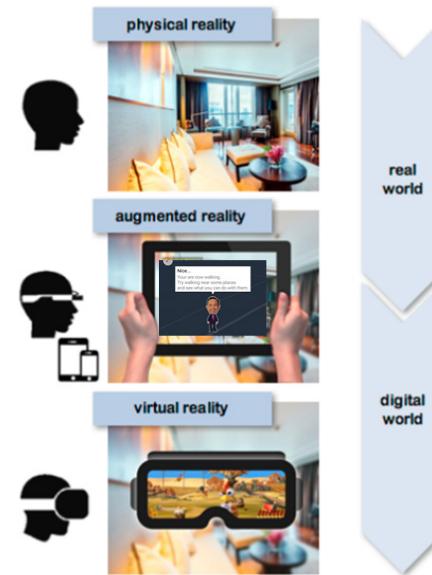


Figure 1: Depiction of physical/augmented/virtual Reality

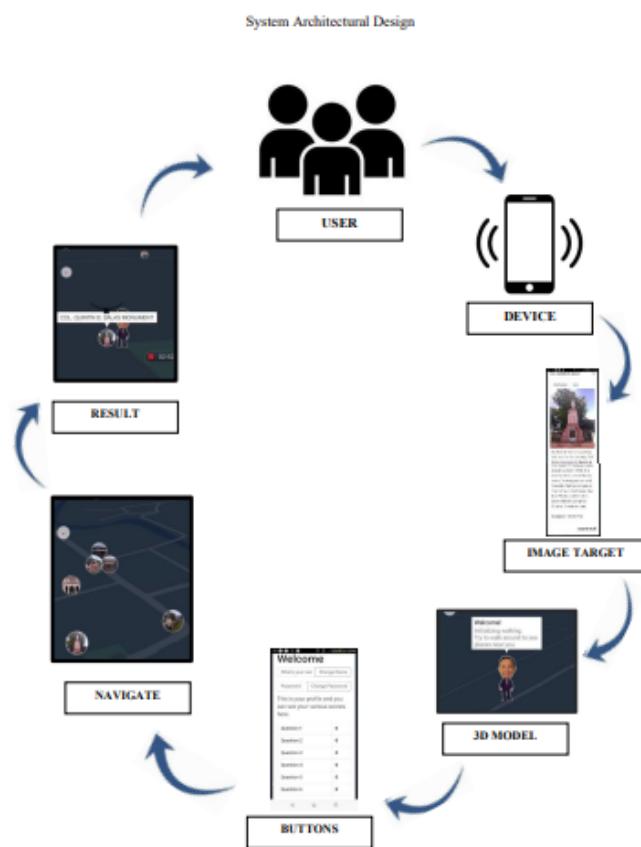


Figure 2: Software Design and Development Component

The significant improvements in software development environments that enable quick production and changing screens and other user interface elements made the RAD technique practicable. The end user can interact with the screens online, just as they would in a production setting. This allows minimal room for interpretation, and many mistakes are discovered using this method. The drawback of RAD is the end user's propensity to make the development effort include scope creep.

Adding a widget or two must be simple because the developer made the basic screen seem so simple to create. The end users and developers were caught in an endless cycle of improvements in most RAD lifecycle failures, with users and developers attempting to satisfy their requests for more and more features.

Due to this, the software development team combines limited prototyping with requirements and design development throughout a traditional waterfall lifecycle rather than using an entire RAD method. The prototypes lack an integrated interface and are narrowly focused on a portion of the application. The use of additional requirements or the addition of user interface options not readily supported by the development environment is actively discouraged because prototypes are used to validate requirements and design elements.

The following phases were observed in the production of the software: Analysis & Quick Design, Prototype Cycles, Testing, and Deployment.

Analysis & Quick Design. This phase analyzes the aspects that were considered before coming up with the concept of the study. Landmark Tourist Spots Using Augmented Reality is one of the most accessible applications. Still, the Unity 3D application made it easier with Cordova Android, especially for Augmented Reality.

Prototypes are much easier compared to other tasks. In this kind of application, the researchers make the design simple yet still aim to impress users as to what the application could do. Minimal buttons were placed to avoid making the screen messy or cluttered.

Troubleshooting was done every time after errors were fixed and maintenance was done. Assembling of the document followed and every progress was recorded for observation and recording.

Deployment of the application included a medium amount of coding since it focused more on its design. The application makes some of the codes function automatically. It runs mainly HTML and Javascript program files or hybrid applications.

The System

The technique converts the 3D model of Landmark tourist Spots in the municipality of Dumangas and popular tourist attractions into virtual augmented Reality. Its features include using a 3D model projected in augmented Reality to create a user-friendly educational application. Additionally, the system has markers that help it locate the image target. The 3D model is automatically displayed after selecting the input location. The system begins as soon as the user chooses to enter the mastery game or the virtual map. When one of the available alternatives is selected, the camera application instantly launches and scans the marker.

Technical Specifications

Software Specifications

The software is developed for the android operating system version starting from Android Nougat and up.

Hardware specifications The application requires an android device with 1 GB or higher Random Access Memory (RAM) 1.0 GHz or higher processor. The higher the RAM and Processor, the faster and better the result. A minimum of 300MHz stock or custom GPU (Graphic Processing Unit).

User specifications The application is accessible to android smartphone users, especially those who want to travel to and know more about Dumangas or who want to install the application.

Systems Implementation Implementing the system involved testing some application prototypes, starting with the Dumangas' Town Plaza. The researcher assessed and evaluated applications for any Landmarks or Tourist Spots for the improvement of the system in terms of rendering display of the Landmarks or Tourist Spots and developing accuracy of marker identification to avoid lags and post errors and to ideally provide the 3D model of the Landmarks and Tourist Spots in Dumangas using

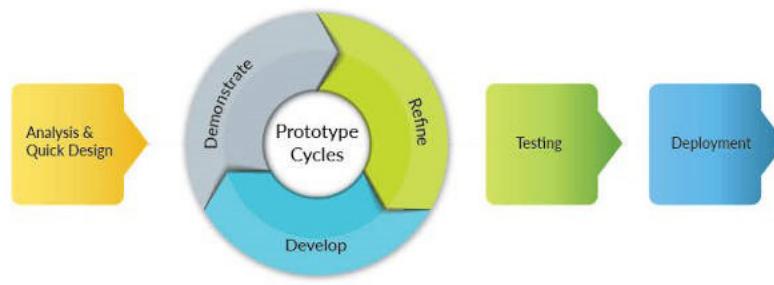


Figure 3: Rapid Application Development Methodologies

Augmented Reality.

Systems Inputs and Outputs The system application is an optical input application. It uses the camera to input identified markers and objects relating to the system input. Input markers are logos. The output is the 3D model of the LandmarkLandmark, rendered on display, which is a visual output that serves information to the user.

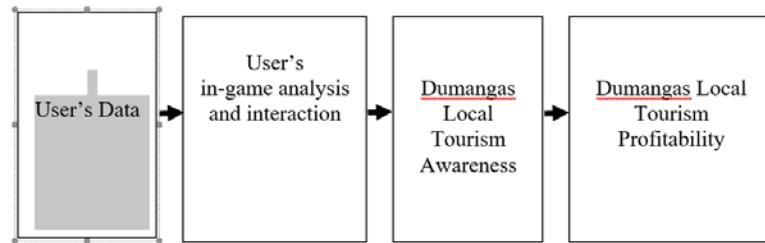


Figure 4: Diagram of Systems Input and Output

RESULTS

The Landmark tourist spots using Augmented Reality computer programs were made utilizing Solidarity and Vuforia after considering a few improvement approaches. Both marker-based and marker-less AR capabilities, as well as client interaction and data assets, were included. Despite the required ease of use and usefulness, this improvement fashion permitted the application to be made in a brief amount of time.

The study was planned to supply information for investigation by highlighting and to be assisted broken down by age gather to decide which include they favored. ISO/IEC 9126-1:25010 Software Quality Model Characteristics were utilized, with reactions perused as 1-2 rating being treated as unfavorable ; 3 being impassive, and 4-5 being treated as favorable. This can be supportive for comparing the comes about in table 2 - The moment address within the study inquired respondents on the off chance that they thought the application would include esteem to their visit. As can be seen from the ISO/IEC 9126-1:25010 Software Quality Model Characteristics, all the scores for all bunches are between 4 and 5, appearing that most individuals who accepted the app would include esteem to their visit. This may show the legitimacy of improvement.

Landmark tourist spots using Augmented Reality might apply anywhere in the country which is not a proper location. So distant, analysts have centered on mixing genuine and virtual pictures and design. In any case, AR may be amplified to incorporate sound.

Table 1 shows the result of the respondents' evaluation using the ISO/IEC 9126-1:25010 Software Quality Model Characteristics.

The Landmark and Tourist Spots Using Augmented Reality has an area mean of 4.330 for functionality, 4.337 for efficiency, 4.335 for compatibility, 4.615 for usability, 4.339 for reliability, 4.678 for security, 4.968 for maintainability, and 4.930 for portability, with the grand mean of 4.5665. This was because the system was suitable for providing appropriate functions to enhance the tourism of the Municipality of Dumangas. Furthermore, the system provides accuracy on the agreed results in functionality stability, performance efficiency, compatibility, usability, reliability, security, maintainability, and portability. As a whole, the result was very high, revealing that the system was stable, efficient, compatible, usable, reliable, secured, maintainable, and portable.

There are some functional factors that the panels suggested for improvement. First, adding the audio function to serve as background music for that the game so the game can also target auditory factors that will lessen instances of developing boredom during the game. They have also suggested that history will automatically appear as soon as the location or GPS of the phone detects the user being within the range of a specific marker. Lastly, they have given suggestions on the appropriate range and location of the markers to ensure the correctness of the user's location.

Table 1: Evaluation of the Respondents of the Landmark and Landmark Tourist Spots Using Augmented Reality

Areas	Mean	Verbal Interpretation
Functionality	4.330	Very High
Efficiency	4.337	Very High
Compatibility	4.335	Very High
Usability	4.615	Very High
Reliability	4.339	Very High
Security	4.678	Very High
Maintainability	4.968	Very High
Portability	4.930	Very High
GRAND MEAN	4.5665	Very High

Table 2 shows the result of the evaluation of IT Experts using McCall's Software Evaluation Criteria for the Software Quality Model.

The Application Landmark and Tourist Spots Using Augmented Reality has a mean of 4.3 for audibility, 4.8 for accuracy, 4.6 for completeness, 4.6 for communication Commonality, 4.7 for conciseness, 4.4 for consistency, 4.3 for observability, 4.4 for Operability, 4.4 for security, 4.8 for Self-documentation, 4.4 for simplicity, 4.6 for Software System Independence, 4.5 for traceability, 4.4 for training, 4.7 for controllability, 4.4 for Data commonality, 4.8 for decomposability, 4.3 for error tolerance, 4.6 for execution efficiency. 4.7

For expendability. 4.8 for generality, 4.7 for hardware independence, 4.5 for instrumentation, and 4.8 for modularity with the grand mean of 4.5625. The evaluation proved to have high results, which proved that the application had met its operational standards and is now prepared to be launched for its targeted users.

The IT experts also suggested several tips for the improvement of the game, such as regulating the difficulty of the application so that the users will not find the game too easy or too difficult for the users/ they have also suggested adding a reward system that would motivate the users to reach a higher level as the game progresses.

Services Data Management System with App Support

It shows that the Landmark and Tourist Spots' generality using Augmented Reality was rated Very High. It is because of the user-friendly design of the system, the ease of its performance, the provided information about the location's history, the accuracy of the location for the user, the game entertainment, and the fast run time of the game.

Generally, the IT experts concluded in their evaluation that the system was easy to use, could manage running on or outside field use, and provide interaction and information to the users.

ANALYSIS/RESULT AND DISCUSSION

This research study was developed to promote local tourism in the Municipality of Dumangas Iloilo. The development method of research was application development which functions to provide information to its users. This research project was made possible using rising technology, specifically augmented Reality. The application was first coded and then launched. Several application troubleshooting was done to fix inconsistencies, and a survey was given to respondents to measure the quality of the application. The application resulted in a grand mean of 4.5665 (Very high) based on the user's evaluation of the application, which proved that the application had given satisfactory results in all eight aspects: functionality, efficiency, compatibility, usability, reliability, security, maintainability, and portability.

This application has also been evaluated by several IT experts using McCall's Software Evaluation Criteria for Software Quality Model. It has resulted in a grand mean of 4.5625 (Very High), which proves that the application runs smoothly and passes the quality standards needed for this type of application, namely audibility, accuracy, completeness, communication

Table 2: Evaluation of IT Experts of the Extension and Community

Criteria	Mean	Verbal Interpretation
Audibility	4.3	Very High
Accuracy	4.8	Very High
Completeness	4.6	Very High
Communication Commonality	4.6	Very High
Conciseness	4.7	Very High
Consistency	4.4	Very High
Observability	4.3	Very High
Operability	4.4	Very High
Security	4.4	Very High
Self-Documentation	4.8	Very High
Simplicity	4.4	Very High
Software System Independence	4.6	Very High
Traceability	4.5	Very High
Training	4.4	Very High
Controllability	4.7	Very High
Data Commonality	4.4	Very High
Decomposability	4.8	Very High
Error Tolerance	4.3	Very High
Execution Efficiency	4.6	Very High
Expandability	4.7	Very High
Generality	4.8	Very High
Hardware Independence	4.7	Very High
Instrumentation	4.5	Very High
Modularity	4.8	Very High
GRAND MEAN	4.5625	Very High

commonality, conciseness, consistency, observability, operability, security, self-documentation, simplicity, software system independence, traceability, training, controllability, data commonality, decomposability, error tolerance, execution efficiency, expandability, generality, hardware independence, instrumentation, and Modularity. The results have shown that the application has shown consistency in its operational factors and has proved to offer user satisfaction to those who have been the pioneer batch of users for the application.

CONCLUSIONS AND RECOMMENDATIONS

The researcher has established an Augmented Reality application that can provide information about the landmarks in Dumangas, Iloilo. After an evaluation, it has been proven that the researcher has successfully developed a proficient Augmented Reality system that could be used by users who want to install the application. This system can contribute to assessing the user's knowledge about the local Municipality of Dumangas, Iloilo, and give them valuable information about the area.

Furthermore, one of the most important contributions of the system is in providing information to users with the power of their mobile devices in the most portable, convenient way, whereby they can interact with models and other contents of the application.

The program was referred to as an integrated travel application. Various concepts could be implemented into the service to produce even more value. Currently, 3D objects are offered. Augmented Reality could be integrated with a mobile phone, and scenarios like the largest Catholic Church in all of Panay, half of the Siete Pecados, or reconstruction of the former Rotunda and the Monument of Col. Quintin D. Salas of Dumangas were generated. A wise decision guide could also be beneficial for the clients. The intention is to work together with the clients so they can offer their ideas and feel involved.

So, this application allows users to view the spots or landmarks of the municipality of Dumangas or as they will be when upcoming modifications are made. Due to changes throughout time, visitors to historical sites such as the oldest and most prominent Catholic Church in Panay. It can be challenging for a modern visitor to envision how these sites know the history of the spots of Dumangas. A visitor with an outdoor AR system could view an artificially created version of Living History. Modern structures and monuments in the distance might be hidden by the HMD head-mounted display and shown directly on a place in the municipality of Dumangas, Iloilo.

But nowadays, AR frameworks are fundamentally found in scholarly and mechanical inquiries about research facilities.

There are some intriguing products on the market, but the technology still has to advance. One issue is that equipment is used in the market penetration process.

Since augmented Reality is still a relatively new concept, it is particularly crucial to develop applications to provide clients with genuine value and introduce it as soon as possible to get a jump start on outperforming rivals and gain market share. Since many individuals already own advanced smartphones, it makes sense to use cheaper means, such as cardboard or similar tools, to reach as many clients as possible.

The most crucial factor in developing applications or services is that they will deliver genuine value to the clients or risk losing their interest fast.

RECOMMENDATIONS

Based on the findings and conclusions derived from the study, the following recommendations were the following:

The developed program may enhance the profitability of the Local Tourism of Dumangas, Iloilo. It can boost the economic status of the municipality of Dumangas, Iloilo, which serves as a great entryway of opportunities for local businesses, job openings, investors, etc.

The program can also be used in schools to help enrich the student's knowledge about the history of the local tourist spots in Dumangas. It is appropriate for today's generation since most students need to become more familiar with the history of Dumangas.

Future researchers may improve the requirements in functional aspects such as the system's input and output and enhance the graphic user interface and detailed 3D models with a compressed size to prevent application lag and maximize user experience. Highlighting possible paths when selecting a target is also recommended to add information about the target. Rigid and smooth animation in camera and character movements will significantly improve the impact of the application on the user.

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**A STUDY ON THE THERAPEUTIC EFFECT OF 5-AZACYTIDINE
TO ATTENUATE THE RAMIFYING REPERCUSSIONS OF ISCHEMIA REPERFUSION INJURY
ON MITOCHONDRIAL MOLECULAR MACHINERY**

Vasisht Yegneshwaran^{1*}, Priyanka N Prem¹, Sri Rahavi Boovarahan¹, and Gino A Kurian^{1,2}

ABSTRACT

5-Azacytidine is a hypomethylating agent that has for long been used in cancer therapy due to its ability to inhibit the protein DNA methyltransferase responsible for hyper-methylating DNA strands. Recently, studies involving in vitro, ex vivo, and in vivo experiments have assessed the cardioprotective effects of 5-Azacytidine during myocardial ischemia-reperfusion injury (IRI). However, the effect of this compound in restoring the damage induced to mitochondrial molecular machinery during IRI has not yet been explored. Understanding this would help us analyze the ways through which mito-targeted therapeutics can be used. The purpose of this study is to investigate the therapeutic impact of 5-Azacytidine, as DNA methylation is a very common epigenetic modification observed during IRI. Furthermore, the protective effect of the compound in alleviating the damage induced to mitochondria during IRI can be identified, as DNA methylation can leave a direct impact on the mitochondrial genes as well. An isolated mitochondria model will be used to determine the effects of 5-Azacydine on mitochondrial molecular machinery as the capacity to generate DNA, RNA, and proteins are preserved in isolated mitochondria. In this study, we focus on the mechanisms of mitochondrial replication, and translation to understand the effect of 5-Azacytidine on the IRI affected mitochondrial system. Mitochondrial dysfunction is also another key turn of events that happens during IRI. The role of 5-Azacyidine in preserving the functionality is also being assessed in our research. The findings of these experiments would help us determine the plasticity the compound imparts on mitochondrial molecular mechanism's integrity and function post-induced IRI.

Keywords: Mitochondria, Ischemia-Reperfusion Injury, In-vitro Replication, Mitochondrial Dysfunction.

INTRODUCTION

Ischemia, which is caused by the blockage of blood vessels, is a condition that leads to infarction of tissue. The best possible treatment identified till date is reperfusion, which involves removing the obstruction to blood flow using drugs and partially or completely invasive surgery depending on the severity of the infarction. Reperfusion of tissues affected by ischemia is contraindicated in its ability to cause ischemia reperfusion injury, which is identified by certain unique pathophysiological hallmarks such as the generation of reactive oxygen species (ROS) due to the reestablishment of circulation, elevated inflammation caused by the excessive ROS, Calcium overload and mitochondrial dysfunction, which includes the abnormal opening of the MPTPs. (Frank et al., 2012; Hausenloy and Yellon, 2013; Sánchez-Hernández et al., 2020)

Researchers have been trying to understand the mechanisms and develop therapeutic measures against this condition. Recent studies implicated epigenetic modifications in regulating the expression of genes involved in pathways, contributing to IRI. A complete understanding of these epigenetic mechanisms is essential to identify an appropriate target to curb the damage caused by IRI, as a prophylactic or as a treatment option. Since they partially control gene expression patterns, these epigenetic mechanisms go beyond genetics. To better understand how ischemia reperfusion damage may be treated or prevented, the exact definition of epigenetics has been employed and controlled with the aid of inhibitors to alter gene expression patterns.

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Epigenetics is the mechanism that influences heritable changes in gene expression and function without altering the genome's sequence. These epigenetic pathways are influenced by external environmental elements as well. These epigenetic mechanisms control various mediators which results in Ischemia Reperfusion Injury. Some of mediators of reperfusion injury are oxygen free radicals (ROS), endothelial dysfunction and microvascular injury, alterations in calcium level and altered myocardial metabolism. Yet these concepts, key events and the complete order of how these mechanisms can be understood is still a work in progress.

Many studies believe that targeting abnormal DNA methylation in Ischemia Reperfusion injury is a key technique for the prevention and treatment of the disease. Methylation of DNA at cytosine phosphate-guanine (CpG) dinucleotides is a typical epigenetic alteration that serves as a link between the genotype and the environment. The methyl group (-CH₃) is added to the 5th position of cytosine residues in Cytosine-phospho-guanine (CpG) dinucleotides during DNA methylation, resulting in chromatin condensation and gene expression changes. Enzymes known as DNA methyltransferases (DNMTs) catalyse this process, which is reversed by enzymes known as Ten-Eleven-Translocation protein 1 (TET1), which converts 5-methyl cytosine (5mc) to 5-hydroxymethyl cytosine (5hmC). (Moore et al., 2012)

DNA methyltransferase inhibitors (DNMTi) have also been demonstrated to be useful in the treatment of ischemia-related illnesses. 5-Azacytidine, a DNMTi is a medication licensed by the US Food and Drug Administration (FDA) for the treatment of acute myeloid leukaemia (AML) and myelodysplastic syndrome (MDS), with an inhibitory impact on DNA methylation as the underlying mechanism. 5-Azacytidine has a long history of clinical use in cancer therapy. Moreover, several studies have been conducted to demonstrate the role of 5-azacytidine as a cardioprotective drug in the treatment of ischemia/reperfusion (I/R) damage. It is done in the animal model of rat. (Boovarahan & Kurian, 2021)

The discovery that 5-azacytidine was integrated into DNA and that it blocked DNA methylation when present in DNA led to its widespread use to show the relationship between the loss of methylation in particular gene areas and the activation of the related genes. (Christman, 2002) Since methylation works on DNA directly, it may work on nuclear and mitochondrial DNA. It also allows for the observation of both direct and indirect effects on epigenetic modulations on various components involved in IRI. An extensive, broad-spectrum target like methylation pattern may provide for the development of therapeutics for ischemic conditioning in the future. The main focus of this paper, will therefore be with respect to 5-Azacytidine and its therapeutic effects on ischemia reperfusion injury.

MATERIALS AND METHODS

Animals

The guidelines from the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India were strictly adhered to throughout all the animal experimental procedures involved in the study. All the rights and terms have been approved by the Institutional Animal Ethical Committee (IAEC) at SASTRA Deemed to be University, Thanjavur, India for the conduct of experiments. Male Wistar Rats of weight between 250-300 grams inbred in the Central Animal Facility at SASTRA Deemed to be University, Thanjavur, India was used in the study. The rats were all housed in a well-ventilated polycarbonate cage in a temperature-controlled room at (22 ± 2°C) with a relative humidity of (60 ± 5%). The animals were exposed to 12 hours of light and dark cycle with ad libitum supply of water and food.

Isolation of Mitochondria

The organelle mitochondria was isolated using density gradient differential centrifugation of the organ homogenate following the guidelines from Palmer, et al. A 10% organ homogenate was prepared momentarily before the process of density gradient differential centrifugation using Isolation Buffer (220 mM mannitol, 70 mM sucrose, 5 mM MOPS, 2 mM EDTA, and 0.2% BSA) with a pH of 7.4. The organ homogenate was then centrifuged at lower speed of 800g for 10 minutes at 4°C to pellet the nuclear fraction. The supernatant was then transferred into a new Eppendorf tube. The supernatant was then centrifuged at 8000g for 10 minutes at 4°C to remove any cellular debris. The supernatant was removed and the pellet was briefly resuspended in Isolation Buffer. This suspension was subjected to high centrifugation of 12,000g for 10 minutes at 4°C to pellet a pure fraction of mitochondria (Graham J. M. et al. 2002). The mitochondria was resuspended in Storage Buffer (100 mM KCl, 100 mM Tris-HCl, 75 mM Sorbitol, 25 mM Sucrose, 10 mM K₂HPO₄, 5 mM MgCl₂, 0.05 mM EDTA, 0.2% BSA) at a pH of 7.4 and their protein concentration was determined with the use of Bradfords reagents (Bio-Rad). A Bovine Serum Albumin standard was used to determine the concentration. After the process of protein estimation, the isolated mitochondria were randomly divided into groups and subjected to Normoxia and Hypoxia Reperfusion using Respiratory Buffer (300 mM Mannitol, 100 mM KCl,

20mM HEPES, 10mM KH₂PO₄, 5mM MgCl₂, 1mM EGTA, 0.2% BSA) at pH 7.1 and Hypoxia Buffer purged with N₂ (75mM NaCl, 25mM HEPES, 20mM Lactate, 16mM KCl, 10mM NaHCO₃, 5mM Deoxy-d-glucose, 1.2mM MgCl₂, 1.2mM CaCl₂, 1mM KH₂PO₄) at pH6.8.

Experimental Groups

Isolated mitochondria from rat hearts after normalisation were randomly divided into six groups and details of each experimental groups are as follows:

1. Normal - After the process of mitochondria isolation, the organelle was subjected to equilibration using Respiratory Buffer for 1 hour 15 minutes to maintain normal respiration.
2. Normal + 5-Azacytidine - After the process of mitochondria isolation, the organelle was subjected to equilibration using Respiratory Buffer for 15 minutes followed by 0.5 µM 5-Azacytidine pre-treatment for another 15 minutes. At the end of drug pre-treatment, the mitochondria were centrifuged at high speed of 12,000g for 10 minutes at 4°C to pellet a pure fraction of mitochondria. Then, the mitochondrial pellet was resuspended in Respiration Buffer for 45 minutes to maintain normal respiration.
3. Normal + DMSO - After the process of mitochondria isolation, the organelle was subjected to equilibration using Respiratory Buffer for 15 minutes followed by 0.5 µM DMSO pre-treatment for another 15 minutes. At the end of drug pre-treatment, the mitochondria were centrifuged at high speed of 12,000g for 10 minutes at 4°C to pellet a pure fraction of mitochondria. Then, the mitochondrial pellet was resuspended in Respiration Buffer for 45 minutes to maintain normal respiration.
4. IR - After the process of mitochondria isolation, the organelle was subjected to equilibration using Respiratory Buffer for 30 minutes followed by highspeed centrifugation at 12,000g for 10 minutes at 4°C to pellet a pure fraction of mitochondria. Then, the mitochondrial pellet was resuspended in Hypoxia Buffer for 15 minutes to hypoxia followed by a 30 minutes incubation in Respiration Buffer to facilitate reperfusion.
5. IR + 5-Azacytidine - After the process of mitochondria isolation, the organelle was subjected to equilibration using Respiratory Buffer for 15 minutes followed by 0.5 µM 5-Azacytidine pre-treatment for another 15 minutes. At the end of drug pre-treatment, the mitochondria were centrifuged at high speed of 12,000g for 10 minutes at 4°C to pellet a pure fraction of mitochondria. Then, the mitochondrial pellet was resuspended in Hypoxia Buffer for 15 minutes to hypoxia followed by a 30 minutes incubation in Respiration Buffer to facilitate reperfusion.
6. IR + DMSO - After the process of mitochondria isolation, the organelle was subjected to equilibration using Respiratory Buffer for 15 minutes followed by 0.5 µM DMSO pre-treatment for another 15 minutes. At the end of drug pre-treatment, the mitochondria were centrifuged at high speed of 12,000g for 10 minutes at 4°C to pellet a pure fraction of mitochondria. Then, the mitochondrial pellet was resuspended in Hypoxia Buffer for 15 minutes to hypoxia followed by a 30 minutes incubation in Respiration Buffer to facilitate reperfusion.

Evaluation of Mitochondrial Function using Mitochondrial Electron Transport Chain Complex Activity

After the estimation of protein and normalisation of mitochondria, the mitochondrial electron transport chain activity was measured spectrophotometrically by employing a specific donor-acceptor oxidoreductase in a 0.1M phosphate buffer (Frazier et al. 2012). For determination of mitochondrial Complex I activity a Rotenone sensitive NADH oxidoreductase was used. A Succinate decyl ubiquinone 2,6-dichlorophenolindophenol (DCPIP) reductase was used determine the mitochondrial Complex II activity. To determine the mitochondrial Complex III and IV activity cytochrome C reductase and cytochrome c oxidase was used as previously described by Ansari et al.

Oxidative Stress Assessment

The antioxidant profile of the isolated mitochondria homogenate was evaluated using Catalase activity, SOD activity and GSH:GSSG ratio. All the experiments used a multimode spectrophotometric plate reader to measure kinetic absorbance as well as endpoint absorbance.

A reaction buffer containing 0.1 M sodium phosphate buffer, pH 7.2, 4 mM H₂O₂, and 5 N H₂SO₄ was added to the isolated mitochondrial samples. The reaction was initiated by the addition of 0.005M KMnO₄ to the reaction buffer containing samples. The change in optical density was measured kinetically measured at 515 nm to assess the catalase activity (Goldblith et al. 1950).

A reaction buffer containing 45mM Tris, 1mM EDTA was added to the isolated mitochondria samples. The reaction was initiated by the addition of 2.5mM Pyrogallol to the reaction buffer containing samples. The change in optical density was measured kinetically at 420 nm to assess the superoxide dismutase activity (Nandi et al. 1988).

A reaction buffer containing 0.25m sodium phosphate and 5% Trichloroacetic acid was added to the isolated mitochondria samples. The reaction was initiated by the addition of Ellman's reagent (5,5'-dithiobis-2-nitro-benzoic acid) to the reaction buffer containing samples. The change in colour due to formation of thionitrobenzoate was measured at 412 nm to assess the GSH activity (Sedlak et al. 1968).

A reaction buffer containing 0.25m sodium phosphate buffer, 0.5mM EDTA, 4mM Oxidised glutathione and 0.2mM NADPH was added to the isolated mitochondria samples. The reaction was initiated by the addition of Ellman's reagent (5,5'-dithiobis-2-nitro-benzoic acid) to the reaction buffer containing samples. The change in optical density due to oxidation of NADPH was measured at 340 nm to assess the GSSG activity (Sedlak et al. 1968).

In vitro Mitochondrial Protein Synthesis

The experiment in vitro mitochondrial protein synthesis was carried out according to the procedures earlier by Fernandez-Silva et al. The isolated mitochondria were briefly suspended in MAITE Buffer (75mM Sorbitol, 25mM Sucrose, 10mM KCl, 10mM K₂HPO₄, 0.05mM Tris-HCl) at pH 7.4 containing 10mM Glutamate, 10mM Succinate, 2.5mM Malate, 1mM ADP and 1mg/ml of BSA. The process of translation was initiated by adding 100 µg/ml emetine, 100 µg/ml cycloheximide, and 10 µM of the 20 L-amino acids to the medium followed by an incubation for 25 minutes in gentle shaker. At the end of incubation process, mitochondria were pelleted by a high-speed centrifugation at 12000g for 10mins at 4°C. The pelleted mitochondria were then suspended in Lysis buffer (137mM NaCl, 20mM Tris-HCl, 50mM EDTA, 1% NP40) with protease inhibitors (2mM Na₂VO₄, 2mM NaF, 0.1 PMSF[Phenylmethylsulfonylfluoride]) followed by Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS PAGE) (Garrido et al., 2008).

A 12% Resolving gel was used for SDS PAGE. After the process of electrophoresis, the gels were stained using Coomassie Brilliant Blue Stain for a period of 4 hours. At the end of 4 hours, a de-staining solution was used to remove the excess stain for a period of 2 hours. The gels were then visualised using Quantity One Software (Bio-Rad, California, USA). The band intensity was quantified using ImageJ software.

In vitro Mitochondrial DNA Synthesis

The experiment in vitro mitochondrial DNA synthesis was carried out according to the procedures earlier by Fernandez-Silva et al. The isolated mitochondria were briefly suspended in MAITE Buffer (75mM Sorbitol, 25mM Sucrose, 10mM KCl, 10mM K₂HPO₄, 0.05mM Tris-HCl) at pH 7.4 containing 10mM Glutamate, 10mM Succinate, 2.5mM Malate, 1mM ADP and 1mg/ml of BSA. The process of translation was initiated by adding 50 µM of each dNTP to the medium followed by an incubation for 5 hours in gentle shaker. At the end of incubation process, mitochondria were pelleted by a high-speed centrifugation at 12000g for 10mins at 4°C. The pelleted mitochondria were then suspended in Lysis buffer (150mM NaCl, 20mM Tris-HCl, 20mM EDTA, 1%SDS) at pH-8.75 in the presence of 10 µM Proteinase K and 10 µM RNase A followed by Agarose gel electrophoresis (Garrido et al., 2008).

Mitochondrial DNA Isolation and DNA Quantification

Intact mitochondrial DNA was isolated by following the exact experimentation procedure mentioned by Martia, et al. Phenol, Cholorform and Isoamyl alcohol was used for biphasic separation followed by precipitation of DNA by 100% ethanol. The precipitated was washed thrice using 75% ethanol and the dried DNA pellet was dissolved in elution buffer AE (5mM Tris-HCl) at pH 8.5. The DNA sample were then quantified using a nanodrop spectrophotometer by Thermo Fisher Scientific (NanoDrop 2000 Spectrophotometer) (Enriquez et al., 1996).

Statistical Analysis

All the statistical analysis involved through the study was carried out using Prism version 8 (Graph Pad Software Inc., San Diego, CA, USA). The data analysis was carried out using Two-way analysis of variance (ANOVA), followed by Dunnet's post-test. The experimental results were expressed as mean ± SD, and a P < 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Results

5-Azacytidine preserves the mitochondrial function during IR in an isolated mitochondrial system

Mitochondrial dysfunction is one of the key events to occur during ischemia reperfusion injury. This has an irreversible impact over the mitochondria even after various therapeutic intervention. So, an ideal therapeutic should overcome permanent mitochondrial dysfunction.

Mitochondrial integrity with 5-Azacytidine pre-treatment was evaluated. From the evaluation we were able to identify

that 0.5 μM 5-Azacytidine was protective against IR. So, an optimal dose 0.5 μM was fixed as standard throughout all the experimentation procedures involving isolated mitochondria. We further evaluated the electron transport chain enzyme activity to assess the mitochondrial function. The IR groups pre-treated with 5-Azacytidine has improved levels of electron transport chain activity. 5-Azacytidine pre-treatment in IR preserved mitochondrial electron transport chain complex activity of complex I, II, III and IV by %, %, % & % in the mitochondria isolated from heart, when compared to mitochondrial groups subjected to IR. 5-Azacytidine pre-treatment in IR preserved mitochondrial electron transport chain complex activity of complex I, II, III and IV by %, %, % & % in the mitochondria isolated from kidney, when compared to mitochondrial groups subjected to IR.

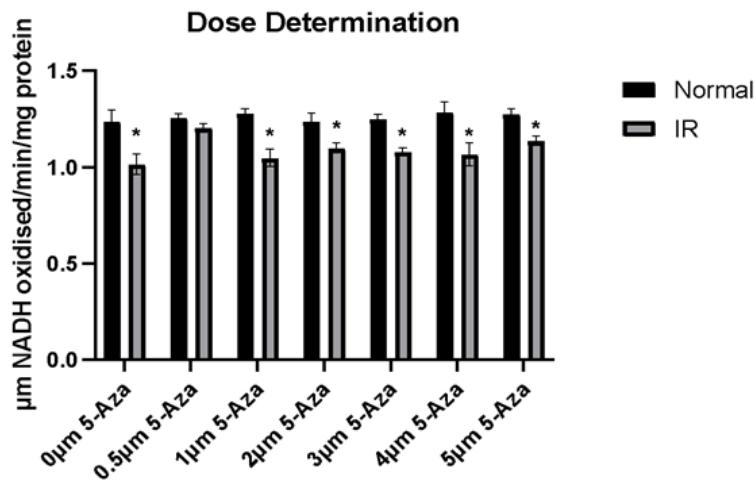


Figure 1: Dosage Determination. Optimal concentration of 5-Azacytidine drug was estimated to be 0.5 μm using NQR assay for an isolated mitochondrial system subjected to IR

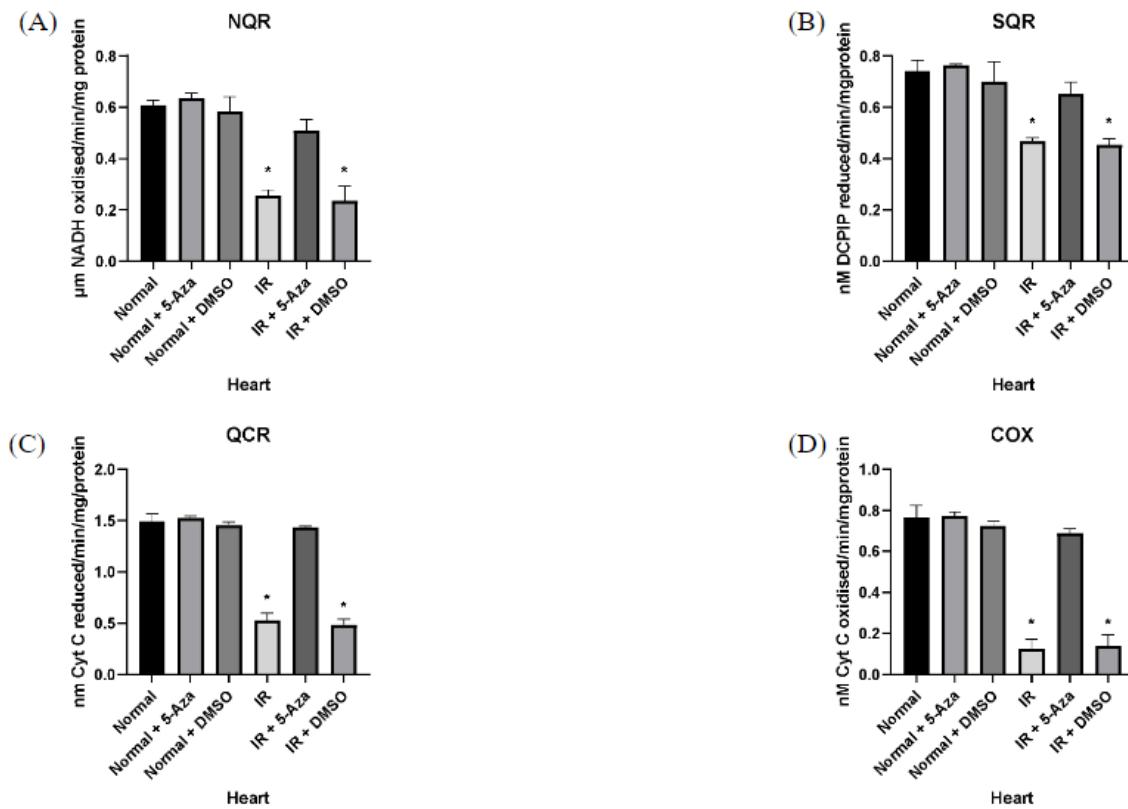


Figure 2: Effect of 5-Azacytidine on mitochondrial electron transport chain complex activity in isolated rat heart mitochondria.

(A) Complex I activity (NQR- NADH dehydrogenase) was measured as $\mu\text{mol NADH oxidized/min/mg protein}$, (B) Complex II activity (SQR- Succinate dehydrogenase) was measured in nmol DCPIP reduced/min/mg protein, (C) Complex III activity (QCR- Cytochrome bc1) was measured in nmol Cytochrome C reduced/min/mg protein, and (D) Complex IV activity (COX- Cytochrome c oxidase) was measured in nmol Cytochrome C oxidized/min/mg protein.(5-Aza,5-Azacytidine; DMSO, Dimethyl sulfoxide; IR, Ischemia-reperfusion.) Data were represented as mean \pm SD. (n=6 per group). *p < 0.05 versus IR.

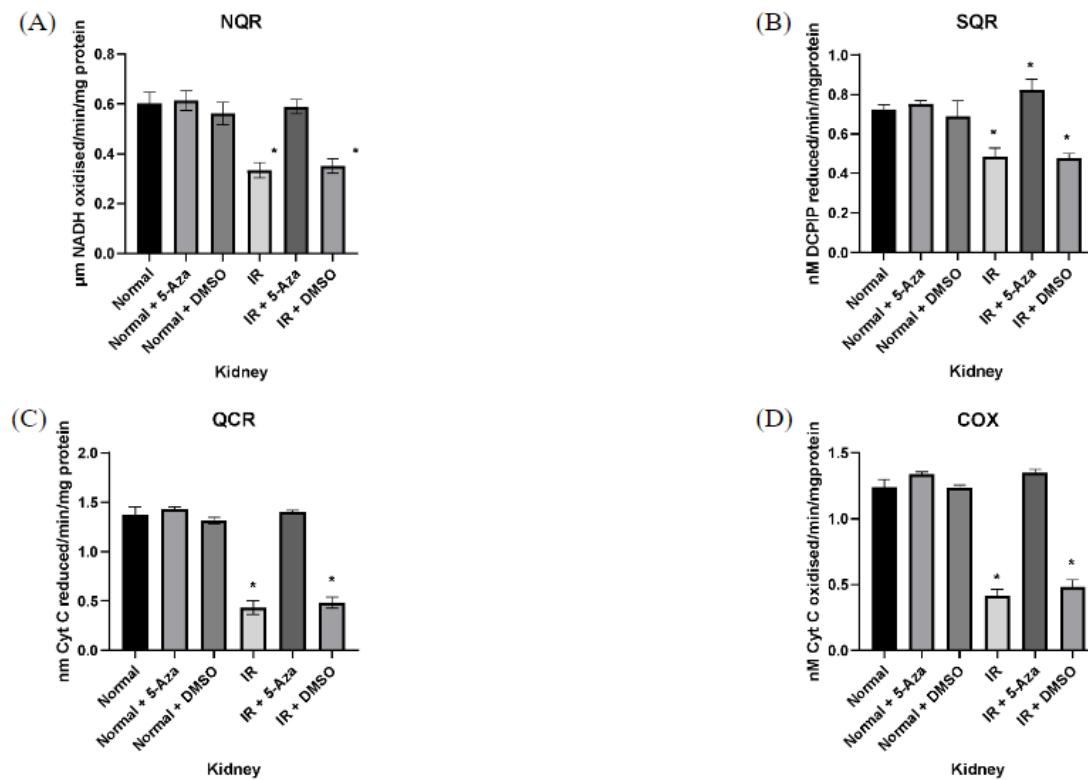


Figure 3: Effect of 5-Azacytidine on mitochondrial electron transport chain complex activity in isolated rat kidney mitochondria.

(A) Complex I activity (NQR- NADH dehydrogenase) was measured as $\mu\text{mol NADH oxidized/min/mg protein}$, (B) Complex II activity (SQR- Succinate dehydrogenase) was measured in nmol DCPIP reduced/min/mg protein, (C) Complex III activity (QCR- Cytochrome bc1) was measured in nmol Cytochrome C reduced/min/mg protein, and (D) Complex IV activity (COX- Cytochrome c oxidase) was measured in nmol Cytochrome C oxidized/min/mg protein.(5-Aza,5-Azacytidine; DMSO, Dimethyl sulfoxide; IR, Ischemia-reperfusion.) Data were represented as mean \pm SD. (n=6 per group). *p < 0.05 versus IR.

Effect of 5-Azacytidine in alleviating damage induced to mitochondria due to oxidative stress during IRI

A robust release of free radicals is one of the most critical events involved in IR injury pathology. This is also one of the key contributors to mitochondrial dysfunction. The therapeutic effect of 5-Azacytidine in overcoming and altering the damages due oxidative stress induced by IR is vital.

Mitochondrial oxidative stress parameters were assessed with 5-Azacytidine pre-treatment. From the evaluation, the IR groups pre-treated with 5-Azacytidine has improved levels of catalase and superoxide dismutase activity by % and % in the mitochondria when compared to mitochondrial groups subjected to IR, isolated from heart. 5-Azacytidine showed improved levels of catalase and superoxide dismutase activity by % and % in the mitochondria when compared to mitochondrial groups subjected to IR, isolated from kidney.

Further experimental evaluation revealed that the IR groups pre-treated with 5-Azacytidine has improved levels of GSH:GSSG ratio by % in the mitochondria when compared to mitochondrial groups subjected to IR, isolated from heart. 5-Azacytidine showed improved levels of GSH:GSSG ratio by % in the mitochondria when compared to mitochondrial groups subjected to IR, isolated from kidney.

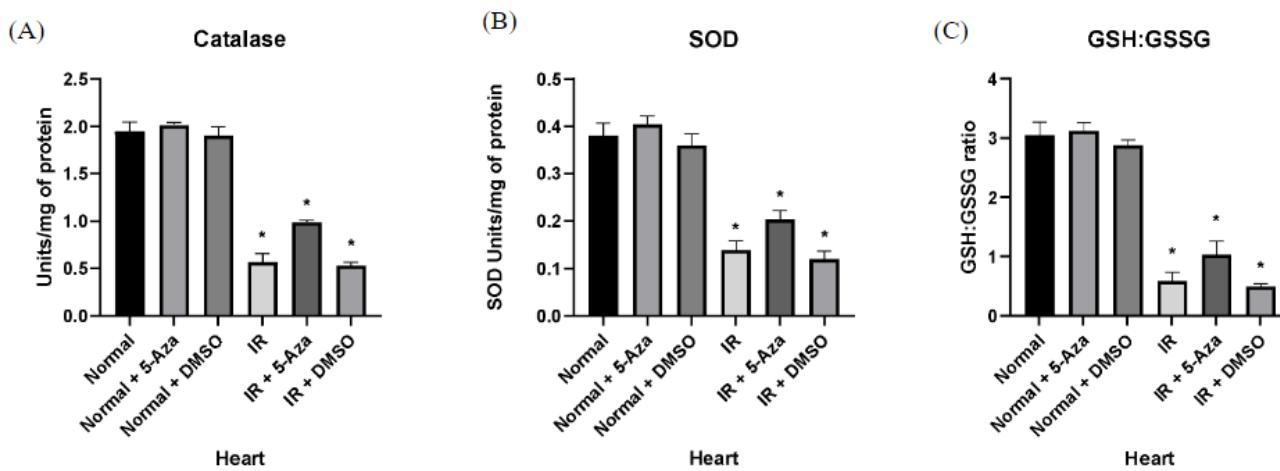


Figure 4: Assessment of oxidative stress damage in cardiac mitochondria subjected to 5-Azacytidine pre-treatment.

(A) Catalase enzyme activity, (B) SuperOxide Dismutase (SOD) enzyme activity, and (C) GSH:GSSG activity; all measured in isolated mitochondrial lysates of rat kidneys. Values are represented as mean \pm SD of six individual animals per group. * $p < 0.05$ versus IR. Abbreviation: 5-Aza, 5-Azacytidine; DMSO, Dimethyl sulfoxide; IR, Ischemia-reperfusion.

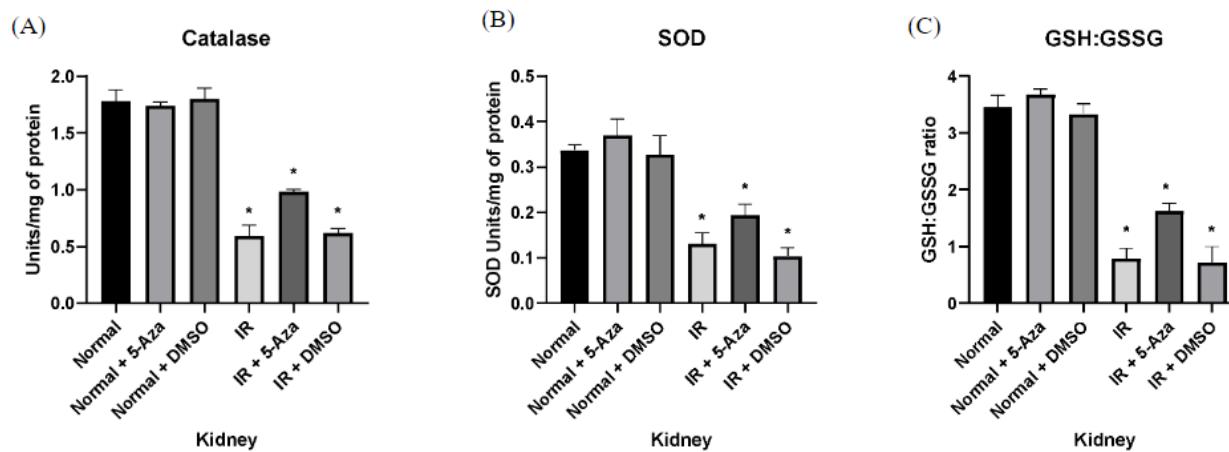


Figure 5: Assessment of oxidative stress damage in renal mitochondria subjected to 5-Azacytidine pre-treatment.

(A) Catalase enzyme activity, (B) SuperOxide Dismutase (SOD) enzyme activity, and (C) GSH:GSSG activity; all measured in isolated mitochondrial lysates of rat kidneys. Values are represented as mean \pm SD of six individual animals per group. * $p < 0.05$ versus IR. Abbreviation: 5-Aza, 5-Azacytidine; DMSO, Dimethyl sulfoxide; IR, Ischemia-reperfusion.

5-Azacytidine pre-treatment overcomes impaired protein translational ability in an isolated mitochondrial system

In vitro protein translation ability of mitochondria in an isolated mitochondria system is assessed and visualised in the experiment. Epigenetic modification during IRI is one of the most reasons for translational inaccuracy. Hampered protein synthesis is one of the main reasons behind irreversible recovery from IRI. The ameliorative effect of 5-Azacytidine in overcoming translational inaccuracy when subjected is explored in our study. The significant improvement in IR groups treated with 5-Azacytidine compared to IR groups can be clearly visualised through the electrograms of the SDS PAGE gels.

Mitochondrial translational ability was assessed after 5-Azacytidine pre-treatment. Before subjecting the mitochondria from heart to in vitro protein translation, the mitochondrial protein was normalised to mg/ml across all groups. After the process of in vitro translation, the IR groups pre-treated with 5-Azacytidine showed an increase in mitochondrial protein by % when compared to mitochondrial groups subjected to IR in heart.

Before subjecting the mitochondria from kidney to in vitro protein translation, the mitochondrial protein was normalised to mg/ml across all groups. After the process of in vitro translation, the IR groups pre-treated with 5-Azacytidine showed an increase

in mitochondrial protein by % when compared to mitochondrial groups subjected to IR in kidney.

Table 1: Quantitative mitochondrial protein estimation by Bradfords method in heart tissue before in vitro protein translation

S.No	Group Name	Protein	Unit
1.	Normal	0.67333333	mg/ml
2.	Normal + 5-Aza	0.67333333	mg/ml
3.	Normal + DMSO	0.67333333	mg/ml
4.	IR	0.67333333	mg/ml
5.	IR + 5-Aza	0.67333333	mg/ml
6.	IR+DMSO	0.67333333	mg/ml

Table 2: Quantitative mitochondrial protein estimation by Bradfords method in heart tissue after in vitro protein translation

S.No	Group Name	Protein	Unit
1.	Normal	0.909	mg/ml
2.	Normal + 5-Aza	0.97	mg/ml
3.	Normal + DMSO	0.612	mg/ml
4.	IR	0.42	mg/ml
5.	IR + 5-Aza	0.873	mg/ml
6.	IR+DMSO	0.359	mg/ml

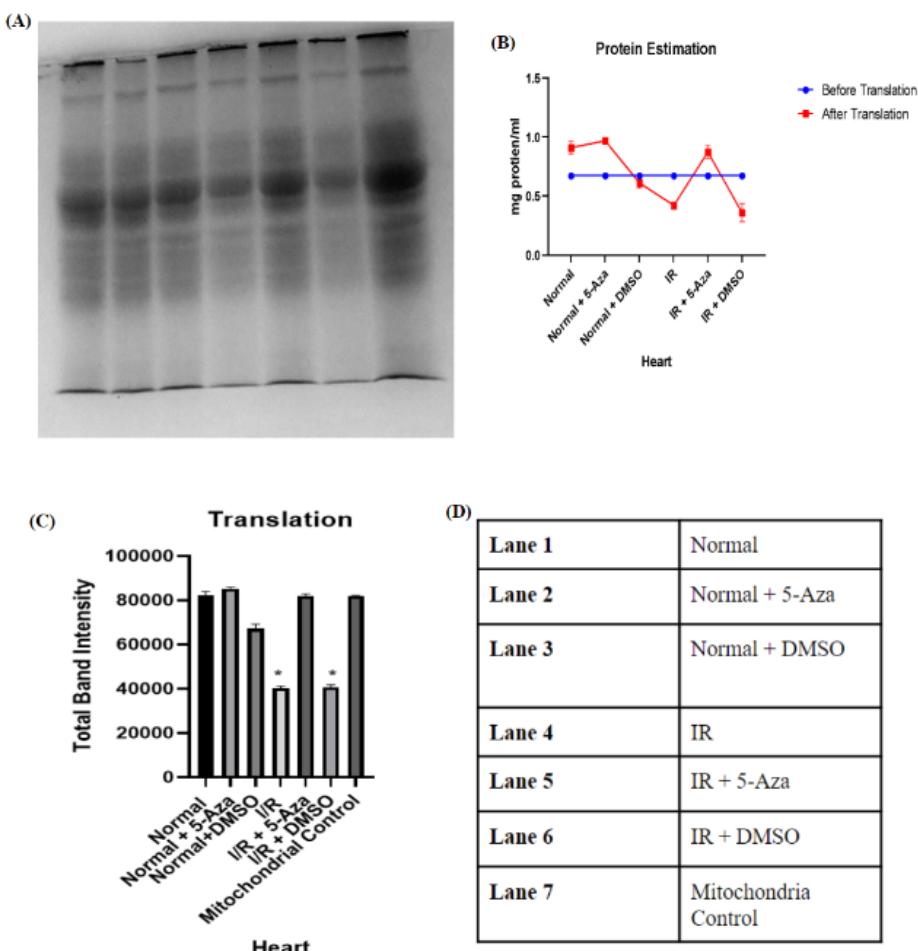


Figure 6: In-vitro cardiac mitochondrial translated proteins analysis using SDS-PAGE gel electrophoresis.

(A) SDS PAGE gel electrogram, (B) Protein estimation, (C) Total Band Intensity, and (D) Representations for gel electrogram lanes. Values are represented as mean \pm SD of six individual animals per group. * $p < 0.05$ versus IR. (5-Aza, 5-Azacytidine; DMSO, Dimethyl sulfoxide; IR, Ischemia-reperfusion.)

Table 3: Quantitative mitochondrial protein estimation by Bradfords method in kidney tissue before in vitro protein translation

S.No	Group Name	Protein	Unit
1.	Normal	0.94833333	mg/ml
2.	Normal + 5-Aza	0.94833333	mg/ml
3.	Normal + DMSO	0.94833333	mg/ml
4.	IR	0.94833333	mg/ml
5.	IR + 5-Aza	0.94833333	mg/ml
6.	IR+DMSO	0.94833333	mg/ml

Table 4: Quantitative mitochondrial protein estimation by Bradfords method in kidney tissue after in vitro protein translation

S.No	Group Name	Protein	Unit
1.	Normal	1.1505	mg/ml
2.	Normal + 5-Aza	1.2545	mg/ml
3.	Normal + DMSO	0.9008	mg/ml
4.	IR	0.69	mg/ml
5.	IR + 5-Aza	1.1896	mg/ml
6.	IR+DMSO	0.67087	mg/ml

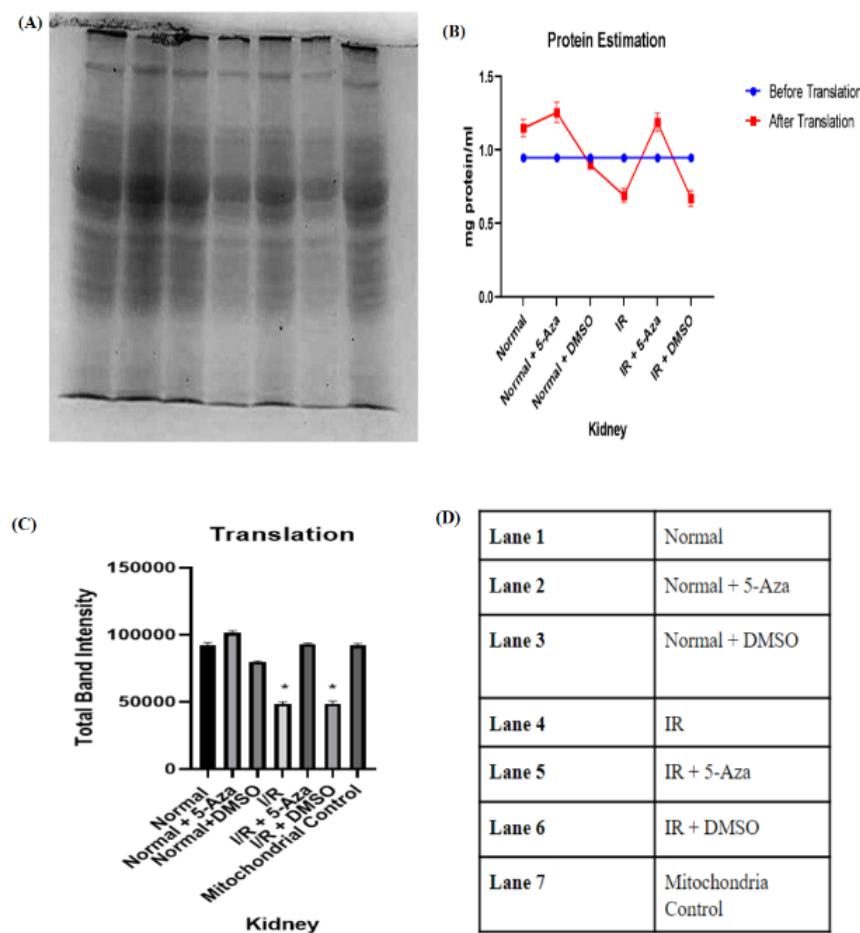


Figure 7: In-vitro renal mitochondrial translated proteins analysis using SDS-PAGE gel electrophoresis.

(A) SDS PAGE gel electrogram, (B) Protein estimation, (C) Total Band Intensity, and (D) Representations for gel electrogram lanes. Values are represented as mean \pm SD of six individual animals per group. * $p < 0.05$ versus IR. (5-Aza,5-Azacytidine; DMSO, Dimethyl sulfoxide; IR, Ischemia-reperfusion.)

5-Azacytidine enhances DNA replication machinery in an isolated mitochondrial system

In vitro DNA replication ability of mitochondria in an isolated mitochondria system is assessed and visualised in the experiment. DNA hypermethylation during IRI is one of the most reasons for transcriptional impairment and translational inaccuracy. Upregulation of DNMT1 during IR is one of the major reasons behind extensive and far-fetched damage. The therapeutic effect of 5-Azacytidine in overcoming impaired DNA replication machinery when subjected is explored in our study. The significant enhancement in mitochondrial DNA copy number in IR groups treated with 5-Azacytidine compared to IR groups can be clearly visualised through the electrograms of the Agarose gels.

Mitochondrial DNA replication ability was assessed after 5-Azacytidine pre-treatment. Before subjecting the mitochondria from heart to in vitro DNA replication, the mitochondrial protein was normalised to mg/ml across all groups. After the process of in vitro DNA replication, the IR groups pre-treated with 5-Azacytidine showed an increase in mitochondrial DNA by % when compared to mitochondrial groups subjected to IR in heart.

Before subjecting the mitochondria from kidney to in vitro DNA replication, the mitochondrial protein was normalised to mg/ml across all groups. After the process of in vitro DNA replication, the IR groups pre-treated with 5-Azacytidine showed an increase in mitochondrial DNA by % when compared to mitochondrial groups subjected to IR in kidney.

Table 5: Quantitative mitochondrial protein estimation by Bradfords method in heart tissue before in vitro DNA replication

S.No	Group Name	Protein	Unit
1.	Normal	0.763	mg/ml
2.	Normal + 5-Aza	0.763	mg/ml
3.	Normal + DMSO	0.763	mg/ml
4.	IR	0.763	mg/ml
5.	IR + 5-Aza	0.763	mg/ml
6.	IR+DMSO	0.763	mg/ml

Table 6: Mitochondrial DNA quantification in Heart using NanoDrop Spectrophotometer after in vitro DNA replication

S.No	Group Name	Nucleic acid	Unit	260/280	260/230	Sample Type
1.	Normal	578.25	ng/ μ l	1.81	2.12	DNA
2.	Normal + 5-Aza	583.14	ng/ μ l	1.82	2.14	DNA
3.	Normal + DMSO	462.69	ng/ μ l	1.9	2.11	DNA
4.	IR	258.18	ng/ μ l	1.9	2.12	DNA
5.	IR + 5-Aza	565.82	ng/ μ l	1.89	2.17	DNA
6.	IR+DMSO	264.28	ng/ μ l	1.83	2.0	DNA

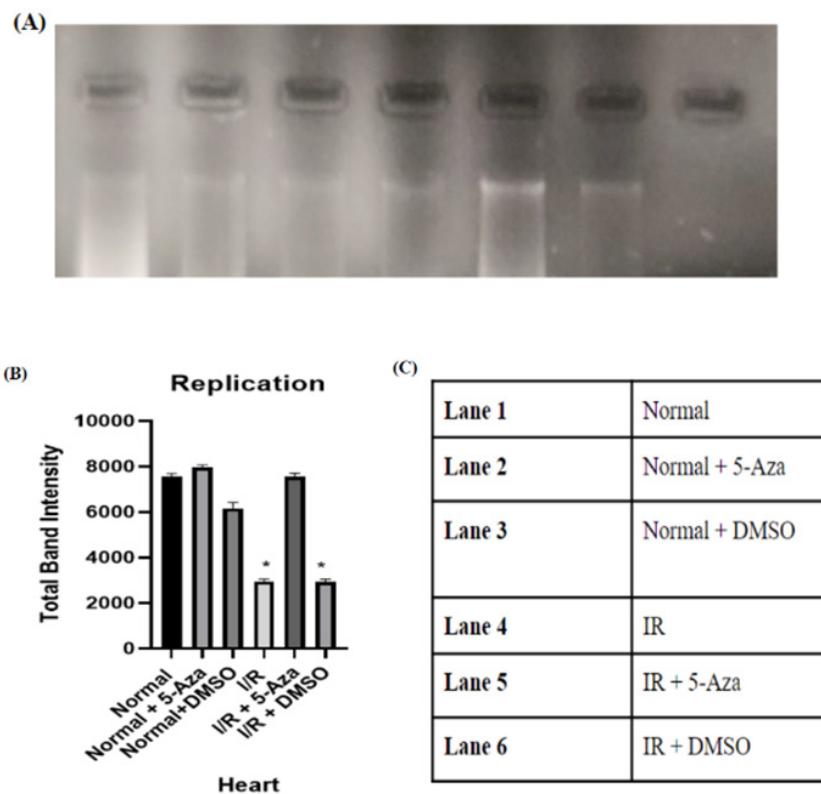


Figure 8: Agarose gel electrophoresis analysis of In-vitro cardiac mitochondrial DNA replication.

(A) Agarose gel electrogram, (B) Total Band Intensity, and (C) Representations for gel electrogram lanes. Data were represented as mean \pm SD. ($n=6$ per group). * $p < 0.05$ versus IR. Abbreviation: 5-Aza, 5-Azacytidine; DMSO, Dimethyl sulfoxide; IR, Ischemia-reperfusion.

Table 7: Quantitative mitochondrial protein estimation by Bradfords method in kidney tissue before in vitro DNA replication

S.No	Group Name	Protein	Unit
1.	Normal	0.984	mg/ml
2.	Normal + 5-Aza	0.984	mg/ml
3.	Normal + DMSO	0.984	mg/ml
4.	IR	0.984	mg/ml
5.	IR + 5-Aza	0.984	mg/ml
6.	IR+DMSO	0.984	mg/ml

Table 8: Mitochondrial DNA quantification in Kidney using NanoDrop Spectrophotometer after in vitro DNA replication

S.No	Group Name	Nucleic acid	Unit	260/280	260/230	Sample Type
1.	Normal	666.9	ng/ μ l	1.89	2.0	DNA
2.	Normal + 5-Aza	679.2	ng/ μ l	1.82	2.1	DNA
3.	Normal + DMSO	589.82	ng/ μ l	1.91	2.1	DNA
4.	IR	326.81	ng/ μ l	1.8	2.12	DNA
5.	IR + 5-Aza	665.7	ng/ μ l	1.83	2.17	DNA
6.	IR+DMSO	387.3	ng/ μ l	1.9	2.0	DNA

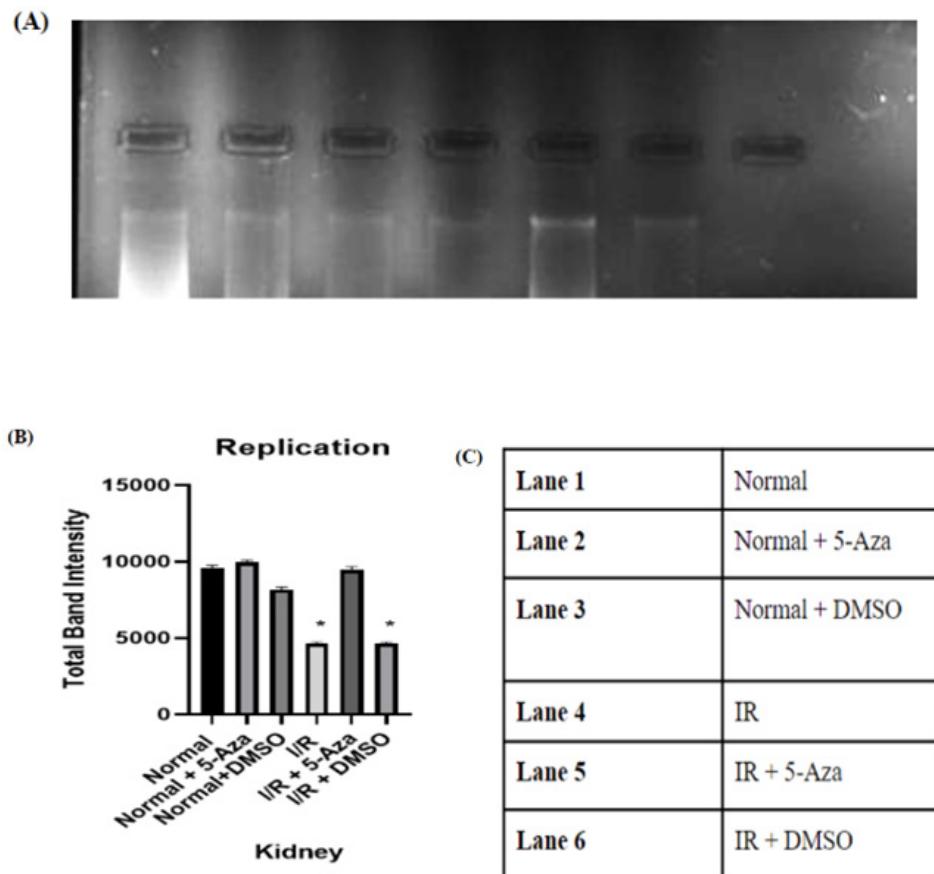


Figure 9: Agarose gel electrophoresis analysis of In-vitro renal mitochondrial DNA replication

(A) Agarose gel electrogram, (B) Total Band Intensity, and (C) Representations for gel electrophoresis lanes. Data were represented as mean \pm SD. (n=6 per group). * $p < 0.05$ versus IR. Abbreviation:5-Aza,5-Azacytidine; DMSO, Dimethyl sulfoxide; IR, Ischemia-reperfusion.

DISCUSSION

Ischemia reperfusion injury is one of the most common and unavoidable forms of injury to occur during a revascularisation procedure or because of therapeutic interventions to an atherosclerotic plaque or an ischemic stroke (Piper et al., 1998). The injury might seem inevitable because of huge number of variable complications arising after interventional therapy. Till date, a lot of different therapeutics have been tested, repurposed and trailed for the treatment of IRI but a lot of them fails in one or many concern. In previous study by Rahavi, et al., has reported the ameliorative effect of 5-Azacytidine against IRI preliminarily due to its inherent ability to combat the DNMT1 by inhibiting this action (A.A.Mangoni et al. 2018). The role of 5-Azacytidine in combating cancer is also well explored. But, the role of 5-Azacytidine in alleviating mitochondrial damage due to IRI is not explored. As mitochondria is one of the key players in regulation and resuscitating cell survival during IRI, it is of immense importance to have a deep understanding of the mechanism through which 5-Azacytidine helps alleviating IRI. The mechanistic action on drug on mitochondria is not well documented and is unexplored. So, having a better understanding of the way through which mitochondria mediates IR protection would help enable a complete overlay (D.Jain et al. 2017). Even though there are multiple drugs to treat IRI, such multifaceted view on the action of drug is absent thereby most drugs fail to transition even into clinical trials. Therefore, phrenological manipulative ability without enough substantial evidence is not fruitful yield (I.Andreadou et al. 2020).

Many drugs like methotrexate, hydroxychloroquine which are used for the treatment of various diseases like cancer, malaria have been repurposed to treat cardiovascular diseases as well (A.Daiver et al. 2021). 5-Azacytidine mediated epigenetic reprogramming is used extensively in cancer research. The mechanism of action and epigenetic modulation mediated by

5-Azacytidine is clearly established and well documented (E.Hervout et al. 2013). Also, many studies have reported the cardioprotective, nephroprotective and vasculo-protective nature of the compound. But the effect of this compound on cardiac IRI and renal IRI are not well established (S.Sou et al, 2016). Therefore, a proper evidence-based study would help us repurpose the drug effectively to manage many cardiovascular and renal complications in a clinical scenario as a potent pharmacological agent (L.Badimon et al. 2019). From our study, 5-Azacytidine has been shown to provide an ameliorative effect against IRI in an Isolated Mitochondrial system thereby, providing a promising role as a pharmacological intervention.

The organs heart and kidney have been shown to exhibit an elevated levels of mitochondrial dysfunction because of the free radical stack and extensive calcium overload (H.Ma et al. 2011). This immediately results in the change in functionality of the mitochondria leading to loss of mitochondrial bioenergetics (K.Yang et al. 2017). Based on these evidences, we identified a significantly decreased mitochondrial electron transport chain activity due to deterioration in function of mitochondrial complexes I, II, III, IV. There was also decreased tendency to scavenge the free radical resulting increased oxidative stress. Our studies have shown that pre-treatment with 5-Azacytidine resulted in improved mitochondrial electron transport chain activity. The results have also indicated that the scavenging effect of 5-Azacytidine but it was not that considerable when compared to its other therapeutic effect. However, the compound enhanced the survival key for mitochondria when subjected to IRI by considerably enhancing the mitochondrial functionality and improved resistance to oxidative stress induced mitochondrial damage.

Translational inaccuracy and reduced mitochondrial copy number are another key deteriorative feature of IRI. So, a pharmacological agent that has the ability to modify the epigenetics of a cellular system is vital (P.E.Nikolaou et al. 2019). Often times we fail to realise that mitochondria is a system of its own and it has a trivial role to play in cellular epigenetics as well (B.A.Hemmings et al. 2015). When subjected to IRI, the levels of mitochondrial protein translation were significantly reduced and it also resulted in decreased mitochondrial copy number. However, once the groups were pre-treated with 5-Azacytidine and when subjected to IRI they showed improved resistance to translational inaccuracy by enhanced protein synthesis. The epigenetic modification that 5-Azacytidine imparts on the DNA replication machinery also resulted improved mitochondrial copy number even when to IRI. Based on the results obtained from these independent experiments throughout the long run we could demonstrate the therapeutic effect of 5-Azacytidine to Attenuate the Ramifying Repercussions of Ischemia-Reperfusion Injury on Mitochondrial Molecular Machinery.

CONCLUSION

Ischemia reperfusion injury mediated mitochondrial damage is inevitable and without proper pharmacological intervention it can be irreversible. This study shows the therapeutic effect of 5-Azacytidine in attenuating IRI mediated mitochondrial dysfunction. 5-Azacytidine pre-treated groups showed improved mitochondrial translational accuracy and increased mitochondrial copy number. These evidences indicated the rooted ability of 5-Azacytidine to act on mitochondria to ameliorate IRI induced mitochondrial damage and thereby contributing towards cellular homeostasis.

FUTURE PROSPECTS

From our findings pre-treatment with 5- Azacytidine has enhanced the ability of protein translation, increased mitochondrial copy number and have restored the damage caused to mitochondrial function when subjected to ischemia reperfusion injury in an isolated mitochondrial system. However, extensive animal studies are required to further the dosage concentration that is required for pre-clinical and clinical applications. Also, the mitochondrial gene expression alterations associated with ischemia reperfusion need to be studied. Study can be extended to analyse the efficacy of 5-Azacytidine drug in distant organ ischemia reperfusion injury.

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SOLID WASTE DISPOSAL SCENARIO OF THREE LADIES' HALLS OF THE CHITTAGONG UNIVERSITY CAMPUS IN CHITTAGONG, BANGLADESH

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ABSTRACT

Solid wastes disposed from three ladies' halls (Shamsun Nahar, Pritilata and Desnetri Begum Khaleda Zia) of Chittagong University campus, Chittagong, Bangladesh, were identified and classified from June 2012 to March 2013. Data were collected from kitchen, dining, canteen, bathroom, and premises of these three halls. Overall, 18,505.0 kg wastes were disposed from different sources and among them 10,406.0 kg (i.e., 56.24%) were from kitchen, 1,218.5 kg (i.e., 6.59 %) from dining and canteen, 1,570.5 kg (i.e., 8.49 %) from bathroom and 5,310.0 kg (i.e., 28.68 %) from others sources. On an average each student of these 3 ladies halls disposed 0.029 kg waste per day that gave a total of 8.520 kg wastes during the 10 months of study period. Waste eating animals from the dumping sides were recorded.

Keywords: Chittagong University, Dispose, Ladies Halls, Solid Waste, Wastes' Feeding Animals.

INTRODUCTION

Waste is a material, which is thrown away or aside as worthless (Cointreau, 1982). Solid waste can be defined as: the useless and unwanted products in the solid state derived from the activities of and discarded by society. It is produced either by - product of production processes or arise from the domestic or commercial sector when objects or materials are discarded after use. (<http://www.smartranger.net/index.cfm?&mnuid=3>).

Solid waste includes highly heterogeneous mass of discarded material from residential, commercial, industrial, agricultural mining activities (Alam et al., 2002). The University of Chittagong (CU) is the third largest and one of the multidisciplinary universities in Bangladesh with 24,283 students, 862 faculty members and 1904 supportive staff. It was established on 18 November 1966 at Fatehpur under Hathazari upazila (sub-district), 22 km north of Chittagong city and 3 km southwest of the upazila headquarter (22°16'48"N and 91°28'24.6"E). Total area of the CU campus is about 709 ha and it is well known for its beautiful green scenario. However, due to lack of a proper waste management plan, wastes are found to be scattered in the open environment and this filth the aesthetic beauty of the campus. So, it has become an essential task to manage the solid wastes of the CU campus in a scientific way. For the protection of environmental quality and recreation potential of the CU campus there must be a specific strategy for the disposal of solid wastes in the CU campus. (Ahsan and Chowdhury, 2008).

MATERIALS AND METHODS

The study was carried out at 3 residential ladies halls of CU Campus from June 2012 to March 2013. The total study area was divided into 3 sites (S-1 to S-3). Site 1: Shamsun Nahar (SN) hall covers an area of 1.417 ha. It is a five storied building and 741 students live in this hall. SN hall has 12 units of bathroom, 11 kitchens, 1 dining and 1 canteen. Site 2: Pritilata (PL) hall comprises an area of 1.417 ha. It is a four storied building and 731 students

live there. PL hall has 16 units of bathroom, 16 kitchens, 1 dining and 1 canteen. Site 3: Deshnetri Begum Khaleda Zia (KZ) hall includes an area of 1.417 ha. It is a four storied building and 700 students live there. KZ hall has 20 units of bathroom, 20 kitchens, 1 dining and 1 canteen.

been placed in every spot such as kitchen, dining and canteen, bathroom, toilet and corridor. Kitchen, dining and canteen, and others wastes were measured weekly but bathroom wastes were measured in the last week of each month because these were cleaned once or twice in a month. All wastes were measured at every morning from 8.00 to 11.00. In KZ hall the surrounding wastes (including garden) were measured with the others wastes but not for the other two halls. It should be mentioned here that the garden wastes of SN and PL are disposed outside the hall premises, but in KZ hall these were dumped inside the hall

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premises. Wastes were put in a tray and verified with naked eyes to separate them into different categories by using one pair of forceps. Major and minor wastes dumping spots (major- huge amount of wastes dumping place and minor-small amount of wastes dumping place) were located in and around the studied halls.

Wastes were disposed by the students of these 3 ladies halls in the buckets, which had SN had 1 major (big) and 14 minor (small) dumping spots, PL had 1 major and 2 minor, and KZ had 3 major and 2 minor spots. Waste feeding animals in the major dumping spots were observed with naked eyes.

RESULTS AND DISCUSSION

The sources, types and physical nature of the disposed wastes (Table.1) from 3 ladies halls were basically similar and mostly contained general wastes, and there were little hazardous wastes also. Overall, 18,505.0 kg wastes were disposed from different sources of 3 ladies halls, of which kitchen wastes were maximum (10,406 kg i.e., 56.26%), and dining and canteen wastes were minimum (1,218 kg i.e., 6.59%) (Fig.2).

Based on sources, the wastes were mainly four categories (Table 1): (1) kitchen waste, (2) dining and canteen wastes, (3) bathroom waste, and (4) others (veranda, corridor, and in and around the halls' premises) wastes.

Kitchen waste: Among the 3 halls, Shamsun Nahar (SN) hall has 11 small kitchen units, Pritilata (PL) hall has 16 units, and Khaleda Zia (KZ) hall has 20 units in different blocks for the students to provide kitchen/cooking facilities. Kitchen

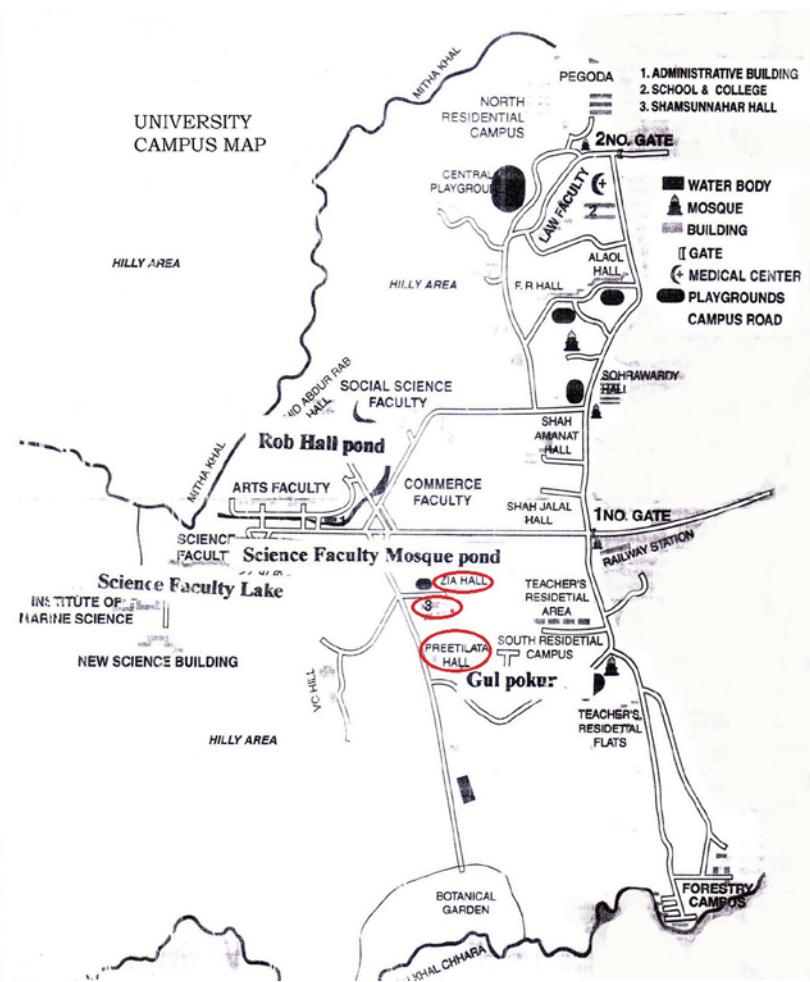


Figure 1: Map of the University of Chittagong showing the study area (edited by illustrator).

Months	Kitchen waste	Dining and Canteen waste	Bathroom waste	Others waste	Total Waste
June 2012	1007.5	101.5	166.5	449.0	1724.5
July 2012	1252.0	128.5	182.5	472.0	2035.0
August 2012	565.0	66.0	104.0	295.0	1030.0
September 2012	1085.5	128.5	151.5	527.5	1893.0
October 2012	1193.0	142.5	177.5	586.0	2099.0
November 2012	1059.0	127.5	167.5	603.0	1957.0
December 2012	1321.0	154.5	162.5	727.5	2365.5
January 2013	1048.0	133.0	158.0	597.0	1936.0
February 2013	1052.0	137.0	173.0	632.0	1994.0
March 2013	823.0	99.5	127.5	421.0	1471.0
Total	10,406.0	1,218.5	1,570.5	5,310.0	18,505.0

wastes include inedible parts of raw and cooked food items, carrying packets from markets and useless and/or broken containers. The sweepers clean these wastes every morning from 8.00 to 11.00 hours.

Table 1: Different types of waste (kg) disposed from 3 ladies halls of CU campus from June 2012 to March 2013

From the 3 ladies halls of CU campus, 10,406 kg kitchen wastes were disposed during the study period and each hall on an average disposed 221.41 kg of wastes. Among these 3

halls, SN hall produced 23.86%, PL hall 39.18%, and KZ hall produced 36.96% of total disposed wastes. PL hall disposed maximum kitchen wastes (4,076.5 kg), on the other hand SN hall disposed minimum (2,483 kg). Usually, the highest amount of wastes was disposed in the first week and the lowest in the last week of each month. The highest amount of wastes (354.5 kg) was disposed in the first week of July (Fig.3) because after one month summer vacation most of the students aggregated in the halls. On the other hand, the lowest amount of wastes (92 kg) was disposed in September (Fig.3) as the University classes were closed from 16 September to 1 November.

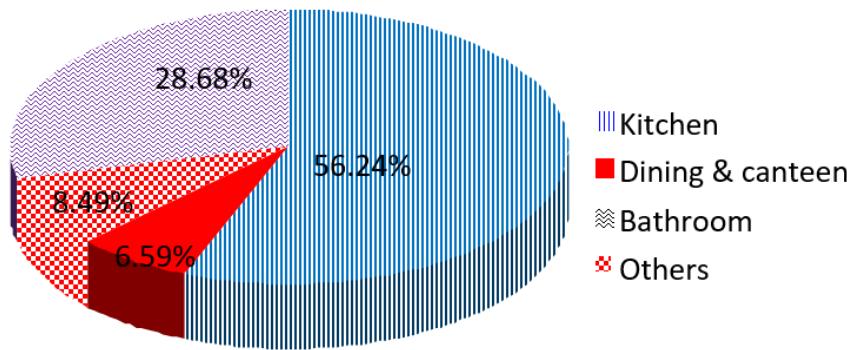


Figure 2: Wastes disposed from different sources of 3 ladies halls in CU campus from June 2012 to March 2013.

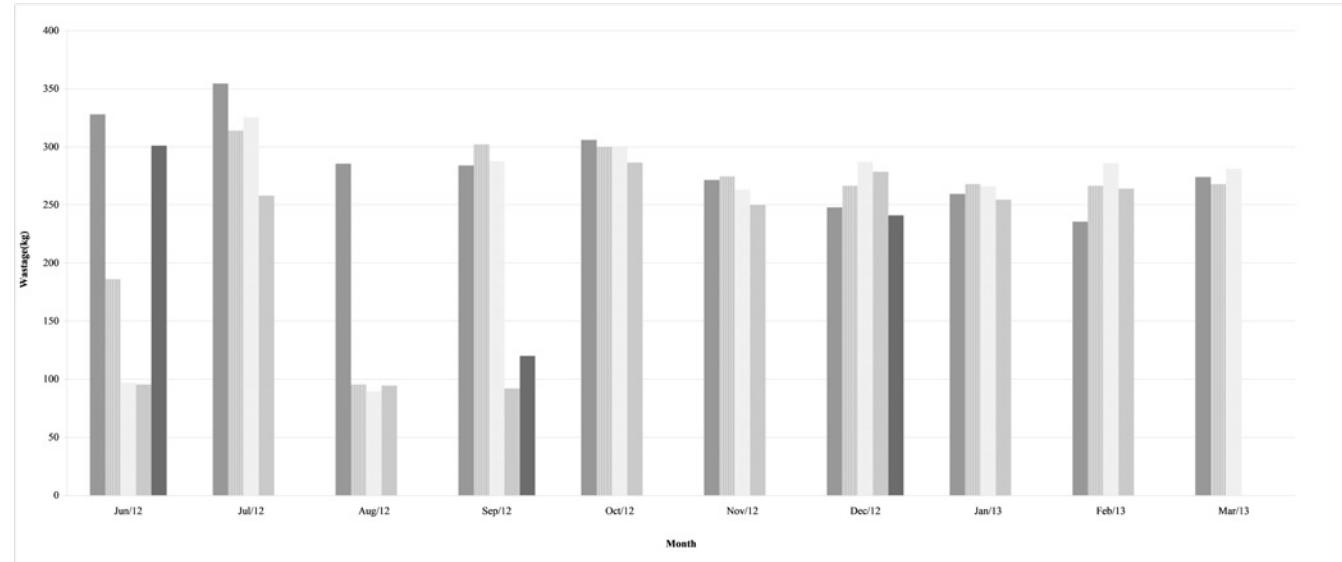


Figure 3: Weekly disposed kitchen wastes (kg) from 3 ladies halls of CU campus.

Dining and canteen wastes: Overall 1218.5 kg Dining and canteen wastes were disposed from 3 ladies halls, and each hall produced 406.167 kg wastes (i.e., 1.38 kg per day), SN hall's dining and canteen disposed 404 kg (i.e., 33.15%) wastes, PL hall 411 kg (i.e., 33.73%) and KZ hall 403.5 kg (i.e., 33.11%) wastes. There was a little variation in the amount of wastes disposed in different months, it was minimum (66 kg) in August during the vacation of CU campus. On contrary in the month of December most of the students gathered in these halls to attend in the admission test of first year honors, as a result the amount of disposed wastes were maximum (154.5 kg) in this month.

Bathroom waste: Bathroom wastes were mainly sanitary napkins, packets and containers of toiletries and cosmetics. In total 1,570.5 kg of bathroom wastes were disposed from 3 ladies halls during the study period. Among the halls, KZ hall turned out maximum amount of wastes 781 kg (i.e. 49.73%) and minimum SN hall 326.5 kg (i.e. 20.79%). When we looked at the monthly

calculation it was found that on an average 523.5 kg of bathroom wastes were disposed from each hall. During the study period the maximum bathroom wastes were disposed in July (182.5 kg) because after summer vacation (02-30 June 2012) most of the students were present in the halls due to their yearly final examination, and the minimum disposal was in August (104 kg) when a smaller number of students stayed in halls because of Ramadan and Eid-ul-Fitr vacation (08–30 August 2012).

During the study period, on an average each student of SN hall disposed 0.441 kg wastes i.e., 0.002 kg wastes per day. Similarly, on an average each student of PL hall disposed 0.634 kg of wastes during the study period i.e., 0.003 kg waste per day; and each student of KZ hall disposed 1.116 kg of wastes i.e., 0.004 kg waste per day. Moreover, each student of 3 halls produced an average of 0.724 kg bathroom wastes during the study period i.e., 0.003 kg per day. When we compared whether the amount of bathroom wastes disposed by each student of 3 halls differed significantly or not, ANOVA showed an overall statistically significant difference among 3 halls except between SN and PL halls ($F = 48$ df = 2/18, $p < 0.01$). On the other hand, there was no significant difference among the amount of wastes disposed by the student of 3 halls in each month ($F = 1.555$, df = 9/18, $p > 0.05$).

Source	SS	Df	MS=SS/df	F=MS/error MS	Table F	p -Value	Comment
Month	0.003	9	0.0003	1.55	2.46(5%)	$p > 0.05$	insignificant
Hall	0.02	2	0.01	48	3.55 (5%)	$p < 0.01$	significant
Error	0.004	18	0.0002				

ANOVA Table (Bathroom wastes): -

DF = degrees of freedom in the source

SS = sum of squares due to the source

MS = mean sum of squares due to the source.

F= the F-statistic.

Table F= table value

Other wastes (from veranda, corridor, and in and around the halls' campus): The wastes, which could not be placed in any one of the above categories, are called other wastes. These included unusable footwear and used pen and pencil. Overall, 5,310 kg of other wastes were disposed during the study period. Among them SN hall produced 201.5 kg wastes (i.e., 3.79%), PL hall 468.5 kg (i.e., 8.82%) and KZ hall 4,640 kg (i.e., 87.38%). It was lowest (285 kg) in amount in the month of August (2012). And in December, it was the highest (727.5 kg). Overall, each student of 3 halls produced an average of 2.445 kg of other wastes during the study period i.e., 0.008 kg per day. Each student of SN hall disposed 0.272 kg of other wastes i.e., 0.641 kg per day, PL hall set out 6.629 kg of other wastes i.e., 0.001 kg per day and each student of KZ hall disposed 0.003 kg of other wastes i.e., 0.023 kg per day in that period. There was a significant difference between the amount of others wastes disposed by each student of SN and PL halls ($F = 62.472$ df = 1/9, $p < 0.01$) and among the months ($F = 3.544$, df = 9/9, $p < 0.05$).

Anova Table (other wastes):

Source	SS	Df	MS=SS/df	F=MS/error MS	Table F	p -Value	Comment
Month	0.003	9	0.0003	3.54	3.18 (5%)	$P < 0.05$	significant
Hall	0.006	1	0.0068	62.47	10.56 (5%)	$P < 0.01$	significant
Error	0.0009	9	0.0001				

Types of the waste based on physical nature:

On the basis of physical nature wastes could be classified into the following categories:

(1) Pills of Vegetables: Among all of wastes, on an average 55.26 % pill of vegetables were disposed from 3 halls.

(2) Plastic: The students dispose bottle of soybean oil, coconut oil, biscuit packets, shopping bags, polythene, sandals and shoes. Among the all wastes of these 3 halls, 10.51% plastics were disposed.

(3) Paper: There are many types of paper needed in the daily life such as newspapers, magazines, cardboard, writing papers,

and colored papers. Among the 3 halls, PL hall disposed the highest amount (13.76%) of papers and SN hall the lowest (9.62%).

(4) Clothes: The students disposed their old and useless clothes of cotton, linen and silk and all together it was on an average 3.44%, which are not easily degradable.

(5) Metal: Aluminum (mostly discarded cooking pans) and steel cans (mostly soft-drink cans) are the two common metallic waste items that are found in the FS halls. These two waste materials (3.99%) are recyclable.

(6) Glass: Different kinds of broken glasses (mostly from broken mug, plate, flower vase and other utensils) were found in waste dumping sites. On an average 10.51% glasses were disposed from these halls.

(7) Others: Old useless bags and shoes made up of leathers, rubbers, and bones of animals (fish, chicken and cattle) were found in the waste dumping sites. On an average 4.16% other wastes were found. Mainly after the vacation of Eid-ul-Azha some students throw bones of cattle and offal that emit bad smell.

Table 2: Category of the wastes, disposed from 3 ladies halls of CU campus.

Categories of the wastes	Description of the wastes
Kitchen waste	Pills of vegetable, eggshell, boiled rice, food-waste; packets of biscuit, coffee; polythene; lunch box; broken glass, mug, bucket, bottle; newspapers; and other papers.
Dining and canteen waste	Pills of vegetable, eggshell, boiled rice; and food waste.
Bathroom waste	Sanitary napkin; tissue; hair; packet/bottle of shampoo, packet of soap; and empty containers of face wash and others cosmetics.
Other wastes	Sandals, shoes, clothes, dolls rubbers, used one-time pens, and pencil.

Waste feeding animals

Seven species of birds and two species of mammals were seen to eat foods from waste dumping sites and two other mammals were reported to do so (Table 3), of which crows and mynas were common. Sometimes 1 or 2 pariah dogs and domestic cats were also seen. Security guards of CU informed that sometimes wild boars (*Sus scrofa*) and jackals (*Canis aureus*) come to the waste dumping site of PL hall at night to eat foods from dumping wastes. Sometimes rock doves (*Columba livia*) were seen at SN hall besides dining hall but they never ate anything from the dumping sites.

Table 3: Mammals and birds seen in the waste dump areas of 3 ladies halls of CU campus.

No.	Common name	Scientific name	Observed (O)/Reported (R)
1	Pariah dog	<i>Canis familiaris</i>	O
2	Domestic/Feral cat	<i>Felis catus</i>	O
3	Jackal	<i>Canis aureus</i>	R
4	Wild pig	<i>Sus scrofa</i>	R
5	Jungle crow	<i>Corvus macrorhynchos</i>	O
6	House crow	<i>Corvus splendens</i>	O
7	House sparrow	<i>Passer domesticus</i>	O
8	Blue rock-dove	<i>Columba livia</i>	O
9	Jungle myna	<i>Acridotheres fuscus</i>	O
10	Common myna	<i>Acridotheres tristis</i>	O
11	Pied myna	<i>Sturnus contra</i>	O

CONCLUSION

The scenario of 3 female student halls of Chittagong University is beautiful, but the disposed wastes making them to some extent dirty and they lose their attraction day by day. The students of SN hall throw wastes from upstairs on cemented floor adjacent to their verandas within the hall premises that making the area dirty, slippery due to the accumulation of water and growing mosses, algae and fungi. Most of the students of PL hall complain about solid waste disposal problem and they blame the university authority because the wastes are dumped at about 10 meters from the southern boundary of the Hall.

That dumping site emits obnoxious smell and provokes vomiting for passerby. The waste burnt smokes also make unpleasant problem for the residential halls. If these solid wastes are not disposed in a safe distance, then these residential halls will face problem of environmental pollution. In fact, wastes are ruining the panoramic beauty of the Chittagong University Campus.

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LABORATORY DIAGNOSIS OF NOVEL HUMAN CORONAVIRUS (SARS-COV-2) INFECTIONS-A REVIEW

Aswathy C Ashok^{1*}, and R. Harish¹

ABSTRACT

COVID-19 is a pandemic, highly contagious infectious disease caused by the Severe Acute Respiratory Syndrome Corona virus-2 (SARS-CoV-2). The World Health Organization has declared the ongoing outbreak a global public health emergency. This disease has spread rapidly and affected millions of people worldwide. Currently, there are no specific clinical signs or symptoms of SARS that can be used to differentiate it from other causes of community- or hospital-acquired viral pneumonia. Accurate diagnosis of cases holds the key to managing any pandemic through identification, isolation, and treatment of patients while defining the epidemiology of the pathogen. Because an increasing number of asymptomatic symptomatic individuals must be tested for COVID-19, a safe and efficient screening system is required. The diagnosis of suspected cases is presently confirmed by nucleic acid assays with real-time PCR using respiratory samples. On the other side, serological tests are comparatively easier to perform, but their utility may be limited by their ease of performance and the fact that antibodies appear later in the disease course. This review is aimed at summarizing the currently available information on different methods used for screening and diagnosing COVID-19 infections.

Keywords: SARS-CoV2, RTPCR, immunological rapid assay

INTRODUCTION

Severe acute respiratory syndrome coronavirus (SARS-CoV-2) is a novel virus that caused the first major pandemic disease in the family Coronaviridae. Many coronavirus infections in the past 20 years were not regarded as highly pathogenic to human beings until the outbreaks of SARS (Severe Acute Respiratory Syndrome) and MERS (Middle East Respiratory Syndrome) (Zhong et al., 2003; Drosten et al., 2003; Fouchier et al., 2003). At the end of 2019, the China Office of the WHO (World Health Organization) reported a cluster of pneumonia cases in Wuhan City, China, and the causative pathogen was identified as Novel Coronavirus (nCoV 2019); the WHO named this disease COVID-19 (Wu et al., 2020; Zhou et al., 2020; Wang et al., 2020). COVID-19 is an extremely infectious disease. Coronaviruses are enveloped RNA viruses belonging to the Coronaviridae family and the order Nidovirales, which contain approximately 27–32 kilobytes of positive-sense single-stranded RNA. This subfamily consists of four genera: Alpha coronavirus, Beta coronavirus, Gamma coronavirus, and Delta coronavirus, on the basis of their phylogenetic relationships and genomic structures. These subfamilies are broadly distributed and cause infections in humans and other mammals. Alpha and beta coronaviruses infect only mammals. Gamma and delta coronaviruses infect birds, but some of them can also infect mammals. Although the source of the beta coronavirus 2019 SARS Cov-2 is unknown, initial cases have been linked to the South Hunan seafood market. The infected people may have severe symptoms in the respiratory and digestive organs. Like other coronaviruses, the SARS-CoV-2 has at least six open reading frames (ORFs) and many other accessory genes. There are two open reading frames (ORFs) at the 5' terminal two-thirds of the genome, ORFs1 and ORF2. These ORF encode two polyproteins, namely pp1a and pp1ab, which are further cleaved into 11 and 16 proteins, respectively. Nucleocapsid (N), membrane protein (M), envelope protein (E), and spike (S) are among the structural proteins found at 3' terminals. In the case of COVID-19, the spike protein appears to be the primary protein interacting with host cells. Hence, the spike protein is likely the protein to which antibodies are raised, but this is not clear at this time. These viruses also contain some accessory proteins, which aid in virus replication. The S gene aids SARS-CoV-2 in host specificity and receptor binding, and some virion may also contain hemagglutinin esterase (HE) protein. (Cui, J., et al., 2019; Huang C et

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al., 2020 and Miller et al., 2016) Clinical manifestations of COVID-19 infection include fever and cough as primary clinical manifestations, as well as shortness of breath and myalgia. Some patients have serious complications such as acute respiratory distress syndrome (ARDS) and cytokine storm, which may lead to death. (Malik et al., 2020). It has the capacity for human-to-human transmission. The lack of awareness in hospital infection control and international air travel facilitated the rapid global dissemination of this agent.

The collection of appropriate specimens is very crucial for the detection of most of the infected cases of COVID-19. Nasopharyngeal swabs are typically collected, but we may miss the detection in some cases; therefore, lower respiratory tract specimens such as sputum and bronchoalveolar lavage (BAL) may be an alternative choice. Therefore, there is an urgent need to have an accurate, rapid, readily available, and reliable diagnostic test for SARS-CoV-2 infection. Various immunological, nucleic acid, and amplification diagnostic tests have been developed and are widely available to date. Various integrated point-of-care molecular devices are currently under development, and some are available to provide accurate and fast diagnostic services for SARS-CoV-2 infections. (Loeffelholz et al., 2020).

In view of the present crisis of the COVID-19 pandemic, fast and reliable testing strategies are imperative. In this review, we attempt to learn more about the current diagnostic methods for SARS and Cov-2 infections. The different methods used for screening and sample collection will also be discussed.

Screening and Specimen collection of patients with COVID-19 pneumonia.

Accurate diagnoses of cases hold the key to managing any pandemic through identification, isolation, and treatment of patients while defining the epidemiology of the pathogen. Because an increasing number of asymptomatic symptomatic individuals must be tested for COVID-19, a safe and efficient screening system is required. Presently, no specific clinical signs or symptoms of COVID-19 can be used to differentiate it from other causes of community- or hospital-acquired pneumonia. In order to decide if a patient should be tested, WHO published case definitions for surveillance but encouraged countries to adapt these depending on their local epidemiological situation and other factors. (WHO 2020). A suspect case is defined as (i) a patient with acute respiratory illness (fever and at least one sign or symptom of respiratory disease, such as cough or shortness of breath) and a history of travel to or residence in a location reporting community transmission of COVID-19 disease within 14 days of the onset of symptoms (ii) a patient with severe acute respiratory illness (fever and at least one sign or symptom of respiratory disease, e.g., cough, shortness of breath, and requiring hospitalization) and in the absence of an alternative diagnosis that fully explains the clinical presentation.

As per the WHO guidelines, a "probable case" is a suspect case for whom testing for the SARS-CoV-2 is inconclusive or for whom testing could not be performed for any reason. WHO further defines a "contact" as a person who experienced any one of the following exposures during the 2 days before and the 14 days after the onset of symptoms of a probable or confirmed case: (i) face-to-face contact with a probable or confirmed case within 1 meter and for more than 15 min. (ii) direct physical contact with a probable or confirmed case. (iii) direct care for a patient with probable or confirmed COVID-19 disease without using proper personal protective equipment. For confirmed asymptomatic cases, the period of contact is measured as the 2 days before through the 14 days after the date on which the sample was taken, which led to confirmation. (Venter, M., & Richter, 2020). Specific and real-time diagnostic tests should be performed not only for the identification of potential cases but also for contacts who need to be quarantined and guided on epidemiological questions around the infection. Selection of the relevant specimen and knowledge of the incubation period, viremia, and shedding period are important criteria in diagnosing individual cases and defining transmissibility to inform the extent of isolation periods for patients. Nucleic acid testing (reverse transcriptase PCR) is recommended for the diagnosis of acute cases. Serological assays have an important role in answering epidemiological questions, including determining the exposure rate and assessing community spreads, but are not relevant for accurate diagnoses of acute cases. Only laboratory confirmation can be used to make an etiological diagnosis and differentiate atypical pneumonia from other causes. Proper collection of samples is the most important step in the laboratory diagnosis of infectious diseases. A specimen that is not collected correctly may lead to a negative result.

As per the Center for Disease Control and Prevention (CDC) recommendations, the upper respiratory specimen should be collected for RT-PCR-based testing of COVID-19, and especially the nasopharyngeal exudate is the preferred choice (CDC, 2020). Within a week from the onset of symptoms, patients with COVID-19 usually possess high viral loads in their upper and lower respiratory tracts. (Zhou et al., 2020). A nasopharyngeal swab and/or an oropharyngeal swab are often recommended for screening or diagnosing early infections. Nasopharyngeal swabs usually reach the correct area to be tested in the nasal cavity (Wang et al., 2020) reported that oropharyngeal swabs were used much more frequently than nasal swabs in China

during the current COVID-19 outbreak; however, the SARS-CoV-2 RNA was detected in only 32% of oropharyngeal swabs, which was significantly lower than the level in nasal swabs (63%). For the late detection and monitoring of patients with severe COVID-19 pneumonia. Ideally, sputum sampling or bronchoalveolar lavage (BAL) has been used for collecting lower respiratory tract specimens as they have yielded the highest viral loads for the diagnosis of COVID-19 (Li et al., 2020; Yu et al., 2020). A recent study revealed that samples of bronchoalveolar lavage fluid yielded the highest SARS-CoV-2 RNA rate, although this study did not compare or evaluate results from nasopharyngeal swabs (Wang et al., 2020). Patients who present with severe pneumonia and acute respiratory distress syndrome may require emergent intubation as well as respiratory isolation in a negative-pressure room. If possible, a lower respiratory tract sputum specimen should be collected during the intubation procedure. Alternatively, sputum and/or bronchoalveolar lavage fluid specimens may be collected after intubation (Pan et al., 2020). Collecting a respiratory swab specimen may carry a theoretical risk of transmitting SARS-CoV-2, particularly if chances of airborne transmission are demonstrated. Currently, biosafety level (BSL)-2 conditions are recommended for the handling of specimens for molecular testing. While collecting specimens, health care professionals should follow WHO infection prevention and control guidelines and wear personal protective equipment (PPE) such as gloves, gowns, eye protection, and N95 masks (WHO 2020). All specimens collected for laboratory investigations are regarded as potentially infectious.

Testing of specimens from multiple sites (e.g., upper and lower respiratory tracts) may improve the sensitivity of the RT-PCR and reduce false-negative test results, especially during the second week of illness. For asymptomatic patients and patients with mild symptoms, the collection of both nasopharyngeal swabs and oropharyngeal swabs is recommended; these should be placed together in the same viral transport medium (VTM) to increase the sensitivity (WHO 2020, CDC 2020). Additional clinical specimens can be collected as the COVID-19 virus has been detected in blood, urine, and stool. In the case of deceased patients, the collection of autopsy material, including lung tissue, is also considered. (Kaijin et al., 2020). As serological assays become available retrospectively in recovered patients, paired serum (acute and convalescent) can be useful in defining cases (Zhang et al., 2020; ShiX et al., 2005; Ding Y. et al., 2004). Several studies reported the detection of higher viral loads in older patients. However, the viral load did not positively correlate with disease severity (Tsang et al., 2020; Pung et al., 2020; Wang et al., 2020). Since SARS-CoV-2 and most other respiratory viruses are RNA-based, care should be taken to select extraction and inactivation protocols that will not damage RNA. A recent study suggests that heat inactivation at 56 °C for 30 min may result in false negatives for samples with low viral loads (Pan et al., 2020).

Specimens for virus detection should reach the laboratory as soon as possible after collection. Correct handling of specimens during transportation is essential. Specimens that can be delivered to the laboratory can be stored and shipped at 2–8 °C. When there is likely to be a delay in specimens reaching the laboratory, the use of viral transport medium (VTM) is strongly recommended in the laboratory biosafety guidance related to the novel coronavirus (2019-nCoV). (Guidance on regulations for the transport of infectious substances, 2019–2020) The primary container should be sealed with a screw cap. The container should be made of plastic that has a low risk of breakage. Patient information should be recorded on the specimen container using two or more identifiers (e.g., name, patient number), along with the necessary information for testing requests. The outer surface of the primary container should be disinfected using appropriate disinfectants, such as 70% ethanol. The container should be packed in a zipper bag and placed in a secondary container before transportation. The secondary container should be sealed and shock-resistant, and it should be labelled to indicate that it contains infectious substances. When transporting specimens to a laboratory within the same institution, the specimens should be transported in person; the pneumatic tube system should not be used. A separate route should be used for transportation. The personnel transporting the specimen should be trained in spill decontamination procedures in case of specimen leakage. Specimens may be frozen to -20°C or, ideally, -70°C and shipped on dry ice if further delays are expected. It is important to avoid repeated freezing and thawing of specimens (Kwon et al., 2020; Korea Center for Disease Control and Prevention, 2020; CDC, 2020). WHO documents a summary of the optimum sample collection procedures and storage, which are similar to those for influenza. The specimen collection and storage temperature protocol to investigate emerging acute respiratory diseases is summarized in Table 1.

Table 1: The specimen collection and storage protocol to investigate non-seasonal influenza and other emerging acute respiratory diseases (Druce, J., et al., 2012),

Specimen types	Collection material	Storage temperature
Nasopharyngeal and oropharyngeal swabs	Dacron or polyester flocked swabs	2-8°C
Sputum	Sterile container	2-8°C

Bronchoalveolar lavage	Sterile container	2-8°C
Nasopharyngeal or nasal aspirates or washes	Sterile container	2-8°C
Tissue from biopsy or autopsy, including lung tissue	Sterile container with saline or VTM	2-8°C
Serum	Serum separator tubes	2-8°C
Whole blood	Collection tube	2-8°C
Stool	Stool container	2-8°C
Urine	Urine collection container	2-8°C

Surveillance and Sampling Strategies

Individual case definition mechanisms (2.1), as mentioned above, help choose whether a patient should be tested or not. However, a pandemic like COVID-19 necessitates robust surveillance systems at local and global levels, which are required to strengthen the effective implementation of control measures. The aim of these surveillance systems is to limit the spread of disease, enable public health authorities to manage the risk, and thereby enable economic and social activity to resume to the extent possible. Surveillance is also necessary to predict the longer-term trends of COVID-19 transmission and the changes ahead (WHO 2020). A "case sample" is considered a subset of individuals from a larger population. Sampling is simply stated as selecting a portion of the infected population that will actually collect evidence from the research area under surveillance. Samples are used to make inferences about populations. (Landreneau, K. J., et al., 2009).

There are two types of sampling methods employed in epidemiological surveillance:

Probability sampling: This includes some form of random selection in choosing the elements. Greater confidence can be placed in the representativeness of probability samples. This type of sampling involves a selection process in which each element in the population has an equal and independent chance of being selected.

- a) Simple random sampling: in a simple random sample, every member of the population has an equal chance of being selected.
- b) Stratified random sampling: this sampling method is appropriate when the population has mixed characteristics (like age, demography, etc.) and you want to ensure that every characteristic is proportionally represented in the sample.
- c) Cluster sampling: cluster sampling also involves dividing the population into subgroups, but each subgroup should have similar characteristics to the whole sample. Instead of sampling individuals from each subgroup, entire subgroups are randomly selected.
- d) Systematic Sampling: This is similar to simple random sampling, but it is usually slightly easier to conduct. Every member of the population is listed with a number, but instead of randomly generating numbers, individuals are chosen at regular intervals for sampling.

Non-probability sampling: The elements that make up the sample are selected by nonrandom methods. This type of sampling is less likely than probability sampling to produce representative samples. Even though this is true, researchers can and do use non-probability samples. The three main methods are:

- a. Convenience Sampling: A convenience sample includes the individuals who happen to be most accessible to the researcher.
- b. Quota sampling is primarily motivated by ease of access. Instead of the researcher choosing participants and directly contacting them, people volunteer themselves (e.g., by responding to a public online survey).
- c. Purposive Sampling: This type of sampling involves the researcher using their judgement to select a sample that is most useful for the purposes of the research.

Any COVID-19 surveillance system placed should be geographically comprehensive and include all people and communities at risk. Surveillance for vulnerable or high-risk populations should be enhanced. This will require a combination of surveillance systems, including contact tracing, in the entire health care system, at the community level, as well as in closed residential settings and for vulnerable groups. Surveillance at the primary care level is needed to detect cases and clusters in the community. Where possible, testing facilities are made available at primary care clinics. A complementary option is to establish dedicated COVID-19 community testing facilities. Patients with probable and confirmed COVID-19 cases are notified within 24 hours of identification. Fast data reporting and analysis are critical to detecting new cases and clusters. Therefore, only the minimum number of data variables are to be collected (e.g., age, sex, date of illness onset, date of sample taken, test result, location of testing site, etc.). Data reporting to local or national public health authorities is done on a daily

basis. Patients with probable or confirmed COVID-19 diagnoses in hospitals are notified within 24 hours of identification. All COVID-19 deaths are reported within 24 hours of the death. The minimum essential data from hospital settings includes: age, sex/gender, and place of residence; date of onset; date of sample collection; date of admission; laboratory test result; severity on admission: admitted to the intensive care unit (ICU); treated with ventilation, if the case is a health care worker; outcome (discharge or death); etc. Existing sentinel surveillance of influenza-like illness (ILI) or acute respiratory infections (ARI) is useful to monitor trends in community transmission of the COVID-19 virus and to ensure that other priority respiratory diseases are being detected. Integration of COVID-19 with the Global Influenza Surveillance and Response System (GISRS) is described in operational considerations for COVID-19 surveillance using GISRS. Virologic sentinel surveillance of COVID-19 is conducted using clinical specimens obtained through sentinel surveillance of ILI, ARI, and SARI (Severe Acute Respiratory Infection). Integrated epidemiological and virological surveillance will play a significant role in monitoring the spread and evolution of COVID-19 virus, understanding the cocirculation of COVID-19 virus with influenza and other respiratory viruses, and subsequent interpretation of respiratory epidemiological and disease observations in relation to COVID-19, as well as supporting the update of diagnostic tests. Infections in health workers should, at a minimum, be systematically integrated into the national surveillance system. Dedicated enhanced surveillance for some high-risk groups is necessary to ensure the prompt detection of cases and clusters, faster than through primary care or hospital-based surveillance. People who live in closed environments, such as prisons, or residential facilities, such as retirement communities or care homes for persons with disabilities, can be especially vulnerable because they may not be able to seek help themselves. Vulnerable groups may also live in settings where the probability of transmission is higher than in the general population or have health conditions or predisposing factors that increase their risk of severe illness. Enhanced surveillance includes the use of active case finding, such as through daily screening of signs and symptoms, including daily temperature monitoring, and daily zero reporting for all individuals in high-risk groups under surveillance.

While surveillance systems will typically capture the number of COVID-19 cases, it is also important to collect information on the total number of laboratory tests conducted for the COVID-19 virus. Data on the number of tests conducted for SARS-CoV-2 are collected from all relevant laboratories. Knowing the testing denominator can indicate the level of surveillance activity, and the proportion of positive tests can indicate the intensity of transmission among symptomatic individuals. Presently, reverse transcriptase polymerase chain reaction (RT-PCR) testing (explained in 2.3) is the most common and reliable laboratory diagnostic method. If other diagnostic methods are used, the number of tests conducted and cases confirmed by different laboratory diagnostic methods need to be recorded.

It is widely accepted that a large fraction of COVID-19 cases goes undetected. However, this is subject to significant ascertainment bias because tests are typically ordered only from symptomatic cases, whereas a large proportion of infected people may show little to no symptoms (Mizumoto et al., 2020). Non-symptomatic infections can still shed the SARS-CoV-2 virus and are therefore detectable by RT-PCR-based tests. It is therefore possible to test randomly selected individuals to estimate the true disease prevalence in a population. Recent technical advances have enabled high-throughput PCR, in which multiple samples are pooled into one tube. Combining probes from several individuals and testing them together reduces the total amount of testing needed. This method is known as "pooling" or "group testing." The main idea is that when samples from several people are mixed together and tested, the test will report negative when everyone is healthy and positive when at least one is positive (Abdalhamid et al., 2020). The other ideas include using ten-fold fewer tests (Verdun et al., 2021) and clearing 20 times the number of people from isolation with the same number of tests (Gollier and Gossner, 2020). However, their efficiency is highly dependent on the frequency of positive samples, which varies significantly across regions and even within regions as testing criteria and conditions change. Two possible optimized pooling strategies are currently employed for diagnostic SARS-CoV-2 testing on large scales; both address dynamic conditions. In the first, an estimate of the target frequency determines the initial pool size, and any subsequent pools found positive are re-pooled at half size and tested again. The second method is a simpler approach of optimized one-time pooling followed by individual tests on positive pools. These strategies are convenient, and they offer a significant reduction in the number of materials, equipment, and time needed to test large numbers of samples. (Shani et al., 2020). On the other hand, a pool testing strategy could potentially increase worldwide testing capacity many times over, thus boosting a country's capacity to test mildly to asymptotically affected individuals. This strategy proposes that instead of individually testing patients with low clinical suspicion of SARS-CoV-2 infections, samples are pooled together in what is called a "minipool" and then tested together, running a single RT-PCR for all the unified samples. Preliminary results showed that there is no dilution and no decrease in test sensitivity when minipools of five samples each are used. Since the

RT-PCR looks directly at the viral RNA, a negative result in a pool test is reliable. Thus, the infection was discarded in all the patients included in the pooled sample.

Nucleic Acid Amplification Assay

SARS-CoV-2 is a single-stranded, positive-sense RNA virus. The availability of sequence data has facilitated the design of primers and probes needed for the development of SARS-CoV-2-specific testing. Routine confirmation of cases of COVID-19 is based on the detection of unique sequences of virus RNA by real-time reverse transcription polymerase chain reaction (RT-PCR), with confirmation by nucleic acid sequencing when necessary. The majority of molecular diagnostic tests have utilized real-time RT-PCR technology targeting different SARS-CoV-2 genomic regions, including the ORF1b or ORF8 regions and the nucleocapsid (N), spike (S) protein, RNA-dependent RNA polymerase (RdRP), or envelope (E) genes. The most widely used method of COVID-19 diagnostics is a reverse transcription quantitative polymerase chain reaction (RT-qPCR) assay to detect the presence of SARS-CoV-2 RNA in patient samples, typically nasopharyngeal swabs. RNA extraction is a major bottleneck in current COVID-19 testing. (Shen et al., 2020; Smyrlaki et al., 2020). Because of its high sensitivity and specificity, polymerase chain reaction (PCR) is regarded as the gold standard test for the molecular diagnosis of viral and bacterial infections. Isothermal nucleic acid amplification, including transcription-mediated amplification and CRISPR-based methodologies, is considered a promising alternative assay due to its fundamental advantage in quick procedure time at constant temperature without thermocycler operations. As such, real-time reverse transcriptase-PCR (RT-PCR) is of great interest today for the detection of SARS-CoV-2 due to its benefits as a specific and simple qualitative assay. Furthermore, real-time RT-PCR has sufficient sensitivity to aid us in early infection diagnosis. Therefore, the "criterion-referenced" real-time RT-PCR assay can be considered as the main method to be applied to detect the causative agent of COVID-19, i.e., SARS-CoV-2 (Carter et al., 2020; Shen et al., 2020; Wan et al., 2016; Noh et al., 2017; etc.).

Reverse Transcription-Polymerase Chain Reaction (RT-PCR):

In acute respiratory infections, RT-PCR is routinely used to detect causative viruses from respiratory secretions in nucleic acid testing assays. The real-time reverse transcription (PCR) method is one of the best and most accurate laboratory methods for detecting, tracking, and studying the coronavirus. Real-time RT-PCR is a method by which the presence of specific target genetic material can be detected (Sethuraman et al., 2020). This reaction relies on small DNA sequence primers designed to specifically recognize complementary sequences on the viral RNA genome and the reverse transcriptase to generate a short complementary DNA copy (cDNA) of the viral RNA. In real-time RT-PCR, the amplification of DNA is monitored in real time as the PCR reaction progresses. This is done using a fluorescent dye or a sequence-specific DNA probe labelled with a fluorescent molecule until the viral cDNA can be detected. Coronaviruses have a number of molecular targets within their positive-sense, single-stranded RNA genome that can be used for PCR assays (Corman et al., 2020). These include genes encoding structural proteins, including envelope glycoproteins spike (S), envelope (E), transmembrane (M), helicase (Hel), and nucleocapsid (N) (Chan et al., 2020). In addition to the genes that encode structural proteins, there are species-specific accessory genes that are required for viral replication, like RNA-dependent RNA polymerase (RdRp), hemagglutinin esterase (HE), and open reading frames ORF1a and ORF1b (Corman et al., 2020; Lan et al., 2020). The assay includes at least two molecular targets to avoid potential cross-reaction with other endemic coronaviruses as well as potential genetic drift of SARS-CoV-2. In the United States, the CDC recommends two nucleocapsid protein targets (N1 and N2) (Holshue et al., 2020), while the WHO recommends first-line screening with an E gene assay followed by a confirmatory assay using the RdRp gene (Corman et al., 2020). RT-PCR has traditionally been carried out as a one-step or two-step procedure. One-step real-time RT-PCR uses a single tube containing the necessary primers to run the entire RT-PCR reaction. Two-step real-time RT-PCR involves more than one tube to run the separate reverse transcription and amplification reactions but offers greater flexibility and higher sensitivity than the one-step procedure. (VanGuilder, H. D., et al., 2008; Wong et al., 2005). Positive test results from a single sample must be confirmed by a repeat test detecting a different region of the SARS-CoV-2 genome on the same sample. If possible, another repeat sample should also be tested to exclude false positive results due to amplicon carryover. Since the viral load in nasopharyngeal aspirate usually peaks on the 10th day after the onset of symptoms, suspected SARS cases must have the tests repeated as the disease evolves to avoid false-negative results. Stool specimens should be sent for testing on a regular basis, as a high percentage of patients develop diarrhoea and shed virus during the second week of illness. Viral load determination of nasopharyngeal specimens or serum upon presentation might have clinical value as it is an important prognostic factor. Any treatment regimen would benefit from long-term monitoring of viral load. (Cheng et al., 2004; Peiris et al., 2003; Chan K. et al., 2004; Chu et al., 2020; etc.). Apart from sensitivity issues, RT-PCR has some other drawbacks, such as possible biological safety hazards that

may occur during transport and sample processing, nucleic acid extraction, and the requirement of sophisticated laboratory equipment like biosafety cabinets. Technical expertise, along with sample transportation, which is inevitable, makes the overall process time-consuming. All these drawbacks could make the process less useful in case of a health emergency or global outbreak situation. Moreover, in PCR, we are able to detect not only the target virus, but it can also perform codetection of several other respiratory viruses, which leads to an increase in false positive or negative results (Cho et al., 2014).

Isothermal nucleic acid amplification

Isothermal nucleic acid amplification is an alternative strategy that allows amplification at a constant temperature and eliminates the need for a thermal cycler. Therefore, several methods based on this principle have been developed. Isothermal amplification techniques are conducted at a single temperature and do not need specialized laboratory equipment to provide similar analytical sensitivities to PCR. Isothermal amplification techniques can be multiplexed during the amplification or readout stage. This is done by using polymeric beads encoded with unique optical signatures like organic fluorescent molecules for each gene. Multiplexing increases the amount of information gained from a single test and improves clinical sensitivity and specificity. Loop-Mediated Isothermal Amplification (LAMP) is a relatively new molecular amplification point-of-care technique that is widely used for COVID-19 diagnosis. The technique is based on the synthesis of target DNA at a constant temperature of 60–65°C using a specially designed primer and DNA polymerase that has strand displacement activity instead of heat denaturation as in other PCR techniques. This novel technique can amplify any genomic material with high efficiency and in a shorter amount of time. RT-LAMP has been developed as a rapid and cost-effective testing alternative for SARS-CoV-2. It requires a set of four primers specific for the target gene or region to enhance the sensitivity and combines LAMP with a reverse transcription step to allow for the detection of RNA. Photometry can be used to detect the amplification product by measuring the turbidity caused by magnesium pyrophosphate precipitate in the solution as a byproduct of amplification. The reaction can be followed in real time either by measuring the turbidity or by fluorescence using intercalating dyes. Since real-time RT-LAMP diagnostic testing requires only heating and visual inspection, its simplicity and sensitivity make it a promising candidate for virus detection. The RT-LAMP test uses reverse transcriptase to convert the viral RNA to cDNA, which is subsequently amplified by the DNA-dependent DNA polymerase for rapid colorimetric detection with a DNA-binding dye.

LAMP has been shown to be effective at detecting viral RNA in cell lysates at levels of approximately 480 RNA copies without interference, providing an alternative to RT-PCR for rapid and simple detection of SARS-CoV-2 RNA. (Notomi et al., 2000; Thai et al., 2004). Transcription-Mediated Amplification (TMA) is a patented single-tube, isothermal amplification technology modelled after retroviral replication that can be used to amplify specific regions of either RNA or DNA much more efficiently than RT-PCR. It uses a retroviral reverse transcriptase and T7 RNA polymerase and has been used for the detection of nucleic acids from multiple pathogens. The initial step involves hybridization of the viral RNA target to a specific capture probe and an additional oligonucleotide containing a T7 promoter primer, which are captured onto magnetic microparticles. Then, the captured RNA target hybridized to the T7 promoter primer is reverse transcribed into a complementary cDNA. The RNase activity of the reverse transcriptase subsequently degrades the target RNA strand from the hybrid RNA cDNA duplex, leaving a single-stranded cDNA, which includes the T7 promoter. An additional primer is used to generate double-stranded DNA, which is subsequently transcribed into RNA amplicons by T7 RNA polymerase. These new RNA amplicons then reenter the TMA process, allowing this exponential amplification to generate billions of RNA amplicons. The detection process involves the use of single-stranded nucleic acid pyrotechnics that hybridize specifically to the RNA amplicon in real time. Each torch is conjugated to a fluorophore and a quencher. When the torch hybridizes with the RNA amplicon, the fluorophore is able to emit a signal upon excitation. (Kacian et al., 1999) Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) represents a family of nucleic acid sequences found in prokaryotic organisms, such as bacteria. These sequences can be recognized and cut by a set of bacterial enzymes called CRISPR-associated enzymes. Certain enzymes in these families can be programmed to target and cut viral RNA sequences. The CRISPR-based methods do not require complex instrumentation and can be read using paper strips to detect the presence of the SARS-CoV-2 virus without loss of sensitivity or specificity. These tests are both low-cost and can be performed in as little as an hour. These tests have great potential for point-of-care diagnosis. (Zhang et al., 2020; Broughton et al., 2020). The rolling circle amplification (RCA) method has attracted considerable attention in nucleic acid determination. In isothermal conditions, RCA is capable of a 109-fold signal amplification of each circle within 90 minutes. An efficient assay for the detection of SARS-CoV-2 by RCA has been set up in both liquid and solid phases and has yielded preliminary results on a small number of clinical respiratory specimens. The main advantage of RCA is that it can be performed under isothermal conditions with minimal reagents and avoids the generation of false-positive results, which are

frequently encountered in PCR-based assays. (Chapin et al., 2011, Xu et al., 2019, Wang et al., 2005, etc.).

Cartridge Based Nucleic Acid Amplification test (CB-NAAT) and True NAAT

Unlike traditional RT-PCR tests, the sample preparation in CB-NAAT tests is automated, and the results are available within half an hour. The test uses nose- or throat-swab samples, which are collected from patients and dipped in a solution that inactivates the virus. A few drops of the solution are then placed on a cartridge. On inserting this cartridge into a machine, a preprogrammed reaction is initiated, which extracts the nucleic acids or the genetic material from the samples. This has to be followed by RT-PCR. The purified nucleic acid is added into a microtube containing freeze-dried RT-PCR reagents and allowed to stand for a minute, then applied to a microchip and then inserted into another machine, where the reverse transcription and PCR take place. The advantage of this test is that it is quick and portable. This allows the easy setup of mobile testing centers or kiosks in containment zones instead of having to transport samples to labs. True NAT is an indigenously developed, portable version of CB-NAAT, also known as the GeneXpert test. Both of these tests were originally designed to screen for tuberculosis.

Immunological assays

Immunological tests measure the antibodies generated by the host body's immune response against the virus infection (antibody test) or the proteins of COVID-19 virus present in the respiratory specimens (antigen test). When a virus enters the human body, it triggers an immune response that results in the production of an antibody against the virus; detecting such an antibody in an infected person is extremely useful regardless of whether the person has symptoms. Antibody tests are blood-based tests that can be used to identify whether people have been exposed to a particular pathogen. The serum includes antibodies to specific components of pathogens, called antigens. These antigens are recognized by the immune system as foreign and are targeted by the immune response. These types of tests are often used in viral infections to see if the patient has an immune response to a pathogen of interest, such as SARS-CoV-2. The role of serological assays to detect IgG, IgA, or IgM anti-SARSCoV-2 antibodies in serum, plasma, or capillary blood provides a clear picture of the outbreak size in each country and helps to assess the degree of immunization. (Okba et al., 2020). Serological testing for COVID-19 is particularly attractive because of the relatively short time to diagnosis and the ability to test for an active immune response against the virus. Serological tests have variable sensitivity and specificity. Research has demonstrated that the spike (S) and nucleocapsid (N) proteins are the primary viral antigens against which antibodies are raised. (Chan et al., 2009; Kumar et al., 2020). These antigens are the most commonly used in serological tests. During infection, several types of antibodies are raised against the virus. IgM antibodies emerge first, after 5 days of post-symptom onset. IgG antibodies typically emerge after 10 days of post-symptom onset. Many serology tests detect both IgG and IgM simultaneously, which increases the specificity of the test. IgA antibodies may also increase during infection and are typically found in mucous. While serological tests are now widely available, the correlates of immunity are still poorly understood. The presence of antibodies only indicates a previous SARS-CoV-2 infection. The results of serological tests can then be used to estimate the true spread of the virus through a population, even if individuals were asymptomatic or were never diagnosed. The presence of antibodies does not indicate that an individual is protected from reinfection since there is limited understanding of the levels and persistence of antibodies necessary for protective immunity. Therefore, serological tests cannot inform an individual of their immunity to reinfection (Yu et al., 2020). However, the test results may also help in choosing convalescent plasma, which can be used as a promising treatment option for COVID-19-infected individuals.

Antibody tests

The determination of SARS-CoV-2 exposure relies largely on the detection of either IgM or IgG antibodies that are specific for various viral antigens, including the spike glycoprotein (S1 and S2 subunits) and nucleocapsid protein. The methodology for these determinations includes the traditional enzyme-linked immunosorbent assay (ELISA), immunochromatographic lateral flow assay, neutralization bioassay, and specific luminescent immunoassays. Each of these formats brings advantages (speed, multiplexing, automation) and disadvantages (trained personnel, dedicated laboratory requirements, etc.). Rapid antigen tests, which use antibodies to detect the presence of viral antigen(s) in serological samples, are complementary to these. Major diagnostic companies are currently focusing on the development of high-throughput serology tests.

- Enzyme-linked immunosorbent assays (ELISA): a faster serological test performed in a laboratory that provides a readout of antigen-antibody interactions.

Essentially, patient antibodies are "sandwiched" between the viral protein of interest and reporter antibodies so that any active patient antibodies are detected. A serological assay was performed using an ELISA kit that was developed for detecting IgM or IgG antibodies against the N proteins of SARS-CoV-2. For IgM detection, ELISA plates were previously coated with mouse and anti-human antibodies. This test can be qualitative or quantitative and is generally a lab-based test that is obtainable within

a few hours. These tests usually use whole blood, plasma, or serum samples from patients. The test relies on a plate that is coated with a viral protein of interest, such as spike protein. Patient samples are then incubated with the protein, and if the patient has antibodies to the viral protein, they bind together. The bound antibody-protein complex can then be detected with another wash of antibodies that produce a colored or fluorescent-based readout. (Liu et al., 2020). ELISA is speedy, has the ability to test multiple samples, and is adaptable to automation for increased throughput, but can be variable in sensitivity and is suitable for point-of-care determinations.

- Lateral flow assays (LFAs): Lateral flow assays (LFAs), also called rapid diagnostic tests (RDTs), display a colorimetric, qualitative readout of the presence of antibodies. These are often used in point-of-care settings. The patient sample is passed through a membrane on which the target antigen is anchored. If the sample contains antibodies specific to that antigen, they form a complex that results in a colored band on the strip. These are similar to pregnancy tests. This is facilitated by a recombinant antigen present on immunochromatographic paper, on which the test sera are applied, and antigen antibody binding is detected visually by a color change on a membrane. The results can be obtained within 15–20 minutes. The test is inexpensive and requires no trained personnel, but it provides only qualitative results. When used in conjunction with rapid antigen tests, where anti-SARS-CoV2 antibodies are used in place of immobilized viral antigen, they allow for a more direct assessment of ongoing infection.
- Neutralization assays: determine an antibody's ability to prevent virus infection of cultured cells and the cytopathic effects of viral replication. For this assay, patient samples of whole blood, serum, or plasma are diluted and added at decreasing concentrations to the cell cultures. If neutralizing antibodies are present, their levels can be measured by determining the threshold at which they are able to prevent viral replication in the infected cell cultures. The time to results for neutralization assays is typically 3–5 days, but recent advances have reduced this to hours. This type of testing requires cell culture facilities, and in the case of the SARS coronavirus, Biosafety Level 3 (BSL3) laboratories are required. Despite these limitations, the determination of neutralizing antibodies is important in the short term for the therapeutic application of convalescent plasma and in the long term for vaccine development.
- Luminescent immunoassay: This test shows whether a patient has antibodies to a pathogen by displaying a fluorescent signal when patient antibodies interact with virus proteins. Luminescent immunoassays comprise methods that lower the limits of detection for antibody-based reagents. Generally, they involve chemiluminescence and fluorescence. For SARS-CoV-2, two-step chemiluminescent immunoassays for the detection of IgG and IgM SARS-CoV-2 antibodies in human serum or plasma have been demonstrated. Samples react with paramagnetic microparticles coated with SARS-CoV-2-specific antigens (recombinant N- and S-proteins), and alkaline phosphate-labeled anti-human IgG or IgM monoclonal antibodies are added to the reaction mixture, resulting in a chemiluminescent emission, measured as relative light units (RLU) by a photomultiplier built into the system. After 25 minutes, the first results were generated (Nuccetelli et al., 2020).

Antigen Detection Assays

Antigen detection with monoclonal antibodies or monospecific polyclonal antibodies against the protein was found to be a sensitive and specific test for the diagnosis of SARS (Kumar et al., 2020). In a large study with sera collected from 317 SARS patients at different time points of illness, the detection of SARS-N antigen was performed using a panel of three monoclonal antibodies. Over 80% of SARS cases were discovered within the first 7 days of illness. As serum antibody levels started to rise at day 7, the sensitivity of the serum antigen assay progressively decreased to 0% at day 21 (Chan et al., 2004, Hsueh et al., 2003).

Rapid assay Methods

Rapid tests are non-automated, primarily qualitative (but also quantitative in some cases), and used for in vitro diagnostics. These tests can provide results within 10–30 min, so their results are considered instant as compared to the molecular tests, which generally take 4–6 h. Moreover, these tests are user-friendly, so they won't require any extensive training or expertise to operate and can be used either in a hospital environment or in the field without any difficulty. The manifestation of the COVID-19 infection is highly nonspecific, including respiratory symptoms such as fever, cough, dyspnea, and viral pneumonia (Huang et al., 2020). Thus, extensive diagnostic tests specific to this infection are urgently required to confirm suspected cases, screen patients, and conduct virus surveillance. Rapid tests are used to diagnose patients without sending samples to centralized facilities, thereby enabling communities without laboratory infrastructure to detect infected patients. In this, a point-of-care (PoC) device, i.e., a rapid, robust, and cost-efficient device that can be used onsite and, in the field, and which does not necessarily require a trained technician to operate (Nguyen et al., 2018),

LAMP assays (explained in 2.3.2) in PoC devices have high specificity and sensitivity and are simple to perform; hence, soon after their initial development, they became an enormously popular isothermal amplification method in molecular biology, with applications in pathogen detection. LAMP uses strand-displacement polymerases instead of heat denaturation to generate a single-stranded template; hence, it has the advantage. LAMP technology is proven to be more stable and more sensitive in detection compared to PCR (Francois et al., 2011). Lateral flow antigen detection (explained in 2.4.1) for SARS-CoV-2 is another point-of-care approach under development for diagnosing COVID-19. In commercial lateral flow assays, a paper-like membrane strip is coated with gold nanoparticle-antibody conjugate, and capture antibodies are used. These assays have previously demonstrated reliable clinical sensitivity (57%), specificity (100%), and accuracy (69) for IgM and 81%, 100%, and 86% for IgG, respectively. A test that detected both IgM and IgG yielded a clinical sensitivity of 82% (Xiang et al., 2020). However, lateral flow assays do not directly confirm virus presence; instead, they provide serological evidence of recent infections (Li et al., 2020). Microarray assays have been used for rapid, high-throughput detection of SARS-CoV-2 nucleic acids. They rely on the generation of cDNA from viral RNA using reverse transcription and the subsequent labelling of cDNA with specific probes loaded into the wells of microarray trays. The microarray assay has proven useful in identifying mutations associated with SARS-CoV-2 and has been used to detect up to 24 single nucleotide polymorphisms (SNP) associated with mutations in the spike (S) gene of SARS-CoV-2 with 100% accuracy. The ability to detect different emergent strains of SARS-CoV-2 may become necessary as the COVID-19 pandemic evolves, and microarray assays provide a platform for rapid detection of those strains as a result of mutational variations. A next-generation shotgun metagenomics sequencing platform has been developed by Illumina with the ability not only to detect the presence of multiple strains of coronaviruses but also to comprehensively examine multiple pathogenic organisms present in a complex sample.

Chest CT images from patients with COVID-19 typically demonstrate bilateral, peripheral ground glass opacities. Because this chest CT imaging pattern is nonspecific and overlaps with other infections, the diagnostic value of chest CT imaging for COVID-19 may be low and dependent upon other interpretations. Given the variability in chest imaging findings, a chest radiograph or CT alone is not recommended for the diagnosis of COVID-19. The American College of Radiology also does not recommend CT for screening or as a first-line test for the diagnosis of COVID-19. Lymphopenia is the most common laboratory finding in COVID-19 and is found in as many as 83% of hospitalized patients. (Huang et al., 2020) Lymphopenia, neutrophilia, elevated serum alanine aminotransferase and aspartate aminotransferase levels, elevated lactate dehydrogenase, high CRP, and high ferritin levels may be associated with greater illness severity (Chen et al., 2020). Patients with critical illness had high plasma levels of inflammatory markers, suggesting potential immune dysregulation (Wang et al., 2020).

This review summarizes various diagnostic methods used for the identification of COVID-19 infections. While RT-PCR has been the dominant technique for detection of viral RNA, other nucleic acid assays, including isothermal amplification assays, hybridization microarray assays, amplicon-based metagenomics sequencing, and the cutting-edge CRISPR-related technologies, are also under development or have resulted in approved tests. A comparative account of different diagnostic methods available for COVID-19 is given in table 2. The urgent need for accurate and rapid diagnosis of SARS-CoV-2 infection remains critical as global healthcare systems continue to operate during the course of the COVID-19 pandemic. In particular, serological and immunological testing of infected asymptomatic and symptomatic individuals, and their close contacts, is expected to be in high demand.

Table 2: A comparative account of different diagnostic methods used for detection of COVID-19

Method	Working Principle	Advantage	Time Required	Disadvantage
Next generation sequencing (NGS)	Whole genome sequencing, shotgun metagenomics etc.	Highly sensitive and specific, could provide all related information; can identify novel strain. Helps to examine multiple pathogenic organisms present in a complex sample.	1-2 days	Require high expertise & cost. Highly sophisticated Lab required.

RT-PCR	Specific primer-probe based detection	Fast results, higher sensitivity, well established methodology in viral diagnostics.	3-4 hrs.	High cost due to expensive consumables. Expensive lab equipment. RNA extraction is highly tedious and sensitive.
Isothermal nucleic acid amplification	Synthesis of target DNA at constant temperature of 60–65°C using specially designed primer	No thermal cycler required. Can be easily detected by color change or turbidity	1-2 hrs.	Too sensitive, highly prone to false positive results due to carry-over or cross contamination
LAMP	More than two sets of specific primers pair-based detection.	Highly repeatable and accurate. No thermal cycler required	1 hr.	Primer designing is complex
CB NAAT/True NAAT	Cartridge Based Nucleic Acid Amplification and detection	Automated sample preparation, pre-programmed reaction. Can be used in a PoC devices	15–30 min	
CRISPR based methods	Enzymes programmed to target and cut viral RNA sequences	low-cost, do not require complex instrumentation, can be read using paper strips.	15–30 min	Not properly standardized for SARS Cov2 detection
Serological (traditional)	Antigen/Antibodies IgG/ IgM/ELISA etc.	Sensitive and specific. useful for choosing the convalescent plasma therapy.	4-6 hrs	Samples taken after 3-4 days of infection.
Rapid Serological (traditional)	Antigen/Antibodies IgG/ IgM	Convenient, can be used in a PoC devices	15-30 min	Samples taken after 3-4 days of infection.
Lateral flow assay	colorimetric, qualitative detection of the presence of antibodies	Inexpensive and convenient, can be used in a PoC devices	10-15 min	Nature of the sample affects capillary action, (e.g. blood clotting) pre-treat meant is required.
Luminescent immune assay	Chemiluminescence or fluorescence labelled antibody or antigen	Highly sensitive, Quantitative	1-2 hrs.	Closed analytical systems required for detection
Microarray	rapid high-throughput detection of viral RNA using cDNA	Highly sensitive, longer target sequences can be detected	1 hr.	Fabrication of cDNA micro array is labor intensive.

CT scan	Computerized tomography of Chest images	Enhance sensitivity of detection if findings combined with RT-PCR results	1 hr.	Indistinguishability from other viral pneumonia other chest complications
Biochemical tests	Blood counts, Lymphopenia, elevated serum enzyme levels, high plasma levels etc.	Could support routine symptomatic treatment regimens of COVID 19	1-24 hrs	Not conclusive evidence for COVID 19 infections

CONCLUSION

The COVID-19 pandemic, caused by the SARS-CoV-2, has resulted in over 17 million confirmed cases and over 7 lakh deaths worldwide in less than six months. The living and working conditions of billions of people worldwide have been significantly disrupted due to different forms of social distancing and lockdowns in many cities. The widespread availability of accurate and rapid testing procedures is extremely valuable in unravelling the complex dynamics involved in SARS-CoV-2 infection and immunity. One of the many challenges in containing the spread of COVID-19 is the inability to identify asymptomatic cases that result in the virus spreading to close contacts. Thus, the global outbreak of COVID-19 has emphasized the importance of the laboratory diagnosis of human coronavirus infections in order to limit the spread as well as appropriately treat those patients who have serious complications. WHO has published a uniform case definition for surveillance and testing for COVID-19 infections. However, the focus on implementing the most reliable diagnostic tools varies in different places. Since COVID-19 is a new nosological entity, there are no data as of yet that would enable the determination of standards for the interpretation of specific diagnostic tests. As with any other infectious disease, the accuracy of each method depends on the method of collecting the material, the quality of the sample, and the equipment applied. Although RT-PCR testing plays a crucial role in accurately detecting SARS-CoV-2 on a case-by-case basis, it also has inherent problems that limit its utility. Current obstacles to the widespread use of RT-PCR testing include a shortage of testing kits and an extended processing period of several hours before results are obtained. Moreover, the results of real-time RT-PCR tests must be cautiously interpreted. A combination of real-time RT-PCR and clinical features, especially CT images, could facilitate better disease management. Proper sampling procedures, good laboratory practice standards, and using high-quality extraction and a real-time RT-PCR kit could improve the approach and reduce inaccurate results. Loop-Mediated Isothermal Amplification (LAMP) is a relatively new, convenient molecular amplification point-of-care technique that is widely used for COVID-19 diagnosis. The technique does require sophisticated laboratory equipment to provide similar analytical sensitivity to RT-PCR. Serological testing for COVID-19 is particularly attractive because of the relatively short time to diagnosis and the ability to test for an active immune response against the virus. A lot remains to be understood regarding the value of serological testing in COVID-19 diagnosis and monitoring. The results of serological tests can then be used to estimate the true spread of the virus through a population, even if individuals were asymptomatic or were never diagnosed. More comprehensive evaluations of the performance of serology tests are rapidly under way. Considerations for the use of serology methods for COVID-19 require the correct and appropriate interpretation of the results and understanding the strengths and limitations of such tests.

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AN OVERVIEW FOR THE POTENTIALS OF ADDITIVE MANUFACTURING POLYMERS RECYCLING PROJECT IN EGYPT

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ABSTRACT

Additive manufacturing is a star-rising technology around the globe. It satisfies key needs in different fields of interest on a global scale. For both ongoing industrial revolutions, it is considered an enabler as well as for achieving certain Sustainable Development Goals. Regarding the manufacturing sector, the technology serves well for different delicate fields like aerospace, automotive and medicine. Polymers are amongst the wide range of materials that are utilized in additive manufacturing. They provide indispensable mechanical properties in different fields as they possess light weight with high strength and toughness. The existence of polymers in the form of waste is massive, especially after the outbreak of COVID-19. The calls for sustainable development are global. Exploitation of polymer wastes as a feedstock material for additive manufacturing can be considered an opportunity to be seized by Egyptian manufacturers. Such that producing new products, reducing wastes and costs. These piles of waste may now be viewed as wealth. Under the umbrella of the current reforms and developments taking place in the country this can be called upon to be a national project. The paper presents an overview for the opportunity of establishing such a national recycling project in Egypt that depends on 3d printing.

Keywords: Additive Manufacturing, Egypt Vision 2030, Polymers waste, Recycling Products, Sustainability.

INTRODUCTION

Around the globe, there are several major fields of keen interest are currently taking place. Sustainability, Industry 4.0 and Industry 5.0 have all emerged simultaneously with no notable time gaps, and countries worldwide are trying their best to adopt them all in parallel. The Egyptian political perspective after the revolution on June 30th has dramatically changed. It has created several pathways to put Egypt on the global track of development. The Egyptian strategy “Egypt Vision 2030” is a national representation of the United Nations Sustainable Development Goals (Amin, 2021). This strategy represents how seriously Egypt beats the grand, rapid and complicated changes taking place around the world. Several initiatives have been called upon to support the implementation of the strategy. “Digital Egypt” initiative that drafts new developmental conditions to facilitate Industry 4.0 adoption in Egypt (Moghaieb, 2019). This initiative was raised by the Egyptian Ministry of Communication. Aiming to transform the entire ecosystem of the Egyptian society alongside all the existing government services to be completely digitized. The initiative of “Decent Life” can be considered as the Egyptian context for the Japanese movement “Society 5.0” to adopt Industry 5.0. The main goal of “Decent Life” is human-centered, as it aims to enhance the daily living conditions of Egyptian citizens, especially in the countryside (Amin, 2021).

Given this plenitude of strategies aiming towards Egypt’s development, multiple challenges are still to be faced. Challenges like climate change, water scarcity, and COVID-19 are global. While an issue like waste management is definitely local and significant. Egypt is a populous country with an estimated amount of about 1.2 Kg of a daily solid waste/person/day generated. Thus resulting in massive amounts of wastes roaming through the Egyptian streets (Development, 2021). The pandemic has magnified the waste problem, especially that of polymeric material (Yee Van Fan, 2021). Polymeric waste piles are accumulating. Proper waste management and recycling projects are among the Egyptian goals to achieve in its vision for 2030 (Development, 2021). The opportunity for using additive manufacturing to recycle polymeric waste and produce new products is widely open. This paper aims to provide a closer lens about the current situation of using additive manufacturing technology in the Egyptian

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market. And to promote for the call for a national polymeric waste recycling project. To produce new products with lowered costs in parallel to abated wastes.

Additive manufacturing and global trends

A. Additive Manufacturing- A Review

Additive manufacturing is the process of building up products layers' from a certain material rather than cutting from it. It is used to be called rapid prototyping, free fabrication and 3d printing. The technique was developed initially to test prototypes and validate the authenticity of designs. The term additive-manufacturing is the official term used in the ASTM International Standards (Mahesh Mani, 2014). The process of production starts with a computer aided design for the product. Then it passes through a printer software that slices the design into several layers. Afterward, the machine starts building the layers incrementally to finally obtain the 3d physical product (Heinrich, 2021).

Additive Manufacturing as a technology includes several techniques. Those techniques do utilize wide range of materials. Exclusive attention is being directed towards those techniques associated with plastics. In order to help solve the plastic waste problem. Polymers dependent additive manufacturing has been used in the production of various application parts. From medical implants, optical products, architectural parts, sports equipment, smart textiles, soft robotics to custom tools for aerospace industry. They all benefit the high ability of customization and complex shapes production that additive manufacturing provide.

- Stereolithography Apparatus, is a vat photopolymerization technology, in which a vat of the polymer resin is cured using an ultraviolet light source. The principle depends on a computerized building stage on which the light illuminates the pattern to be printed (Md. Sarower Tareq, 2021), (Ferry P.W. Melchels, 2010).
- Selective Laser Sintering, which depends on using an infra-red laser beam to appropriately heat powdery substances. The powder used is a pre-polymer thermoplastic. With the help of a roller, the powder is spread over the surface of a built cylinder. Then a piston moves downward for a distance equal to one object layer such that the powder layer is accommodated. The fabrication chamber is entirely sealed and its temperature is maintained at (the thermoplastic glass transition temperature) just below the melting temperature (Mazzoli, 2013), (Ian Gibson, 1997)].
- Poly Jet Printing and Multi Jet Fusion are polymer-based additive manufacturing techniques for producing prototypes and finished products. Poly Jet Printing is similar to Stereolithography. While Multi Jet Fusion is greatly comparable to Selective Laser Sintering.
- Fused Deposition Modeling is the revolutionary digital form of conventional extrusion and injection molding (Md. Sarower Tareq, 2021). This is the most popular polymer-based additive manufacturing technology. Its principle is based on the availability of uninterrupted feed of the polymer filament into the liquefying chamber. The liquefying chamber transforms the filament from the solid state into a semi-liquid state ready for deposition through the extrusion nozzle over the building platform (Ankita Jaisingh Sheoran, 2020).

The process of additive manufacturing generally faces a set of provocations that require improvements. Processing conditions affecting the structure and properties of the product are under investigations for further enhancement (Arit Dasa, 2020). Also, size limitations of the produced part is another issue. As larger parts are unsuitable for the technology due to amplified processing time (K.Satish Prakasha, 2018).

B. Additive Manufacturing and Industry 4.0

Industry 4.0 is the 4th industrial revolution. It firstly appeared in 2014 by the German government (Xun Xu, 2021). It is named the Digital Revolution, as it aims to digitizing the entire manufacturing sector. This digitization constitutes the integration of the physical along with digital technologies (Rojko, 2017). Cyber-Physical Production Systems represent its core. Smart factories concept is considered a target for Industry 4.0. These smart factories depend on remote decision-making and self-managed operations, as well as mass customization (Marina Crnjac, 2017).

Based on these concepts associated with Industry 4.0, additive manufacturing is considered a strong enabler for the revolution. Additive manufacturing is a technology with strong capabilities that promote Industry 4.0 adoption (Ugur M Dilberoglu, 2017). It can handle a wide range of materials through the process. It has the merit of producing intricate, sophisticated and highly customized/personalized products. It also works with computer-aided designs with minimal human intervention (Javeed, 2019). It is typically a programmable machine that can be self-organized/optimized.

C. Additive Manufacturing and Industry 5.0

Industry 5.0 is also called "Society" according to the Japanese government. It is a value-driven revolution, rather than a technological one (Yuqian Lu, 2022). Industry 5.0 according to the European Commission is driven by 3 main values:

Sustainability, Human Centricity and Resilience (Maija Breque, 2021). Industry 5.0 is not initiated to replace Industry 4.0 but to complement it. It is supposed to value and appreciate the human role. Human centricity in Industry 5.0 is not limited to the fear of unemployment that is going to spread due to digitization brought by Industry 4.0. It is a much wider concept that cares for personnel health and opinions as well (Yuqian Lu, 2022). It is also meant to promote resilience measures and encourage sustainability efforts. Industry 5.0 is an approach that maintains the engine of prosperity through the transition to digital green manufacturing.

Additive manufacturing is a customized technology that promotes human centricity. The technology values customers' personal requirements and delights (Javeed, 2019). The resilience of manufacturing allows fast response to any changes in the demand (Bardia Naghshineh, 2022). Additive manufacturing can stand disruptions in orders with minimal production disturbance. Additive manufacturing has higher impacts on achieving sustainability performances than subtractive methods (Sam Solaimani, 2021).

D. Additive Manufacturing and Sustainability

Profound problems regarding the environment have been intensively discussed since late 1980's. Calamities in terms of climate change, scarcity of water and sparse resources are alarming (Hossam A. Kishawy, 2018). Greenhouse gasses and water pollution are spreading diseases. So, the criterion for evaluating an organization's success is no longer limited to economic means. The criterion has widened to include the environment as well as the social impacts of the business (Correia, 2019). Activities that minimize energy consumption, raw material consumption and pollution are promoted. By 2015, the United Nations issued a set of 17 goals known as Sustainable Development Goals. These are globally obligatory on developing and developed countries to be achieved by 2030 (Nations, 2017).

Additive manufacturing when measured against the three new lines of business success, or what is known as the Triple Bottom Lines (TBL) of sustainability (Correia, 2019), the following results can be demonstrated.:

Table 1: Additive Manufacturing against Sustainability Triple Bottom Lines

Triple Bottom Lines	Gains	Inadequacies
Environment Line	<ul style="list-style-type: none"> - Fewer waste - Higher material utilization - High possibilities of recycling 	<ul style="list-style-type: none"> - Higher energy intensity
Social Line	<ul style="list-style-type: none"> - Enhanced customer satisfaction - Potential benefits on workers' health 	<ul style="list-style-type: none"> - Undetermined effect on unemployment
Economic Line	<ul style="list-style-type: none"> - Short lead times - Less assembly - On-demand manufacturing 	<ul style="list-style-type: none"> - Long time - Costs/part - Limited dimensions

Generally, additive manufacturing is acclaimed to enhance sustainable practices. Environmentally, it is meant to eliminate resource demands and minimize energy consumption. Along with decreased carbon footprint related to the process. Since it uses no tooling system as well as no environmentally damaging lubricants. Socially, it may be argued to decrease human involvement such that increasing unemployment. On the other hand it is a computer aided manufacturing method that values designers and increases their capacity and welcomes their significant influence. New business models can be built based on additive manufacturing, economically speaking (Muthu, 2016). Additive manufacturing is anticipated to reduce the effects of transportation, eliminate inventory and supply issues. Over the near future, centralized additive manufacturing is viewed as an improved business approach (Danfang Chen, 2015), (Carla Gonclaves Machado, 2019)

E. Additive Manufacturing and COVID-19

The virus was initiated in Wuhan city of China and shortly was declared a pandemic. COVID-19 is a respiratory novel virus that causes severe acute respiratory disease that may lead to death. Scientists and epidemiologists are still studying this 120nm in diameter pathogen (Rigoberto Advincula, 2020). The unprecedented events caused by the virus from its novelty to its vast spread among people have led to certain containment actions. These actions included mainly the usage of personal protective equipment as precautionary measures. These equipment constituents were face shields, surgical masks and latex gloves. Along with frequent hand sanitization (Kuan Shiong Khoo, 2021). The circumstances that occurred with the virus spread worldwide resulted in curfew inside countries and isolation for some parts of cities. This has slowed down trade on all economic levels

creating an urgent demand for the equipment entailed to help face the virus. Thus, encouraging local resourcing for shortages that occurred in these goods. Additive manufacturing technology stepped in to fill these needs (Rigoberto Advincula, 2020). Additive manufacturing exceeded the expectations in fulfilling this decentralized supply chain.

Additive Manufacturing in Egypt

Since 2014 and after changing the Egyptian regimen, Egypt has started the journey of revitalization. By the issuing of the Egyptian Strategy for Development “Egypt Vision 2030” in 2016, Egypt is aiming to be placed among the top 30 countries worldwide regarding the economic and scientific and innovation statuses (Chen, 2018). Since then, Egypt has been committed to implement reforms in these sectors in line with the health, educational and housing sectors. Despite the several successes earned due to the reforms, other pressing challenges are yet to be handled. One of them is the identification of innovative development mechanisms while considering the availability of limited resources (Amin, 2021).

From this point further, additive manufacturing can be considered an innovative mechanism that can use wastes to recycle them and produce broadly new products (K. DePalma, 2020). Thus, overcoming the limited resources problem regarding raw materials. Also, there is an ongoing awareness promotion for green manufacturing called “Go Green”. This call was held just prior to the climate conference held in Sharm Elsheikh this November. The call aimed at establishing new idea for green smart manufacturing projects. To identify the applicability of such a proposal the additive manufacturing situation in Egypt is to be studied in the following sections.

A. Additive Manufacturing and Education in Egypt

Both under college and college education are under the spot of reform these days in Egypt. Curriculum updates are carefully managed. Enhancement for universities to join the digital era is taking place by leaps and bounds (Ashour, 2020).

Colleges of Engineering are improving their curriculums. Additive manufacturing is being adhered to where possible, especially for the mechanical departments. Several graduation projects are about executing 3d printers by the students themselves under the proper guidance of the staff (Ashraf Elsafy, 2020). For example, at Pharos University in Alexandria, which is a private university, an additive manufacturing project is accomplished this year. The project is an open source recycling technique. Which integrates an extruder above the nozzle directly. Such that it recycles and manufactures at the same time. The American University in Egypt offers around the year courses regarding additive manufacturing. Also, the public universities have their own labs that include 3d printers that are built in the fulfillment of the student’s graduation project bachelor’s degree.

B. Additive Manufacturing Markets in Egypt

There is a growing market for 3d printers in Egypt. Not only limited to an engineering college education. Dentistry applications are a very large market for the usage of additive manufacturing and are growing widely in Egypt. This is according to the Journal of the American Chamber of Commerce in Egypt (Noureldin, 2019). The pace of the commercial usage of the technology in customized accessories production is at its maximum. A cost drop by 51 % occurred in the costs of the machines/materials assisted its spread (Mehrshad Mehrpouya, 2019).

C. Municipal Solid Wastes in Egypt and Opportunities for Recycling

On local basis, daily amount of 1.2 kg/person/day of solid wastes is produced. Regionally, Egypt is considered chief in polymeric waste generation in the African continent. Egypt owns 18.4% of the total continent’s polymeric wastes available (Otolong Donald Akan, 2021). This massive amount of solid waste exists with a collection efficiency of 40% and a recycling efficiency of no more than 2.5% (Development, 2021). The available wastes offer an adequate feedstock for 3d printers in the Egyptian market. However, it is the quality of the final product that raise concerns here. As to use wastes as feedstock either being chemically/mechanically recycled or not prior to the manufacturing stage might lower the grade of the finally produced part.

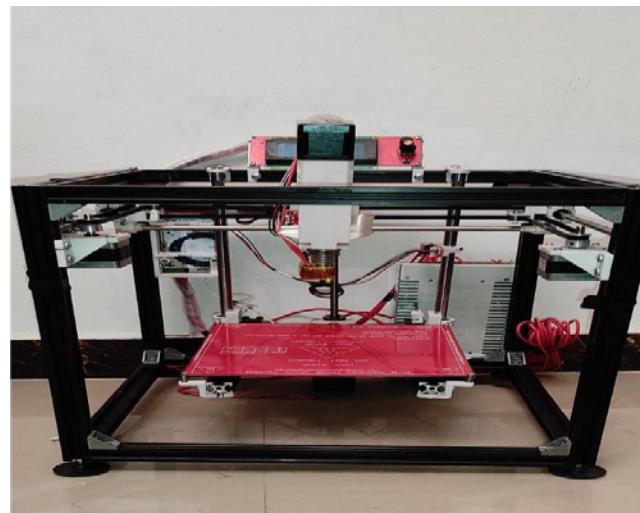


Figure 1: 3D-printer with integrated Direct Filament Extruder made at the PUA as a graduation project

RESULTS AND DISCUSSION

The status of the proposed project can be considered promising. Feasibly speaking, materials and machines are both available for affordable prices. The required experiences to start the business can be easily provided. The developmental track Egypt is riding on now opens horizons for newly suggested projects to be nationally adopted. But there are problems associated with the process. Product quality which is influenced by the materials or the processing conditions. Material concerns do rise due to poor waste management methods. That they do not guarantee sorting. Such that mixed wastes used as feedstock with/without prior recycling would negatively impact the properties of the produced part. Recycling of

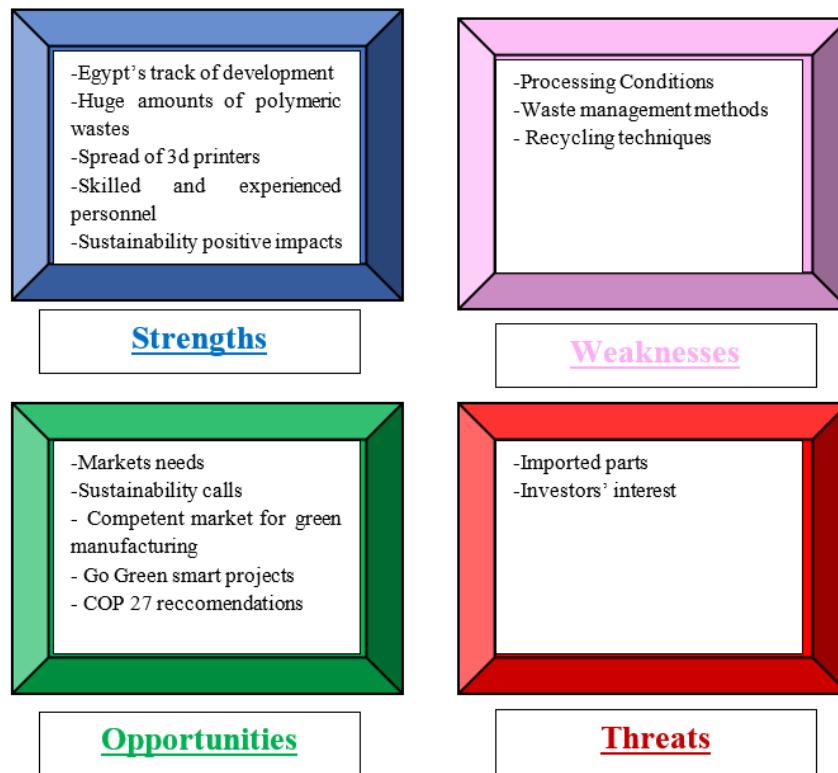


Figure 2: SWOT analysis for the potentials of additive manufacturing in Egypt

sorted wastes before being fed in the additive manufacturing process has its own complications. As recycling processes in themselves might degrade the produced feedstock quality. The additive manufacturing technology has its own spectrum of challenges. From build orientation, voids formation to processing time. However, there are great positive chances for the project success. Availability of a massive market that appreciates the indispensability of plastic products. The encouragements to foster sustainability practices. Which mainly preserve resources, eliminate wastes and decrease Co2 emissions. On the other hand several adversaries for the project do exist; extremely low price imported products as well as the interest of investors to invest in recycling projects. But, hopefully an event like the “Climate of the Planet, COP 27” conference, held in Sharm Elsheikh this November will definitely encourage recycling activities through sustainable means.

CONCLUSION

The additive manufacturing future for recycling practices in Egypt can be considered fortunate. Polymeric Wastes availability with the widespread acknowledgment of the process among people, increase the adoption possibility of the recycling business. Egypt is a dense country with a multi-level skilled population. There are low-skilled, semi-skilled and highly skilled employees in all fields. This allows such a recycling business to find all kinds of needed human resources to begin. Such a project can be deployed as a national project under the umbrella of the Egyptian Sustainable Development Strategy. The “Go Green” call for smart green manufacturing processes can be a supportive initiative for the project. Such that, providing licenses and funds for startups utilizing the idea.

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DESIGN AND IMPLEMENTATION OF SECURE IOT ARCHITECTURE FOR HIGH TECH BUILDINGS

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ABSTRACT

IoT platforms set up according to diverse design principles, computing paradigms, technologies, and aims. This has been done for the purpose of making the development of the internet of things (IoT) ecosystem easier and accelerating its pace. In this paper, we offer a survey of the primary examples that are now populating the vast landscape of IoT platforms, as well as a comparison of those examples in light of the IoT-a reference design. In this way, heterogeneous internet of things platforms (both current and future) can be analyzed regardless of their low-level specifications, but only through the lens of those key functionalities and architectural building blocks that enable the interplay among devices, data flow, software, and stakeholders within the IoT ecosystem. This can be done regardless of whether the platform is currently in use or will be in use in the future. In addition to these, security by design—that is, the incorporation of security design principles, technology, and governance at every level—must be incorporated into each and every tier, component, and application in order to reduce the likelihood of cyber-attacks and maintain the integrity of IoT platforms. This is necessary not only for the individual components themselves, but also for all of the components when they are working together as a whole.

Keywords: IoT, Sensors, Router, Switch, Sniffer

INTRODUCTION

In order to protect sensitive data and infrastructure, security is of the utmost importance for Internet of Things (IoT) systems. However, security challenges have become more expensive in recent years, particularly in Industrial Internet of Things (IIoT) sectors. Despite this, there are a few significant hurdles that must be overcome in order to address these security concerns in IoT domains: In terms of the machine resources required, applications run in decentralized settings like Blockchain, a wide variety of smart devices are utilized, and the number of sensors that can be deployed is constrained(Mohanta, Jena, RamasubbaReddy, Daneshmand, & Gandomi, 2020).

In this sense, conventional security does not make sense for IOT systems. The problem of cybersecurity has become one of the utmost importance in the fields of Internet of Things (IoT) and the Industrial Internet of Things (IIoT), particularly with regard to the reduction of cybersecurity risk for end users and enterprises. The management of Internet of Things security can benefit from newly developed cybersecurity technology and application(Raimundo & Rosário, 2022). However, there is a deficiency in the efficiency of solutions for the cyber risks posed by the internet of things.

Security is one of the basic requirements for Internet of Things (IoT) systems since, it helps to prevent sensitive data as well as infrastructure from being compromised. Nevertheless, the costs associated with addressing security concerns have increased over the past few years, particularly in industries related to the Industrial Internet of Things (IIoT). Despite this, there are several substantial obstacles that need to be cleared in order to handle these security concerns in IoT domains, including the following: When it comes to the necessary computing resources, application run in decentralized environments such as Blockchain, a wide variety of smart devices are utilized, and the number of sensors deployment is limited(Serror, Hack, Henze, Schuba, & Wehrle, 2020). In this sense, traditional security does not make any sense for the systems that make up the IOT. The problem of cybersecurity has become of the utmost importance to the IOT and the IIoT, particularly regarding the reduction IOT and the Industrial IIoT have become intertwined with a wide variety of physical and digital objects. Newly developed cybersecurity technology and applications are useful for improving the management of security for the IOT. On the other hand, the efficacy

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of solutions for the cyber dangers posed by the IOT is still lacking(Lee, 2020).

This study aims to encourage additional interest for everyone who is interested in IOT's cybersecurity risk management field. For instance, the currently available frameworks did not supply managers with any tools for the allocation of resources. Because there are no techniques for resource allocation, any decisions regarding cyber investment were taken based on gut feeling and lacked any justification(Shreeve et al., 2020).

The IoT aspires to combine the digital and physical worlds into a single coherent system, which will create significant new commercial opportunities across a variety of industries, including manufacturing, tourism, and energy sector. It has given rise to a new paradigm in which new processes are being driven by a network of machines and gadgets that are capable of interacting with one another and working in conjunction with one another. The IOT is vulnerable in terms of security breach, however, there are various security concerns that are frequently very demanding due to the IoT's complicated context and its extensive number of tools, both are considered as a weakness in terms of the acquired resources (Tawalbeh, Muheidat, Tawalbeh, & Quwaider, 2020).

The perception layer, network layer, and application layer of the Internet of Things architecture can be broken down into these three categories Figure. 2. Sensors, actuators, and network layer are examples of physical items in the perception layer that attempt to convey data, gather device information, and send it to the target device. Depending on the type of sensors used, data such as temperature, humidity, rain, sunlight, etc., may be collected. The digitalized signal is subsequently transmitted over secure channels to the network layer.

Data is sent from the perception layer to the application layer through the network layer, known as the transmission layer. The application layer

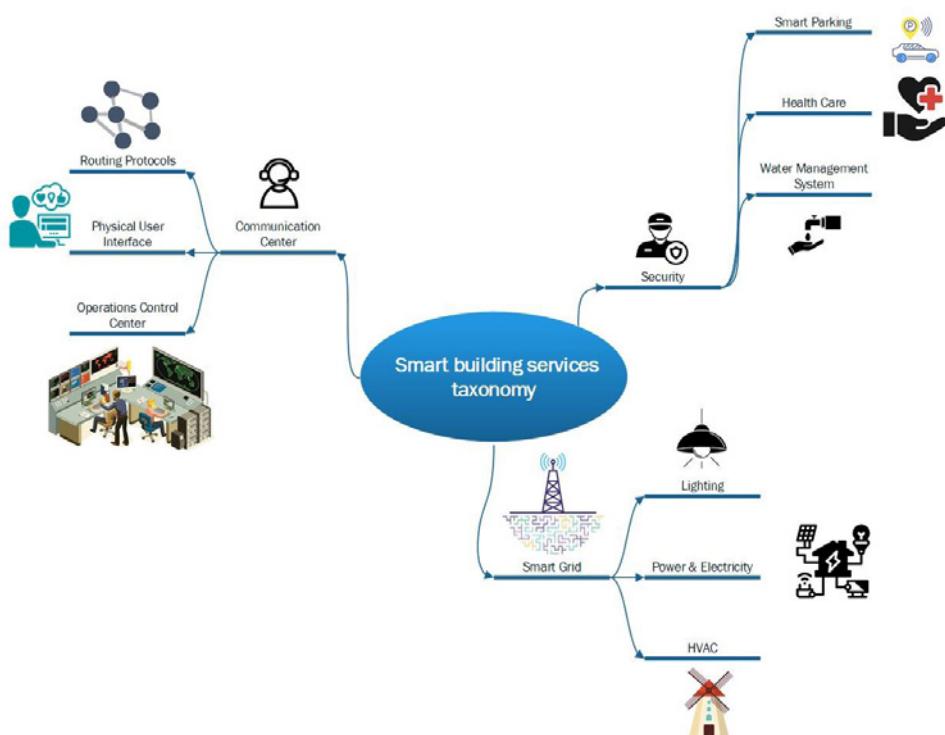


Figure 1: Smart building services taxonomy

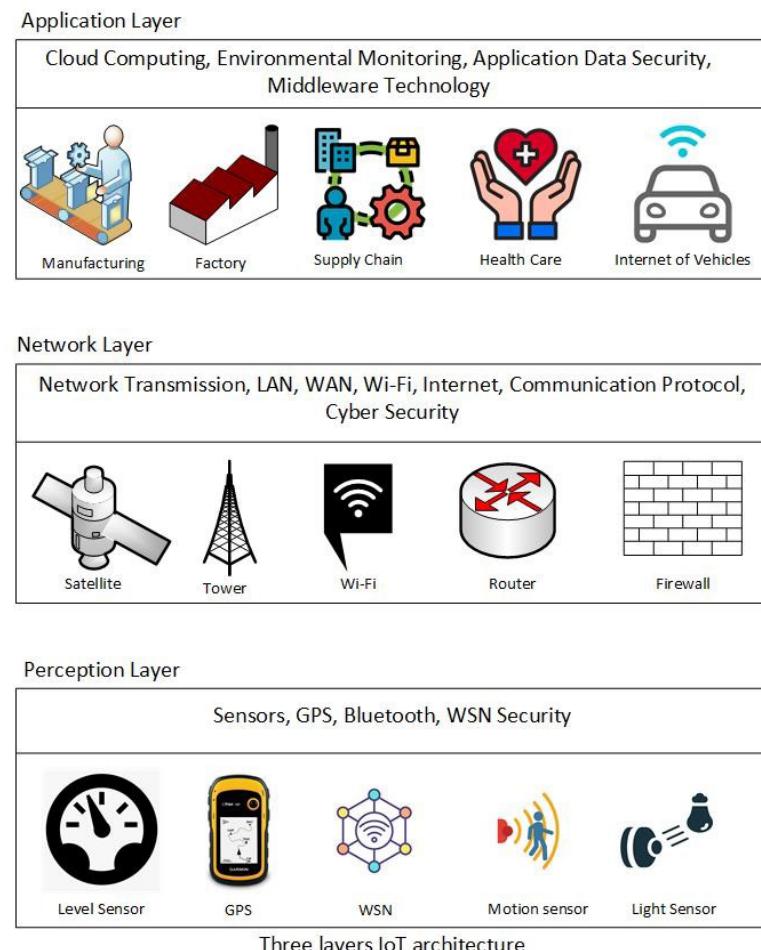


Figure 2: Three layers IoT architecture

makes different high-quality services and applications available to customers or consumers. This layer implements numerous smart applications for smart buildings, smart grids, smart transportation, and smart healthcare. Customers can use this interface to get the data they are looking for or talk to a physical device(Kumar & Mallick, 2018).

The IOT is a network that connects the digital world (the Internet) to the real-world situations via sensors. The IOT extends the capabilities of regular internet to traditionally non-physical items. Some examples of these types of things are automobiles and electric tools(Ammar et al., 2022). The IIoT cloud computing, and big data analytics, including robotics, are all examples of technologies that can be utilized in conjunction with the IOT to facilitate the production of high-quality goods at reduced prices. As a consequence of this, society is becoming increasingly susceptible to cyberattacks e.g. denial-of-service attacks because there are a rising number of IoT-based linked devices already in existence.

In addition, cyberattacks on smart grids, which are essential infrastructure components that are especially vulnerable and entail greater costs, have a significant and negative influence on the safety of both citizens and governments.

Despite the fact that there is a wealth of important data in the healthcare industry at the moment, cyber defences in hospitals are, on average lacking, putting the lives of patients and their trust in the institution in jeopardy(Tufail, Parvez, Batool, & Sarwat, 2021).

It is currently being applied in a variety of fields, including telemedicine, industrial settings, smart homes, cloud computing (thread detection), and health care (biomedical illness), amongst other applications. As part of IOT, wireless sensor network technologies make it possible for smart device connection with other devices and share functionality on a global scale. The wireless smart home automation system of the network comprises of sensors and actuators that can share resources or communicating with one another. This is the most significant piece of technology for developing smart houses. The IOT is a paradigm that strives to combine home automation into the concept of a "smart house," which is a concept that is part of the IOT paradigm(Mocrii, Chen, & Musilek, 2018).

As a result of the prior literature's concentration on the technical aspects of IOT cybersecurity, there is a gap in security frameworks that may be used to handle the complex cybersecurity issues that are present in the IOT. The purpose of this research is to devise an improved strategy for safety of sensitive data on IOT environment and industrial cyber risk management.

LITERATURE REVIEW

There are a lot of issues that are directly tied to the Internet of Things, and its development is still in its early stages. The term "Internet of Things" (IoT) describes a concept that permeates all of reality. Confidentiality, integrity, availability, scalability, confidentiality, and interoperability are just some of the ways in which the Internet of Things stands to drastically improve our environment. While important, securing the Internet of Things is a complex subject. The current level of system security is crucial to the development of the IoT (Abgaryan, 2021). The major issues in today's world are data protection and assembling of smart sensing devices effectively. There is a need to explore the reasons behind using particular security protocols rather than others, while stressing the importance of keeping consumers apprised of the issues at hand and the options available to them. In addition to highlighting cybersecurity's application on IoT devices, it intends to educate people in industry, academia, and student education on why cybersecurity is becoming increasingly important in today's society. This works in tandem with the prior objective. It aims to equip individuals with the tools necessary to make decisions in light of evidence and a range of qualitative approaches, all while inspiring them to consider how best to meet the next challenge on humanity's great journey (Krishnan et al., 2021).

Concerning the elimination of cybersecurity threats to consumers and businesses, the issue of cybersecurity has risen to the top of the list of priorities for the IoT and IIoT. Advanced cybersecurity tools and apps can help improve IoT security management. The cyber hazards posed by the IoT, however, have inadequately effective answers. For this purpose, multiple security issues are studied thorough search of the Scopus database. Rather than providing concrete technical advice for fixing existing network security flaws, it aims to capture the continuing conversation about IoT (Choo, Gai, Chiaraviglio, & Yang, 2021). It is of the utmost importance to ensure the safety of Internet of Things (IoT)-based "smart" systems that incorporate sensors, actuators, and distributed control loops, but doing so is incredibly difficult. Security patterns are the distilled wisdom of many years of accumulated, cross-domain security expertise. The research shows that there has been an uptick in the number of publications over the past three years that concentrate on patterns and designs for IoT security. Neither the architectural nor the network nor the IoT levels of security (or privacy) have been consistently addressed. This research adds to the large-scale efforts already under way to solve the problems highlighted by the OWASP top ten vulnerabilities in the Internet of Things (IoT) (Sharifi,

Allam, Feizizadeh, & Ghamari, 2021).

There are a lot of issues that are directly tied to the Internet of Things, and its development is still in its early stages. The term "Internet of Things" (IoT) describes a concept that permeates all of reality. There is a tremendous potential for the Internet of Things to significantly advance humankind in the areas of accessibility, integrity, availability, scalability, secrecy, and interoperability. However, securing the Internet of Things is a complex challenge. The current level of system security is crucial to the development of the IoT. Fundamental to the paradigm are measures taken to protect and integrate various forms of smart sensors and information and communication technology (ICT) (Lu & Da Xu, 2018). The IoT is no longer hailed as a revolutionary development with far-reaching implications for technology and human existence. Healthcare, food, finance, manufacturing, government, healthcare, oil and gas, transport, postal-services, military, and many other industries have adopted the concept. These industries are capitalizing on the IoT's potential for optimal operation to facilitate a wide range of tasks. IoT (Internet of Things) devices are used in homes, workplaces, and other settings every day, helping its users become more productive and efficient (Lu & Da Xu, 2018).

Cybersecurity has become an extremely important feature of Internet of Things as hacks have become more common and more damaging over time (IoT). The purpose of Internet of Things (IoT) cybersecurity is to lessen the overall cybersecurity risk faced by organizations and consumers through the protection of IoT assets and the privacy of users. Cybersecurity advancements in recent years have opened the door to the prospect of better monitoring and control of IoT security. However, there is a lack of robust framework for managers to apply in the oversight of cyber risk associated with the Internet of Things. In order to help allocate funds to different Internet of Things cybersecurity projects, a four-tiered cyber risk management architecture is outlined here (Lee, 2020). While 5G and 6G networks may provide novel communication network design, the same energy capacity means that hackers can still target the same vulnerabilities in Internet of Things equipment. Next-generation decentralized systems and networks, such as Blockchain, require a system that can detect and counteract threats (Abuhasel & Khan, 2020). A study based on a questionnaire provided to students to assess their proficiency in the areas of IoT, AI, and e-commerce (EC). At the outset of this line of inquiry, college curricula were examined to see how much emphasis was placed on emerging technologies like artificial intelligence, the internet of things, and electronic communication. The poll was conducted utilizing questions developed from the study's conclusions. Students were given access to the questionnaire that was created so that their responses could be collected. There was a total of 563 students included in the study sample for the survey. The results were further analyzed and investigated. The study's findings synthesize a data source that businesses may use to assess the qualifications of workers on the market at present and in the near future. The results also show which topics are more common knowledge among students and which are more common knowledge than others (Zhu, Ota, & Dong, 2021).

The network of connected gadgets known as the Internet of Things (IoT) is expanding rapidly and will soon permeate every aspect of human life. The proliferation of IoT devices and services has not gone unnoticed, and neither has the increase in the number of attacks against them. Despite the fact that cyberattacks on the IoT are nothing new, it is becoming increasingly important that can step up in the area of cyber defense as the IoT grows more deeply intertwined into our lives and society. A thorough familiarity with the threats and attacks that can be made against IoT infrastructure has become essential in light of the pressing need to keep it safe (Zhu et al., 2021). With more and more devices connected to the Internet of Things (IoT), our lives have become more easier. the capacity for two or more parties to exchange information across a network without needing to physically engage with one another. Yet, the number of threats to the IoT infrastructure has grown. There have been numerous security holes and attacks discovered ever since the IoT was first developed. Most security issues in IoT devices may be traced back to an outdated version of the firmware. Identifying and updating the machine's firmware presents a number of challenges. This incapacity has made it far less difficult to break into an IoT device (Cekerevac, Dvorak, Prigoda, & Cekerevac, 2018).

Recent study highlights the need for cyber security in the manufacturing sector and related fields. Manufacturing, oil and gas refining, pharma, food and beverage processing, water treatment, and many others are constantly looking for methods to improve their security measures as more and more machinery and gadgets are connected to the internet. Manufacturers of electronic gadgets and plant managers are under constant pressure to protect their facilities from cyber threats. Furthermore, the data types and topologies of IoT devices, as well as the challenges of threat management and compliance upkeep, vary widely between industries (Mishra & Pandya, 2021). Given the rapid expansion of numerous forms of attacks and threats, conventional methods of securing the Internet of Things are unable to meet the current security concerns. The key to establishing a security system that is dynamically enhanced and up to date for the next-generation Internet of Things system is the use of professionals in artificial intelligence (AI), particularly machine and deep learning solutions. comprehensive understanding of IoT security

intelligence, which is founded on machine and deep learning technologies that extract insights from raw data to intelligently safeguard IoT devices from a variety of cyber assaults (Tahsien, Karimipour, & Spachos, 2020).

Most of the social science research articles include a literature review. It usually contains the significant contribution of past papers to justify the adopted theory and variables. Further to develop hypotheses. It can be divided into subsections. Literature should be written concisely in detail by maintaining continuity of the texts and cited the original work following APA in-text citation format, e.g., Single authored document (Author's Surname, 2021); double authored document (1st Author's Surname & 2nd Author's Surname, 2021); Multiple authored document (1st Author's Surname, et al., 2021).

METHODOLOGY

The simulation is developed in packet tracer 8.2 to visualize smart building features control architecture. The ground floor rooms are well equipped with the smart functions as presented in Fig 4. The ground floor consists of 2 living rooms, kitchen, dining hall, parking area and outdoor front area. The simulation is capable to handle complex design situations with smart visualization tools. This factor enables to develop network configuration and troubleshooting via desktop computer/Android/iOS-based mobile device. Packet tracer is compatible for both the Linux and Windows environment. The features included in smart building are Smoke sensor/detector, Smart lamp, Smart Heating Unit, Smart Cooling Unit, Smart Coffee Maker, Smart Solar system, Temperature Sensor, Smart Window, Smart Door, Smart Garage parking system, monitors cool and heat through IOE4. The internet system is included to operate the smart features remotely from network Home Gateway as shown in figure 3.

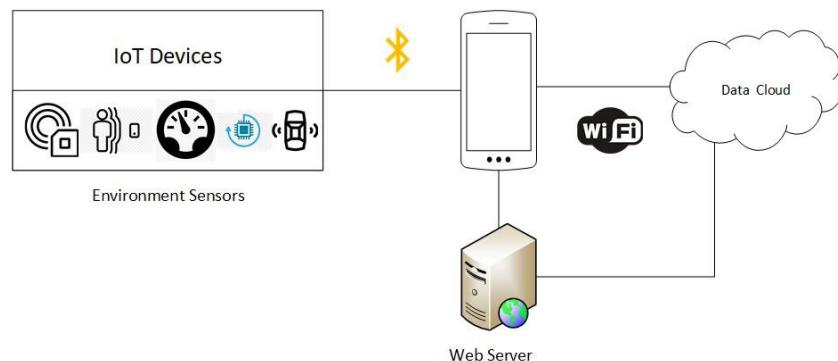


Figure 3: Smart-Building network topology

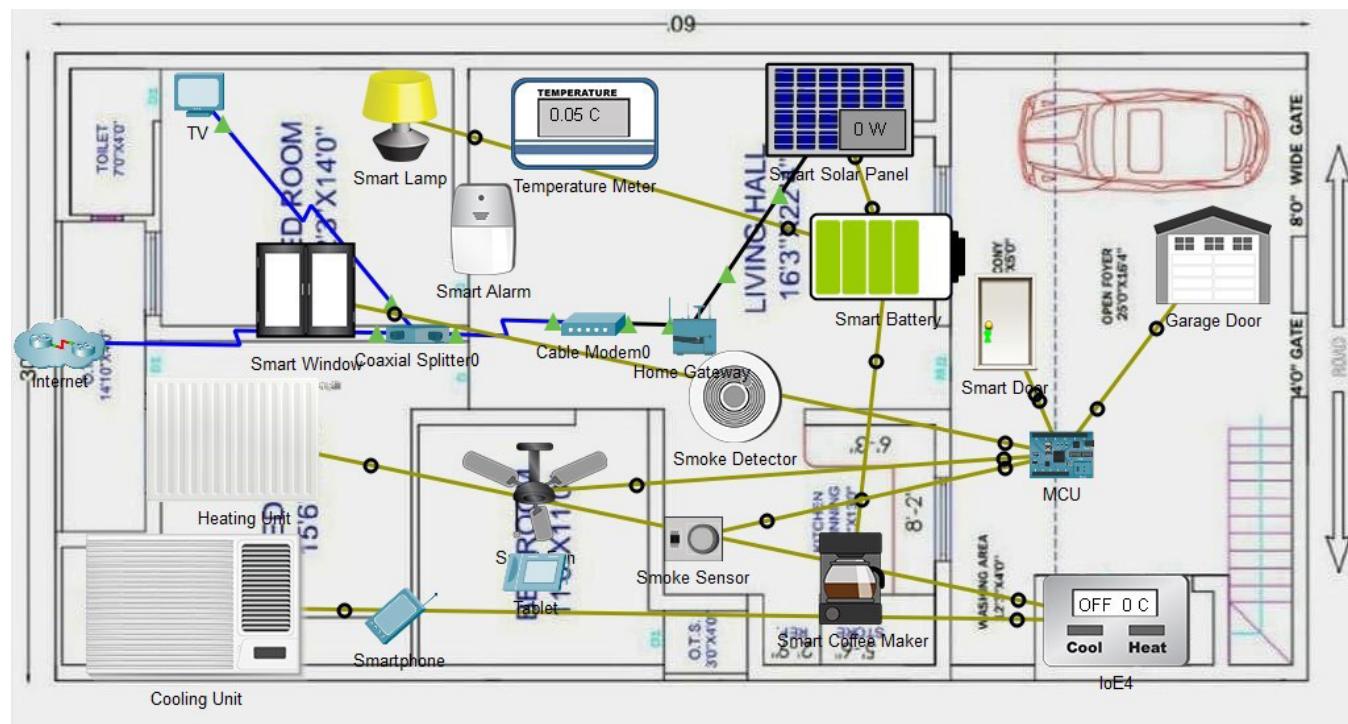


Figure 4: Cisco Packet Tracer layout of Smart-Building simulation.

IOT HARDWARE AND SOFTWARE

In smart building infrastructure, numerous software and hardware components e.g. sensors, switches, server, router, application programming interfaces (APIs) are included for internal and external communication. The major role of IOT hardware is to

enable the smart system and implements smart room features with secure environment. The communication among devices is enabled through APIs. The role of API is to interconnect different smart devices smartly and capable to get commands through cloud (WAN) . The Microcontroller Unit is used to interconnect the smart sensors and operates devices installed at various positions in the building according to the data received from sensors . The LAN protocol in building is implemented through home gateway responsible for all external communication. Cloud is acquired to access the smart features through tablet PC. The devices are connected through IOT custom cable as per communication protocols. The ISPs is responsible for internet services in order to achieve smart features externally. IOT cloud is created to share data gathered from smart sensors with IoT server. The inclusion of smartphone is serving an important purpose to get instant inputs from human beings to adjust the room features according to customer demands efficiently. In case of urgent purpose all smart devices can be controlled via internet. This feature is a remarkable milestone as users can save time and energy from distance. Moreover, the security measures can be checked to handle any emergency situation remotely.

The smart building environment consist of many smart services to control and implement smart energy and utilization accordingly. The smart cooling and heating unit in figure 5 is installed in all the rooms to control the room environment as per atmospheric demands. Both units are connected with IoE4 unit to consistently monitor the heating or cooling operation as per

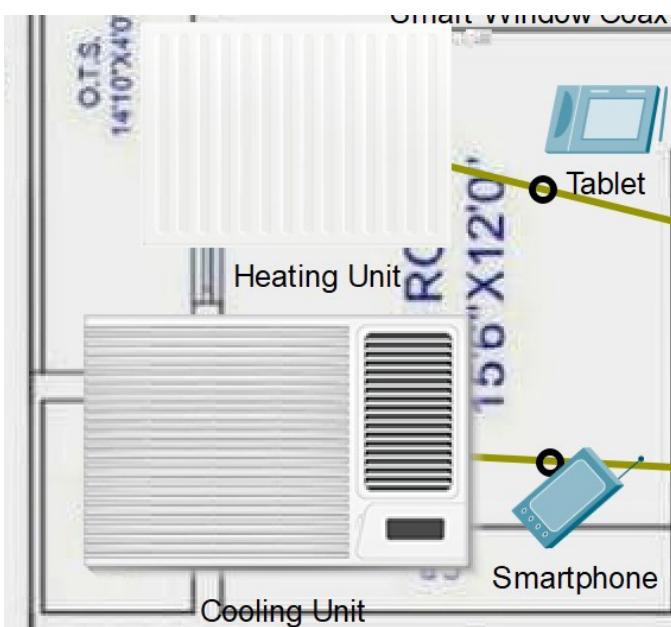


Figure 5: Smart Cooling and Heating Units

desired temperature smartly provided by user.

The Smart Kitchen services

The Window and lamp in figure 6 are operated smartly as per room environment. The air flow can be enhanced by opening the window and window can be closed in case of harsh weather outside. The lamp operation is based on illumination level in building. If visibility is low then lamp turns on and it turns off when things are easily visible in the room. This feature is also saving the electricity cost smartly. Moreover, user can turn on/off the lamp through smartphone. The kitchen of building in figure 7 is a sensitive area of any building. The smoke detector is installed to indicate any sort of smoke and take appropriate action against smoke via smoke alarm and building safety protocols. The smart coffee maker is an advanced option to make coffee for desired number guests in minimum time. The staff is trained to handle the presentation of coffee in meeting rooms and offices.

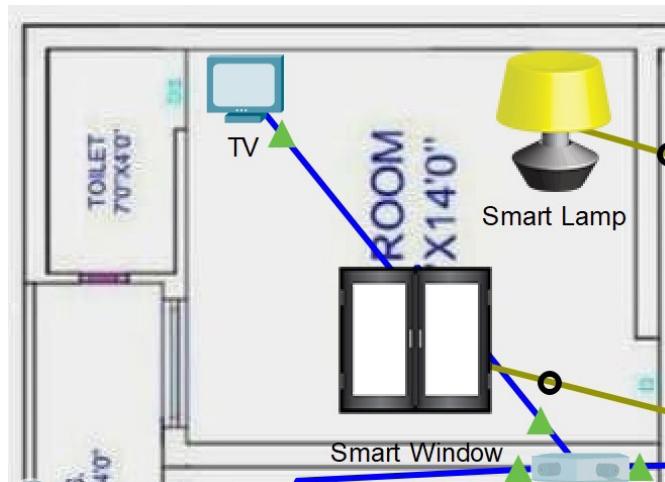


Figure 6: Smart window and Lamp

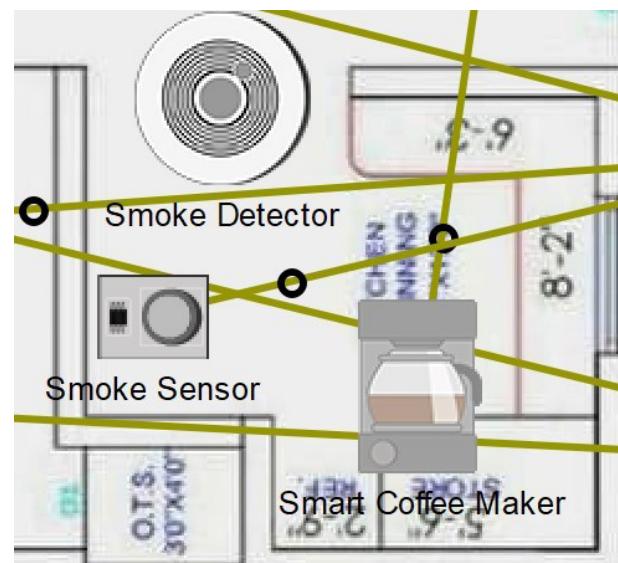


Figure 7: Smart kitchen accessories

The Smart Garage

The figure 8 presents smart door and garage door feature to entertain the persons coming from outside. The door and garage open in case of arrival of car in order to minimize the arrival time of guest from gate to office in building. If a person is present on the gate, then only door opens. If garage is occupied, then garage will not open and the voice recording will guide the guest for alternate parking arrangement.

Security of Smart data

The sniffer is added with IoT custom cable to enhance the security of dataset received from smart sensors. The sniffer role is to handle the data packet delivery as per the IP address. The handling of data via sniffer is providing security of data communication among devices and MCU. If hacker attempts to steal the data packets, then sniffer is capable to detect suspicious activity and blocks the entry and exit of data packets from hacker. The MCU communication cannot be accessed by hackers.

RESULTS

The functionality of smart features provided in simulated model is discussed in this section. The dynamic environment simulation is presented for application of smart features in building environment. The results in figure 9 are indicating the state of devices connected to smart building architecture. The environmental conditions are tested manually to justify the functionality of all smart features offered in smart building architecture. The air conditioner. The IoE4 module constantly monitors the temperature reading to turn on/off the Air conditioner/heater accordingly. If temperature increases beyond 30° then air-conditioner turns on. If temperature drops down below 30° then air-conditioner turns off. If temperature decreases below 15° then heater turns on. If temperature increases beyond 15° then heater turns off. The environmental features are included in figure 10 for smart handling of atmospheric conditions effectively. Figure 10 present the variation in atmospheric pressure and wind speed, Figure 11 shows the variation in humidity, rain and visible light. While, Figure 12 expresses the variation in ambient temperature and sunlight for 12 hours respectively.

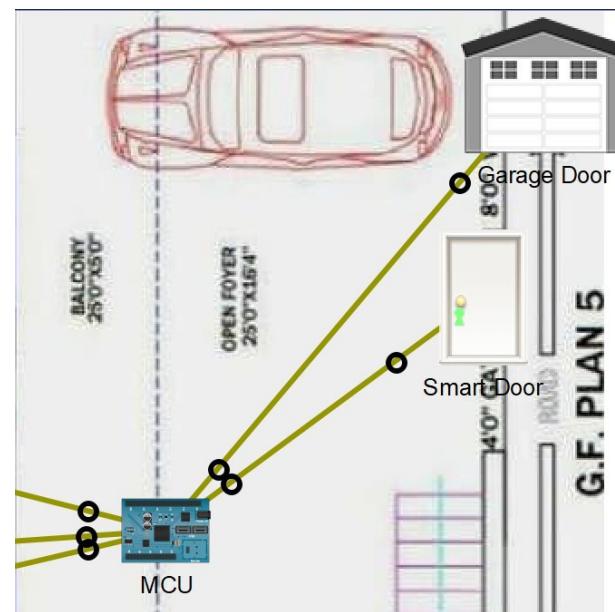


Figure 8: Smart door and garage system with MCU

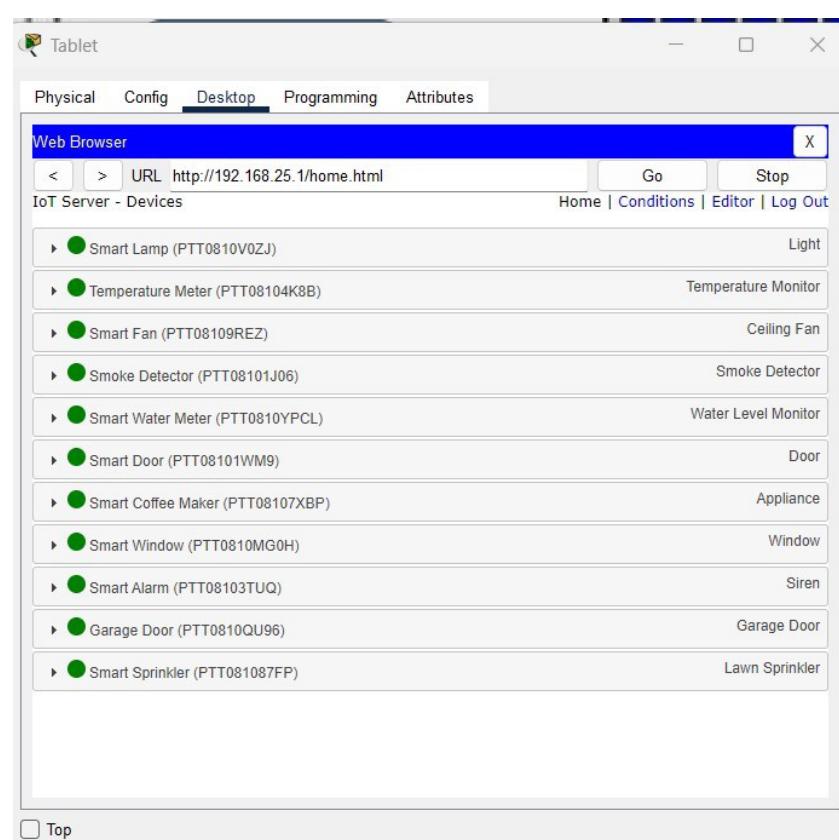


Figure 9: IoT connected things

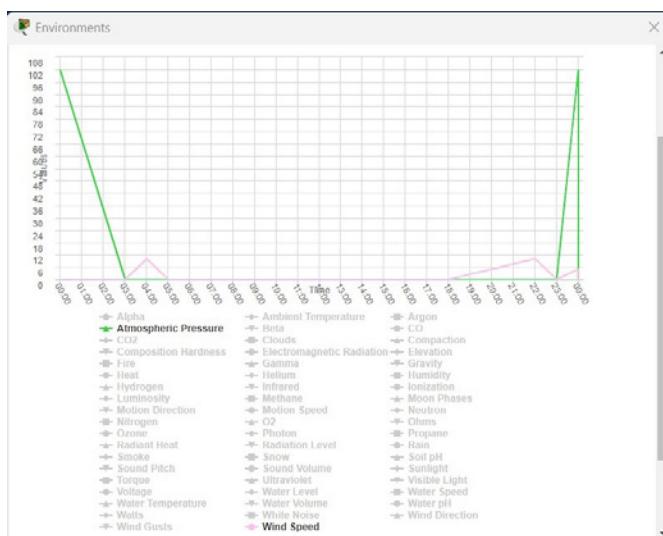


Figure 10: Simulation results of atmospheric pressure and wind speed.

CONCLUSION

The newest Cisco packet tracer version (8.2) is utilised in our article to discuss smart buildings because it has a lot of IoT devices and allows for simulation of real networks with all of its associated components, devices, and protocols. In packet tracer, the Physical Workspace feature enables topology construction and physical device placement in cities, buildings, and electrical closets. The primary component of a smart city is a smart building, which must be built up in accordance with established or forthcoming standards. According to their functions, various services are offered for the various rooms, halls, and spaces of the structure. In an IoT Web-Based smart building simulation prototype, various services are monitored and managed using webpage GUIs on smartphones, laptops, and desktop computers. It may be inferred that a variety of programming languages, including Python, JavaScript, and Blockly, may be employed when developing our product. Since devices and services may be modified, added, or removed, flexibility and scalability are two of the key characteristics of our smart building prototype. The addition of new systems allows scalability to be validated.

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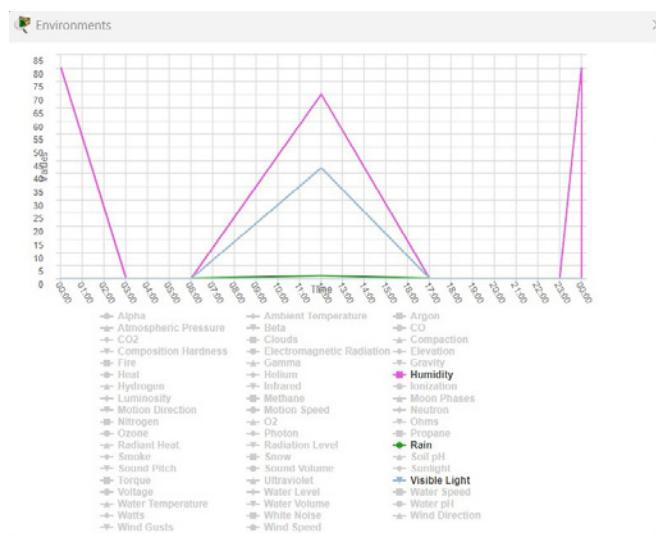


Figure 11: Simulation results of Humidity, Rain and Visible Light.

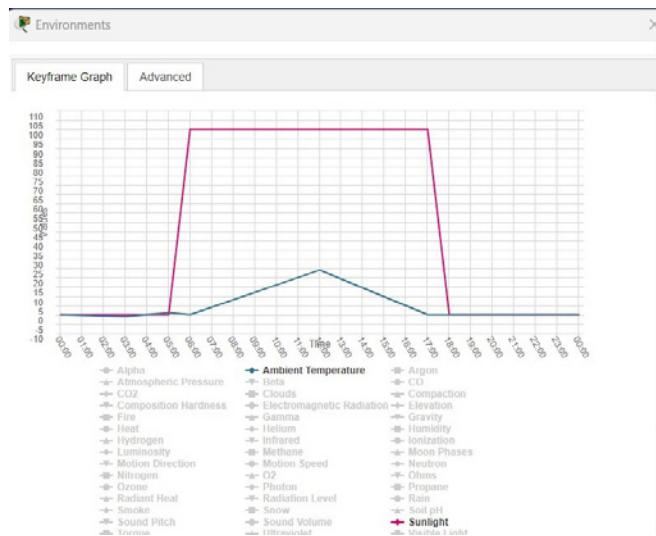


Figure 12: Simulation results of ambient temperature and sunlight.

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**PROMOTING THE USAGE OF ECO-FRIENDLY TERTIARY PACKAGING:
A MARKET RESEARCH ON THE PERCEIVED BEHAVIOR OF
FILIPINO CONSUMERS BASED ON SUSTAINABILITY FACTORS**

Aaron L. Dena¹, Lyza B. Honrado¹, Princess Mica Lin¹, and Emmanuel J. Dotong^{1*}

ABSTRACT

This paper conceptualises the impact of environment, social and economic factors with regards to the behaviour of Filipino consumers in using and buying sustainable tertiary packaging based on their demographic and behavioural profile. Plastic pollution is one of the main issues that we need to care about especially that 40% of the plastic waste came from packaging. This study employed the method of conducting online survey questionnaires through Google forms to be answered by 450 respondents. The researchers found out that using and buying sustainable tertiary packaging is more accepted by the female consumers both in Metro Manila with the age bracket of 29 years old and below. They are using and buying it very often, especially twice a week. That's why this study was used to develop and refine strategies to promote using and buying sustainable tertiary packaging as an alternative to plastic bags. We have so much power to change the world just by being careful in what we are using and buying in terms of the right packaging.

Keywords: Sustainable, Tertiary Packaging, Purchase Frequency, Packaging Preference

INTRODUCTION

Product Packaging is one of the biggest contributors of waste in the world. Each year, about one trillion plastics end up in landfills on a global scale. Due to tight competition in the market, most companies uses non-environmentally friendly materials in their consumer product packaging as they consider the economic and social benefit of it in terms of aesthetics, durability, affordability, and mass production. However, conscious of the fact that climate change is the most pressing problem on the planet and that the consumption of goods drastically increases, there is an urgent need to reduce waste in commercial products. (Zapar, 2021).

Environmental concerns are a priority for a great number of nations. In emerging countries, social media influences consumers to buy sustainable products which significantly help the consumers to have more access to information on product usage and spreads awareness on sustainable packaging. This has not only led to an increased sales of sustainable products, but it has also raised awareness of environmental issues (Nielsen, 2015).

In response, sustainable packaging was brought to the market to make it more environmentally friendly. Sustainable packaging refers to the production and or consumption of environmentally - friendly packaging that is toxic-free, hygienic, and eco-friendly that aim to reduce environmental damages cause by its production (Das, 2019). As a result, more companies have begun to demonstrate their concern for the environment by minimizing the usage of plastic and replacing it with recyclable alternatives.

Numerous research indicate that sustainable packaging has a significant effect in consumer buying decisions (Orzan, 2018; Popovic, 2019 & Rokka, 2008). Consumers nowadays are inspired to use eco-friendly packaging as more and more consumers are conscious on the impact of plastic packaging in the environment (Martins, 2019).

In order to preserve the environment in which we live, consumers are incrementally changing their lifestyles and consumption habits to promote sustainable packaging to public. However, many consumers are not motivated to buy sustainable packaging as they still not recognizing the impact of buying and using plastic packaging. In relation to this, many companies do not have a market intelligence in terms of how consumers perceived the usage of sustainable packaging to convince them to switch

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from producing plastic packaging to eco-friendly packaging. With this, the researchers aimed to conduct a market research to determine the perceived behavior of Filipino consumers in buying and using sustainability packaging by considering the three sustainability pillars.

There are a lot of studies that examine that sustainable packaging plays a vital role in the purchasing decisions of the consumers (Orzan, 2018; Popovic, 2019 & Rokka, 2008). Consumers are continuously changing their lifestyle and behaviour towards the consumption by promoting sustainable packaging in order to protect the environment that we live in. However, a lot of consumers don't have sustainable thinking. That's why they need to be motivated to make them realize that their actions have a positive effect and impact in our environment. This study reviews different literatures that make sustainable tertiary packaging preferable by the consumers due to the compliance in the three sustainability pillars. Consumers believe that sustainable packaging is a more effective way of helping the economy because this is a green product. Some of the buyers are supporting recyclables to protect the world for the future. The researchers encourage consumers to try sustainable packaging, to encourage them to believe what sustainability performance is. The researchers combine existing findings which are mainly related to sustainable packaging.

From these related literature reviews, the researchers were able to gather important information, data and ideas on the study of Factors Affects the Metro Manila Consumers in Using and Buying Sustainable Tertiary Packaging. Some of the foreign studies are more focused on packaging of a product and its uses and about the plastic waste that may have bad outcomes when using it. Therefore, the researchers conclude that there are different factors of sustainable packaging that can impact consumer buying decisions. Based on the gathered reviews, sustainable packaging is preferable for consumers due to its features, quality and designs. Consumers are now being aware of the impact of sustainable packaging on the environment as well as the harmful effect of plastic bags on the environment. The findings that the researchers found in the review related literature will support the study on evaluating the role of sustainability packaging in the consumer buying decisions in the cities of Metro Manila depending on their demographic profile, perceptions, lifestyle and behavior.

The study aims to know the role of sustainable packaging in buying decisions of the consumers. Furthermore, it objects the relationship of environment, social, economic factors, price, quality, designs and personal factors into the consumers' buying decision. The possible solution is to promote the advantages of using sustainable packaging in the consumers in order for them to accept it and to lessen the use of plastic waste since it can harm the environment and species as well.

METHODS AND MATERIALS

Research Design

This study utilized quantitative approach for the research. A descriptive – correlation was used to assess the role of sustainable packaging in consumers buying decisions with regards to environmental, social and economic factors of sustainability as input for the conducted study.

Descriptive design is used in research studies aims to provide static pictures of situations that describes a population, situation, or phenomenon of the study (Formplus, 2018). This study was descriptive in nature that involves investigation and comparison between and among the variables used. Since the study focused on the consumers buying decisions towards sustainability packaging, the quantitative descriptive method of research is the most appropriate to be used as the researchers believed that this method would provide adequate and accurate interpretation of findings.

Survey Instruments

An online survey questionnaire, through Google forms, was used as an instrument for this study. Such is deemed appropriate for compliance with national government and IATF restrictions. Researcher-made questionnaires were put together to meet the objectives of this study with 36 questions that will be answered from 3-5 minutes. In answering the survey questionnaire, the respondents will encounter different questions about their demographic, behavioral, environmental, social and economic factors of sustainability packaging.

The researchers considered gathering data from the consumers that are buying in Metro Manila. To select the sample from the population, the non-probability sampling method will be used, specifically, convenience sampling and voluntary response sampling. The researchers chose this method of sampling as it is easier to access the respondents.

Statistical Treatment

The researchers applied the following statistical treatments for the statistical data presented by the study. A descriptive statistic was used to identify the number of occurrences on the respondents' behavioral profile, age, gender and location using Frequency.

Percentage was applied in capturing the demographic and behavioral profile gathered from the whole number of respondents. Weighted mean was applied to distinguish the average value of the respondents' level of importance, frequency, likelihood and agreement in terms of the factors that affects their in using and buying sustainable tertiary packaging. Standard Deviation was applied to distinguish the dispersion among the respondents' level of importance, frequency, agreement and likelihood towards sustainable packaging. An Inferential Statistics was used to determine the significant difference using Test of Normality using Kolmogorov Smirnov, Mann Whitney and Kruskal Wallis.

Objectives and Hypotheses

The objective of this study was to determine the level of acceptability on the use of sustainable tertiary packaging among the Filipino consumers based on their demographic and behavioural profile.

The following hypotheses below were set to guide this whole study.

H01: There is no significant difference in the level of acceptability on the use of sustainable tertiary packaging among the respondents based on their demographic profile.

H02: There is no significant difference in the level of acceptability on the use of sustainable tertiary packaging among the respondents based on their behavioral profile.

RESULTS AND DISCUSSION

Table 1 shows that the greater percentage of the respondents are at the age bracket of 29 years old and below with a frequency of 285 and 63.3 percent. It also conveys that female have the greater percentage with the frequency of 258 and 57.3 percent. 68.2 percent of the total respondents are residing or consuming convenience products inside Metro Manila. However, the 31.8 percent of the total respondents are residing or consuming convenience products outside Metro Manila.

Table 1: Demographic Profile of the Respondents

DEMOGRAPHICS	VARIABLES	FREQUENCY	PERCENTAGE
Age Group	29 and below	285	63.3
	30-49	108	24.0
	50 and above	57	12.7
Sex	Male	192	42.7
	Female	258	57.3
Residence	Metro Manila	307	68.2
	Outside Metro Manila	143	31.8

Table 2: Mean Summary of Behavioral Profile

Variables	Mean	Interpretation	STD DEV
Frequency of use sustainable packaging in carrying purchased goods	3.24	Often	0.64
Frequency of buying sustainable packaging in a convenient store and shopping mall	2.85	Often	0.73

Based on table 10, consumer use and buy sustainable tertiary packaging often with the mean of 3.24 and 2.85 and standard deviation of 0.64 and 0.73.

In terms of the frequency on the consumption of sustainable tertiary packaging, consumers are likely to buy and use it very often. It means most of the respondents of the study are often using and buying sustainable tertiary packaging in their times of their visit in the convenient stores and shopping malls. Overall, they are the regular users of this kind of sustainable packaging since they are always using this in purchasing goods. (Serrano, et.al, 2017)

Table 3: Kruskal Walis - Frequency of use sustainable packaging in carrying purchased goods

Variable	p-value	Verbal Interpretation
Environment Mean	.001	Significant difference
Social Mean	.000	Significant difference
Economic Mean	.003	Significant difference
Overall Mean	.000	Significant difference

It conveys that there is a significant difference among sustainability factors and frequency of use sustainable packaging in carrying purchased goods. There are consumers that used sustainable tertiary packaging very often since it is comfortable to use, they are influenced by societal norms and accepted the regulations of their cities. Moreover, there are consumers that always used sustainable tertiary packaging every day since they are influenced to all sustainable factors. Consumers are often and always using eco bags and other sustainable tertiary packaging because of its environmental benefits, influenced by others and regulations. (Valenzuela, 2019)

Table 4: Kruskal Walis - Frequency of buying sustainable packaging in a convenient store and shopping mall

Expectation (Before)	p-value	Verbal Interpretation
Environment Mean	.000	Significant difference
Social Mean	.000	Significant difference
Economic Mean	.000	Significant difference
Overall Mean	.000	Significant difference

It shows that there is a significant difference among sustainability factors and frequency of buying sustainable packaging in carrying purchased goods. There are consumers that buy sustainable tertiary packaging very often since it is durable that can last longer, they are influenced by societal norms like social media, family and friends and willing to pay an extra amount in buying sustainable tertiary packaging. Moreover, there are consumers that are rarely buy sustainable tertiary packaging since it is reusable and most of them bring their own eco bags in carrying their purchased goods. Buying sustainable tertiary packaging is also part of the daily lives of the consumers. They usually buy it because they are stronger, can save money because you are not buying them every day, and most of the people are already buying sustainable tertiary packaging. (Kyle, 2019)

CONCLUSION AND RECOMMENDATION

To conclude, using and buying sustainable tertiary packaging is acceptable by the Filipino consumers. However, the consumers with the age of 29 years old and below are more accepted using and buying it unlike the older consumers. Metro Manila and outside Metro Manila consumers are both accepted using and buying sustainable tertiary packaging rather than plastic bags. Nevertheless, it is important to promote more of using and buying sustainable tertiary packaging in order to fully accept it by the Filipino consumers and to continue removing plastic bags in our daily lives.

The researchers recommend that Filipino consumers must consider using and buying sustainable tertiary packaging in carrying their purchased goods to lessen the use of plastic bags.

It will also encourage consumers to create their own reusable and recycled bags is also important to reduce costs and to maintain sustainability. Furthermore, they need to support this campaign to prevent the use of plastic bags since it is non-biodegradable that can last for a long time in streets, oceans and landfills. This campaign will encourage the future entrepreneur to use sustainable packaging in order to prevent the waste of the unused packaging and to prevent harming our surroundings. Moreover, the future researchers can promote sustainable packaging by creating articles and studies about this campaign to show the good impact of using and buying sustainable tertiary packaging since they have an advantage to spread information to all Filipino consumers.

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UNIVERSITY 4.0: DIGITAL TRANSFORMATION OF HIGHER EDUCATION EVOLUTION AND STAKES IN MOROCCO

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ABSTRACT

Facing the challenges of the new industrial revolution, the deep coupling between universities and industry 4.0, the integration of information and communication technologies in education, and the enhancement of the ability to serve society on the basis of internal and external synergy should become the common choice of different types of universities. The university plays an important role in development in any advanced economy. In the age of knowledge and globalisation, there are rapid technological changes that involve new disruptive processes. In this permanent challenge, it is necessary to adapt to the digital transformation, in order to be able to better respond to the needs and challenges of a constantly changing environment. It is necessary to pay attention to technological advances, in order to promote digital transformation in the new university, University 4.0. In order to face the challenges of the technonolgie development and the efficiency of universities in Morocco, it is necessary to introduce modern technologies, blockchain technology, artificial intelligence, chatbots..... into the sphere of Moroccan universities.

Keywords: Morocco, Digital transformation, ICT, digitalisation, University 4.0

INTRODUCTION

This Digital transformation is about focusing the development of universities and higher education institutions on the application of technology, as is the case in other sectors. Dewar (2017) defines University 4.0 as a university that is other-oriented, to primarily serve students, outward-looking, engaged, and connected to the surrounding productive environment, in line with Barnett's (2017) concept of the ecological university, which refers to the interconnection of the university with various ecosystems (knowledge, social institutions, people, economy, learning, culture, and natural environment).

Precisely, in order to follow a logical sequence until reaching version 4.0, Barnett describes the evolution of the university in different phases: a university 1.0, which would be the metaphysical university developed in medieval times, with a strong presence and dominance of spiritual and religious beliefs. Version 2.0 is born in post-industrial societies, more focused on the deployment of research within the university as a driver of technological progress oriented towards economic development. It would correspond to the universities created from the 15th century onwards, with teaching that was increasingly open to different approaches to thought. A few centuries later, version 3.0, which could be called an entrepreneurial university, defined by Barnett, as a university for itself, serving many different functions and communities, but above all concerned with optimising its own interest or strategy in an increasingly competitive world. This university 3.0 is also defined by Pulido, (2019) as an advanced and social university, developing in Europe in the 19th century, combining teaching and research functions, with self-governance and institutional autonomy.

Dewar, (2017) argues that University 4.0 will provide on-demand learning in multiple formats, with continuous transfers between different modalities, with more intense collaboration between universities and the productive fabric in a digitised environment. In this context, Pulido (2019) interprets University 4.0 as a university that undergoes such a disruptive change that it requires a radically new university (4.0) in organisation, technology, and education-research strategy that responds to the needs of a profoundly evolved society. Indeed, digital technologies are driving digital transformation, a new form of

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organisation and increasing and unpredictable changes, generating a wide range of new challenges. It is therefore University 4.0 that corresponds to a modern university, as a metamorphosis of previous versions in a technological environment that is advancing into the digital age and must meet the demands and commitments of a globalised society.

Due to the digital transformation, the university has ceased to be what it used to be. The university today has to follow the trends imposed by globalisation and the increasing use of technology by organisations and individuals. There is a concern about whether the university can survive the intelligent world resulting from the advances in digital technology brought about by the fourth Industrial Revolution. Is it possible to envisage the university of the future as a smart 4.0 university that has begun to emerge?

This article introduces us to the digital era, trying to show the relevance and impact it has on contemporary society. It identifies the main disruptive technologies that are shaping it and how, from these, the models and processes of organisations are being transformed, generating profound, abrupt and at the same time ephemeral changes. From this point of view, the challenges of universities in the digital age, the debate on the future of the university cannot be postponed in the face of the challenges of Industry 4.0. This transformation is seen as a necessity that must be tackled without delay, but with a critical view and taking into account the particularities of each institution. We also try to sketch some ideas on the role that higher education institutions could play in this coupling between university and industry 4.0.

THE DIGITAL TRANSFORMATION OF HIGHER EDUCATION

The economic impact of digitisation is accelerating as countries evolve in their degree of digitisation. The most digitally restrictive economies benefit less, largely because they have not yet developed an ICT ecosystem to reap the benefits of digitalisation (Cerezo, et al., 2017, Tamer, 2022). According to these authors, digital transformation is understood as a relatively new and recent phenomenon, and an organisation cannot be considered to have reached a final state of maturity in this area or to have managed to define it in its entirety. Considered as a new paradigm, digitalisation, as a new way of doing things, has a great impact on the way universities carry out their main missions and functions (Juanes and Rodríguez, 2020).

Universities must also provide students with the skills and knowledge they need for a very different future. In this new educational landscape, the digital transformation of higher education is essential (Tamer, 2022).

Of this conception, very few intend to create 4.0 university models, which suggests that they continue to rely on the current university model in terms of organisational form. It is therefore important to understand that the process of digital transformation implicitly involves a change in the organisational model. Digital transformation represents new opportunities for business strategies, integrating technology, streamlining processes, preparing teams to work and collaborate with digital tools and establishing business logic or processes with the digital economy, thus achieving better performance.

According to Garcia, (2018), it can then be deduced that, digital transformation allows institutions to adapt a socially responsible and ethical business model, allowing them to apply a scalable development model, without forgetting that they influence to reduce environmental impact by streamlining processes and reducing consumption of non-renewable materials.

According to Gaibor, (2020), Digitalisation is the great driver of wealth creation, an important point in this analysis is that digital transformation brings greater productivity, agility, quality, innovation, cost efficiency, as well as many other aspects, both for digital and offline businesses, where the key is to understand how digital techniques and tools can impact and grow a traditional business or institution, but in a joint and strategic way. Digital transformation is not just a technological problem that is solved by an injection of technology.

According to Barro (2018), the digitalisation of a university requires first and foremost an investment effort in ICT infrastructure and resources. However, to make it a 4.0 university, the relationship between teachers and new students (millennials, generation Z) also needs to be reformulated, where traditional channels are no longer a priority but complementary. As explained in the article, the simple use of technology is not enough to take the step towards digital transformation. According to Gaibor, (2020), it is necessary to raise awareness and train the whole team, so that they can make the most of digital tools in their daily work. Looking at the issue from different angles, it is clear that the current rigid educational structures need to be changed, barriers need to be broken down and technology needs to be used to provide educational content at all times. We need to promote a more fluid and flexible education to better adapt to different needs, as the current rigidity of university structures does not, in some cases, promote adequate education (Gaibor, 2020).

FROM UNIVERSITY 1.0 TO UNIVERSITY 4.0

The university has played, and must continue to play with greater intensity, an important role in the development of innovation in any advanced economy. After all, it is the natural space in which knowledge is developed and promoted and, as such, must be

transferred to society. Globalisation, new processes and, consequently, new working methods and rapid technological changes define the changing environment in which the University operates. In this permanent challenge, it is necessary to adapt to the new times and, in particular, to strengthen the role of resilience and adaptation to change in the university context. It is no longer just about the agility with which the organisation evolves in the present moment, but the ability to anticipate the future era, for which we must always be vigilant and constantly reflect.

In this context, this vigilance implies the vision of a digital world, to which the University has already opened its doors but where there is still a long way to go. We are talking about a disruptive era that is changing the world around us or, in global terms, the 4.0 era, which applied to any field denotes a commitment to the digital world, to the digitalisation of processes or to what is known as digital transformation.

The regular use of the internet by millions of people around the world has led to the development of the internet of things, which is a growing advance in connecting digital devices and objects to each other, interacting in such a way that there are no temporal or spatial boundaries. We are witnessing a new industrial revolution that affects the intellectual-intensive jobs of the 21st century, whereas in previous industrial revolutions it was mainly manual activities that were affected. This is the fourth Industrial Revolution, that of the fusion of technologies, where the combination of advances in the development of robotics and artificial intelligence, the collection and processing of massive information or Big Data have and will have an impact on the economy and therefore on the qualification needs of jobs in all productive sectors. The digital transformation implies focusing the development of the university, higher education institutions, on the application of technology, as is happening in other sectors (Tamer, 2022).

Dewar, (2017), defines the 4.0 university as a university that is other-oriented, primarily to serve students, outward-looking, engaged and connected with the surrounding productive environment, which refers to the interconnection of the university with various ecosystems.

Specifically, to fit into a logical sequence until reaching version 4.0, Barnett, (2014), describes the evolution of the university in different phases: a university 1.0 which would be the university developed in the medieval period (the main European universities date back to the 11th century), with a strong presence and dominance of spiritual and religious beliefs and which evolved towards liberal arts type education. Version 2.0 appears in the sphere of post-industrial societies, more focused on the deployment of research within the university as a driver of technological progress oriented towards economic development. It would correspond to the universities created from the 15th century onwards, with teaching that was increasingly open to different approaches to thought.

A few centuries later, version 3.0, which could be described as the entrepreneurial university, defined by Barnett, (2014), as a university for its own sake, serving many diverse functions and communities, but above all concerned with optimising its own interest or strategy in an increasingly competitive world. This University 3.0 is also defined by Pulido, (2019) as an advanced and social university, developing in Europe in the 19th century, combining the teaching function with the research function, with self-governance and institutional autonomy.

Dewar, (2017), argues that University 4.0 will provide on-demand learning in multiple formats, with continuous transfer between different modalities, with more intense collaboration between universities and the productive fabric in a digitised environment. In this context, Pulido, (2019), interprets University 4.0 as a university that undergoes such a disruptive change that it requires a radically new university (4.0) in organisation, technology and education-research strategy that responds to the needs of a profoundly evolved society. Indeed, digital technologies are leading to digital transformation, a new form of organisation and increasing and unpredictable changes, generating a wide range of new challenges. It is therefore University 4.0 that corresponds to a modern university, as a metamorphosis of previous versions in a technological environment that is progressing in the digital age and that must respond to the demands and commitments of a globalised society.

According to Barth, Rieckmann, (2016), the major changes we are seeing with information and communication technologies make traditional approaches to classical pedagogy and its conventional credentials obsolete. Among the soft skills that will soon be indispensable are higher-order cognitive thinking, innovative adaptive thinking, cognitive load management, multiple literacy, complex situation solving, social skills, elastic skills and cross-curricular skills to accomplish tasks of a changing nature. Barth, Rieckmann, (2016), add that digital skills will also lead to a diversity of synaptic and social connections that will become increasingly flexible and adaptive. According to Deward, (2017), the typology of universities provided by Professor Emeritus Barnett, (2014), from the Institute of Education at the University of London presents us with the following classification:

University 1.0: This would be the university, in the service of God, which appeared in medieval times. The first stages of this

university were structured around specialised communities that eventually evolved into the tradition of liberal arts education (Le Goff, 2008).

University 2.0: could be seen as the research university that has emerged in post-industrial societies, where universities have become the focal point of research-led technological progress. The great post-war expansion is clearly focused on research for economic development. Based on the massification of education, with the teacher as the main provider of knowledge and the student as a passive receiver who absorbs the content (Shchedrovitskii, 2011).

University 3.0: is described as the entrepreneurial university, functioning, in Barnett's, (2014), terms, as a university 'for itself', serving many diverse functions and communities, but primarily concerned with maximising its own self-interest. Based on the integration of computers and the internet into teaching and learning, thereby increasing access and equity (Li, 2020).

University 4.0: refers to the green, outward-looking university, deeply connected to industry and the communities around it. It is committed to meeting the needs of its students. It relies on high-speed internet, mobile devices, technology platforms and digital applications, which facilitate personalised learning anytime, anywhere and change the transmission roles of teachers (Efimov, and Lapteva, 2017).

University 4.0 is an apt description of how universities around the world must respond to the new economy and associated trends such as digital disruption and changing labour markets. If universities want to remain relevant, they must undertake revolutionary changes at the organisational, operational, structural, pedagogical, socio-cultural and cognitive levels today (Aladyshkin, Odinokaya, Safonova, & Kalmykova, 2020). University 4.0 is fully in line with the fourth Industrial Revolution. We are talking about new platforms that will use artificial intelligence algorithms in combination with the Internet of Things (IoT) to personalise student learning. This will force traditional professors to take on new teaching roles that transcend the delivery of declarative content (Aladyshkin, Odinokaya, Safonova, & Kalmykova, 2020).

This revolution is centred on the development of new information and communication technologies in education incorporating robotics, automated systems, blockchain, fintech, bots, deep learning, 5G technology and cybersecurity systems. All of these will impact our daily lives, social relationships, work and learning experiences for life.

Today's student is not just limited by a teacher-led educational model, but draws learning from a variety of information sources at a personalised pace. They do not only expect academic excellence, but also desire personalised excellence by expanding their horizons of possibilities. We cannot be satisfied with a university that adapts to new circumstances and tries to integrate emerging technologies (Kazimirov, 2018). We need a radical change and this requires: ending the problems of massification, adopting the procedures for professional promotion, softening the relationship with the social environment, strengthening student engagement, implementing realistic strategic plans (Antonio Pulido, 2019).

For James, (2019), one of the contemporary methodologies is accelerated distance learning, i.e. the idea that students learn theoretical knowledge at a distance through digital means, while ensuring that practical skills are acquired in physical environments. It is a flexible form of learning that requires responsibility and good time management to develop skills based on an increasing economy of autonomy.

At this point, it is a matter of building and investing more in a robust educational ecosystem, not to replace or displace it, but as a form of flexibility and adaptability. According to Aladyshkin, et al, (2020), What is required of education today is not a solid classical education, not because of the modality, but because of the social diversity of the modern world. Higher education institutions are moving towards a more personalised form of learning. Aladyshkin, et al, (2020), add that by using data and tracking student performance, universities will be able to identify students who are struggling and provide them with learning strategies optimised to meet their needs. Data analysis will be used to treat each student, understanding that each student's learning needs and desired outcomes will be different.

According to Villalobos, and Pedroza (2019), the central idea supported in this article is that there is no element of the university that is not undergoing profound changes with the use of new communication and learning technologies. University life is being renewed with its productions, processes and tasks; training, teaching, learning, research, curriculum, etc., are all being changed by the integration of information and communication technologies for teaching. Thus, various documents from universities in different parts of the world have been worked on and creativity has been used to give shape to University 4.0 (Villalobos, and Pedroza 2019).

The virtuous circle of innovation in the university in the transition to the future. The debate on the future of the university cannot be postponed in the face of the challenges of 4th industrial revolution, where developments in technology, physics and biology converge. The archetypal monolithic university, composed of disciplinary islands focused on essentially theoretical teaching,

with atomised contents disconnected from real problems and with informational pedagogical practices that favour repetitive and contemplative learning, which is also of little impact in making contributions to the future (Gueye, and Exposito, 2020). According to Gueye, and Exposito, (2020), the university in the knowledge society is obliged to reinvent itself because otherwise, with its traditional model, it will be unable to meet the needs and challenges of an increasingly dynamic world. Recent experience shows a historical truth: universities that work hand in hand with technological advances are best placed. By investing in research and development, they are creating innovations and acquiring a leading role in the current new technological configuration.

According to Madaliyeva, et al., (2020), it is the faculty that enables the university to develop, to participate in opening up and solving the challenges present in the new fields of knowledge. In order to reinvent itself, the university must implement and encourage the integration of new information and communication technologies for teaching in teaching and learning, always on the basis of scientific research, while promoting new forms of organisation, new methods.

Indeed, according to Lapteva, and Efimov, (2016), the trend of university education in Industry 4.0 is moving towards the innovative research-based university. Lapteva, and Efimov, (2016), indicated that the innovative university is the one that makes research its main development axis. On the one hand, new knowledge is provided and, on the other hand, the learning and teaching system is redefined. The result is a university model characterised by dynamic feedback between these two aspects. The best ranked universities are those that encourage this kind of flow (Lapteva, and Efimov, 2016).

According to, Madaliyeva, et al, (2020), with the upgrading of traditional industries and the advent of Industry 4.0, the economic structure and industrial mode have undergone unprecedented changes, which means that universities have to adapt to the demand and provide responses. Madaliyeva, et al, (2020), add that the construction and exploration of new information and communication technologies for education, on the one hand, actively adapt to the changing demand for profiles in the context of industrial production and technological innovation, and on the other hand, promote institutional reform and internal development of universities.

Today, the university is changing, there is no country that does not rethink the change of the model and function of its university. We can even say that the country that resists change is endangering the existence of this thousand-year-old institution (Madaliyeva, et al., 2020). Various factors require a change in the university, the most representative being technological development, what society expects from the university and that it responds to economic, social and political development, which implies an internal renovation of university processes (Lapteva, and Efimov, 2016).

The university is challenged because technological advances are not always born within it, the dominant dynamic is that of an academic science and technology, that is, it is formed only to reproduce, not to generate new advances (Gueye, and Exposito, 2020). With the fourth Industrial Revolution, the university has to deal in a different way with its dynamics in the training of professions, moving towards teaching and intelligent learning, devoting itself more to scientific research and technological development, with this, its vision is transformed and its model is mobilised towards open and flexible forms. Not all universities are taking the shift in the same way (Lapteva, and Efimov, 2016).

The best-placed universities are at the forefront of change, while others are slow and lagging behind. The best-placed universities are those dedicated to research with models linked to the economic and social development of the country (Gueye, and Exposito, 2020). They generate technological advances and produce economic resources. This is in contrast to poorly positioned universities, which maintain the stagnant practices of the past, dedicated to training based more on academic discipline than on the generation of new knowledge and technical and technological resources. There is an uneven development of the university in the world that coincides with the economic, political and social situation of each country (Gueye, and Exposito, 2020). Therefore, the transformation of the university represents the transformation of the country or vice versa, as there is a reciprocal relationship between the university and national development.

In general, the transformations of the university in the world have an accelerated dynamic, which requires addressing the dimensions that need to change in the direction of progress. Villalobos, and Flores (2019), have identified some axes of university transformation:

- Diversification of university modalities, nowadays pluriversity is a fact, they coexist with face-to-face mobility, alternative and complementary distance, open, digital and mixed modalities that are increasingly positioned.
- With the development and application of information and communication technologies (ICT), curricular modalities are being transformed into flexible, open, networked, integral and individualised itineraries, combined with platforms such as Coursera, Udacyt, Scolartic, Mooc courses and standard university credits.

- With artificial intelligence, pedagogical relationships are being transformed, as there is now a relationship between teachers, robots and students and learning is an unprecedented experience, generating a ramification of learning types: adaptive (Big Data with Learning Analytics), 3D, gamified, flipped learning, adaptive, with virtual reality, multimodal, storytelling-based learning, and so on.
- There is a new academic ecosystem of training, learning and management based on the technology trend of 5G (fifth-generation 5G mobile networks), digital assistants, robots, augmented reality, global educational platforms, communities of practice and the use of blockchain (Schwab, 2017).
- Rethinking the training of professions by strengthening the themes of humanities and human development tending towards the connection and expression of smart, sustainable and coexisting cities.

The building blocks of university transformation are many, the university 4.0 model is super-connected in an interactive environment between humans and new technological species creating a new university ecosystem of teaching co-existence for the continuous development of learning for disruption (Giesenbauer, det Müller-Christ, 2020).

According to Colombo, et al, (2020), The use of hard technological applications derived from intelligent computing allows for the superconnection of knowledge at previously unsuspected levels, artificial intelligence stands out, starting from the use of simple artefacts to the use of machine learning, we observe how in universities the use of apps, robots and virtual intelligence increases its application. At the same time, the forms of learning with soft technologies are multiplying, they are abandoning their traditional form of being secondary in the training processes, they are now the central character, we are in the era of augmented learning acquired by our human skills and simultaneously powered by nanotechnological artificial bodies, for this reason we speak today of a trend towards nano-credentials and no longer of the old university references of diplomas and degrees (Colombo, et al., 2020).

Flores, (2018), note that we are living in a time of crisis that requires the university to move to where the people are, not that the people move to the physical address of the university. Flores, (2018), adds if administrators, faculty and non-teaching staff resist the demands of the millennial society, they will begin to feel the effects of the erosion caused by the proliferation of emerging educational agencies that will launch their attractive academic offerings.

These conventional institutions will not only face the challenge of placement rates or employability of their graduates, but will also have to deal with the socio-cultural challenges and emerging crises of our time (Colombo, et al., 2020). The educational community will have to work together to find solutions to crises on and off campus. According to Colombo, et al. (2020), before long we will see the academic offerings of departments lose their footprint, as social demand will dictate what people want to learn, how to learn it, when, where and with what resources.

Duc, et al., (2018), suggest that universities should partner with the local community, industry and society at large to co-design and co-implement a stronger higher education system. Duc, et al, (2018), add that their operational and pedagogical business model is incompatible with the times we live in. We are now living under the threat of a micro-organism that has crippled the entire world. The initial solution was to save the semester with any video-conferencing technology that would be used for remote academic continuity. As the summer holidays approach, higher education institutions will have to prepare for longer periods of remote administrative support and online teaching. What we know as the physical space for working and learning will never be the same again (Duc, et al., 2018).

According to Giesenbauer, det Müller-Christ, (2020), University 4.0 is not the one that improves on what other universities do, but the one that dares to do things differently. It is the one that looks at the segments that other institutions are unable to look at. An agile educational organisation is one that looks at different latitudes, looking for new learning niches that society needs. It is very clear that no single technology will replace administrators, teachers and non-teaching staff. Those who will replace them will be the expert users of cyber-human interfaces (Gueye, and Exposito, 2020). They will take them out of their fragile safety and comfort zones. Technology is not just about the digital gadgets we acquire in our workplaces and homes. Real technology is about effectively connecting our brains to devices to create new solutions to the emerging crises of our time (Gueye, and Exposito, 2020).

CREATIVE UNIVERSITY

After the text edit has been completed, the paper is ready for the template. Duplicate the template file by using the Save As command, and use the naming convention prescribed by your conference for the name of your paper. In this newly created file, highlight all of the contents and import your prepared text file. You are now ready to style your paper; use the scroll down window on the left of the MS Word Formatting toolbar.

We are now living in a unique time where the digital revolution is changing the way most people live on the planet. Central to this change is the development of digital universities. It has already become a driver of the economy. Digitalisation improves the conditions for doing business, increases the level of education and computer literacy of the population and, in general, the level of competitiveness of the nation. Digital technology has such a profound effect on the competitiveness of countries that nations around the world are looking to modernize the industry. The changes brought about by technology, which are redesigning production processes, are helping to increase the efficiency and quality of services. As the experience of the rest of the world shows, digital technologies make a tangible contribution to GDP growth. This is why some countries have adopted entire national programs for universities. The whole world is now embracing digital transformation.

To date, Morocco country is implementing national digitalisation programs. A first approximation of the state of innovation in the world can be found in the recent report Global Innovation Index 2021 (GII), indeed Morocco occupies the 77th place with a score of 29.3 (Table 1).

Table 1: 2021 Global Innovation Index Ranking

GII RANG	ECONOMY	SCORE	GROUP RANK	REGION RANK
76	OMAN	29.4	47	11
77	MOROCCO	29.3	8	12
78	BAHRAIN	28.8	48	13

The ranking is dominated by Switzerland, Sweden, the United States, the United Kingdom, and South Korea in these top five positions (Table 2). As noted, the performance in investment and digital transformation in Moroccan universities, there is a deficit of innovation at the national level. Moreover, change or improvement towards advanced positions is difficult and slow for a country such as Morocco. It is also interesting to note the reference to the most important regional science and technology clusters in the world, with the understanding that innovation activities tend to be geographically concentrated. In this regard, the United States remains the country with the largest number of innovation clusters (26).

Table 2: Classement de l'indice mondial de l'innovation 2021

GII RANG	ECONOMY	SCORE	GROUP RANK	REGION RANK
1	SWITZERLAND	65.5	1	1
2	SWEDEN	63.1	2	2
3	UNITED STATES OF AMERICA	61.3	3	1

Another source for assessing which countries have the most innovative universities is the Reuters ranking: The World's most innovative universities, which identifies and ranks the educational institutions around the world with the best results in innovation, understood as the best performance in advancing science, inventing new technologies and stimulating new markets and industries. In its latest edition, referring to 2021, Stanford University in the United States leads the ranking of leading universities in scientific and technological innovation, Stanford maintains its top spot year after year because it produces a steady stream of innovations that are cited by other researchers in academia and private industry. This type of influence is a key measure in the ranking of the world's most innovative universities, which was compiled in partnership with Clarivate Analytics and is based on proprietary data and analytics, including patent filings and research paper citations. Followed by Massachusetts Institute of Technology (MIT), and Harvard, all of which have held their positions for seven consecutive years since Reuters began producing the rankings. In fact, no Moroccan university has made it into the top 70 universities, so these results show that Morocco continues to have a low R&D investment effort compared to other economies of similar size, and this circumstance conditions any effective progress. Barnett, (2017) points out that universities are not living up to their potential and responsibilities in a constantly changing and challenging environment. The truth is that in the age of knowledge and globalization, the university must constantly reinvent itself from becoming an obsolete institution, so that it can better respond to the needs and challenges of a changing world. This reinvention implies paying attention to scientific and technological advances, developing them, integrating them and being more active in the innovation strategy.

THEORETICAL AND MANAGERIAL IMPLICATIONS:

From our study we can recommend Moroccan universities to consider adopting the following practices:

as a first link, blockchain which is gradually being implemented not only in all areas of business, but also in higher education, as the interaction between business and science contributes enormously to the growth of innovative products and services. In higher education, the demand for innovation, the possibilities offered by digital technology, are very relevant today. Their necessity is associated with objective processes such as the volume of information that is increasing at an enormous rate, and the capacity of students to absorb it (Vasilieva, 2017). In Morocco, the transition to digital media is progressing; especially in higher education institutions which are increasingly moving away from paper-based media. Indeed, the collection of information on paper creates an additional workload also for administrative staff and allows for changes in documents; reporting forms on the results obtained in the different educational institutions may not match, which reduces the efficiency of the staff; the lack of a comprehensive database on graduates with specific skills makes it difficult for employers to find the right specialists; the lack of an open database on the employment of graduates and their transfer to other jobs does not allow higher education institutions. In order to solve all these problems and increase the efficiency of universities in Morocco, it is necessary to introduce modern technologies, blockchain technology, into the sphere of universities (Tamer, 20).

Secondly, the technologies of augmented reality and virtual reality which constitute fundamentally new means and methods of interaction between teachers and students, which guarantee the effective realization of pedagogical activities in the sphere of higher education. The analysis carried out allows us to conclude that the application of innovative technologies in the educational process contributes not only to the progress of students, but also to their interest in the learning process. In Morocco, the application of augmented reality and virtual reality technologies in the student learning process will, on the one hand, facilitate the task of the teaching staff and, on the other hand, will significantly help students to master knowledge, form their skills and abilities and, overall, will have a positive effect on the training of graduates (Tamer, 2019).

Thirdly, chatbots in higher education, which are conversational assistants or better known as bot or chatbot or chatterbot, which can be defined as a virtual assistant, are a set of computer programs that possess the ability to maintain a conversation with a human being through natural language. Similarly, a conversational agent can be understood as an automatic system capable of emulating a human being in a dialogue with another person, with the aim that the system provides certain information or performs a certain task. The objective of chatbots in higher education is always to achieve interaction based on models similar to those used by humans, which is achieved through dialogues, as they are programmed to have the capacity to analyse the environment and propose solutions to problems, interpreting emotions and contributing to the teaching-learning process to the maximum extent possible.

fourthly, artificial intelligence, which is defined as a technology whose value on the market is incalculable, both in the present and in the future, but we should not only refer to the monetary value, but also analyse the value it has for the optimisation of non-commercial processes, as in the higher education sector, Artificial intelligence to be a turning point in the changes of traditional higher education paradigms, although the pedagogical modalities at all levels of education systems are being adapted, given the current technological tools, virtual teaching modalities are becoming more common in the education policies of developed countries. Finally, artificial intelligence can optimise the use of these valuable resources, as one of the main problems today is the under-use of technological tools or their use in isolation and out of context.

CONCLUSION

The landscape of contemporary education is diverse. Higher education systems are now developing as institutionally complex structures that align learning with the organisations of different professional spheres of society and digital transformation (Aladyshkin, et al., 2020; Karpov, 2013). Socially The most important and economically significant element of this structure is the higher education sector. Its institutional base is composed of scientific institutions, high-tech companies, innovative enterprises, industrial consortia, innovative growth institutes giving rise to University 4.0 (Aladyshkin, et al., 2020). Ecosystems become the place where favourable conditions for the efficient transfer of technologies and scientific and technical innovations are created. University 4.0 becomes the basis of global competitiveness of national economies, and its ecosystem forms new fast-growing industries, promising technological markets, economically advanced administrative-territorial spaces. enfin, sur la base des recommandations de notre recherche, les universités au maroc peuvent promouvoir un apprentissage actif, constructif et réel, tout en provoquant un processus d'innovation.

Finally, based on the recommendations of our research, universities in Morocco can promote active, constructive and real

learning, while provoking a process of innovation.

This requires the involvement of all stakeholders in the process of changing the design of the higher education process in Morocco, which was based on the practical application of the content covered in the subject and on the integration and didactic use of digital technologies.

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MULTIPLE-DRUG RESISTANT SHIGA TOXIN-PRODUCING *E. COLI* IN RAW MILK OF DAIRY BOVINE IN KHYBER PAKHTUNKHWA, PAKISTAN

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ABSTRACT

Shiga-toxin-producing *Escherichia coli* (STEC) also known as verocytotoxin-producing *E. coli* (VTEC), strains are zoonotic pathogens and can lead to severe human diseases, such as diarrhoea, haemolytic uremic syndrome, and hemorrhagic colitis. The most recognized serotype of this class is *E. coli* O157:H7. The bacteria's main reservoir is ruminants, and contaminated raw milk is the most important source of illness. STEC harbours drug-resistant genes in dairy bovine raw milk samples received from street vendors, milk shops and dairy farms in Khyber Pakhtunkhwa, Pakistan. The current study investigated the presence of various STEC pathotypes in raw milk of dairy bovine. Of 800 milk samples, 321(40.5%) were positive for *E. coli*. Further investigation of *E. coli* through multiplex PCR for the presence of four virulence genes, i.e., stx1, stx2, eae, ehxA revealed that 40 (12.46%) out of 321 positive samples belonged to STEC various serotypes and positive virulence genes. STEC isolates were evaluated for susceptibility to 12 antibiotics, and three or more antimicrobial drugs were found resistant. The highest percentages of resistance were detected for ceftriaxone (72%), amoxicillin (68%), and penicillin (59%), followed by Augmentin (59%) and the highest susceptibility was found for Norfloxacin (54%) followed by Enrofloxacin (50%), and florifincol (40%). We phenotypically observed the assays that 1.37% of STEC isolates are produced (ESBL) extended spectrum beta Lactamase and contained the bla Ctxm gene. These results highlight the potential threat to public health and necessitate adopting appropriate control measures to minimize the threat.

Keywords: Raw milk, Shiga toxin-producing *Escherichia coli*, ESBL, Khyber Pakhtunkhwa.

INTRODUCTION

Shiga toxin-producing *Escherichia coli* (STEC), also known as verotoxin-producing *E. coli* (VTEC) strains, are essential food-borne pathogens using unhygienic milk and meat and dairy products of the animal origin. This *E. coli* STEC is an emerging contagious disease in humans, diarrhoea, dysentery, severe enteritis and Haemolytic urinary Syndrome (HUS) (Paton and Paton, 1998). Bacterial Toxins production is the significant activity promoting the toxigenic virulence factors of these pathotypes. STEC Strains are concerned in the most critical food-borne outbreaks belonging to two distinct serogroups, that is, *E. coli* O157 is zoonotic strain and non-O157 groups. Although other strains, i-e., O145, O111, O103, and O26 of non-O157 *E. coli* serotypes, are also occupied in fatal infection, *E. coli* O157 is the maximum recorded sero-pathotypes related to HC and HUS. However (Karch, Tarr. and Bielaszewska, 2005) Primary usual cache of STEC pathotypes are ruminants and the infectious pathogen arrives in the food chain and espacially in dairy milk through contamination dung from the cow (Fink, et al., 2013, Nataro and Kaper, 1998). Different pathotypes of *E. coli* strains (STEC, EIEC, EPEC, DAEC, ETEC, and EAEC) are defined by the nature of the sickness induced by the strain: Shiga toxin-producing/enterohemorrhagic (EHEC/STEC), enteroaggregative (EAEC), Shiga/enteroinvasive (EIEC), enterotoxigenic (ETEC), and diffusely adherent (DAEC) (Badri et al., 2017). *E. coli* serotype O157:H7 was discovered in 1982 (Riley et al., 1983). STEC strains contaminate food primarily

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through unpasteurized raw milk from animals and various dairy products. The food-borne disease also arises frequently in developing countries, including Pakistan, owing to prevailing unhygienic food handling practices, poor food safety laws, weak regulatory control system, inadequate financial resources to spend on safer apparatus and food handlers' lack of education and processors (Haileselassie et al., 2013).

Antibiotics are primarily used in livestock production, which built the rout for the appearance of a different hazard in the dairy value chain system and has developed drug resistance. In addition, renowned data expressed that (STEC) strains identified from raw milk and dairy related products are resistant to various types of antibiotics (Dehkordi, et al., 2014). The frequency of pathogenic strains of multi-drug resistant (MDR) and extended-spectrum β -lactamase (ESBL)-producing *E.coli* is quickly increasing, becoming a global issue (Ali, et al., 2016). The appearance of ESBL-producing strains has limited the therapeutic options and its importance in the last years has revealed a more complex situation. One of the key mechanisms of antimicrobial resistance of *Enterobacteriaceae* groups is the production of Extended Spectrum b-Lactamase (ESBL) enzymes. The ESBL enzymes broadly reported from *E. coli* go to class A of Ambler's categorization and include TEM, SHV, and CTX-M (Jacoby and Munoz-Price, 2005). The involvement of STEC with ESBL genes was also archive standard (Valat, et al., 2012).

STEC infection could be spread via uncooked raw milk and other raw dairy products. Raw Milk produced by dairy farmers and vendors is collected and stored as untreated whole milk. The unpasteurized milk and others dairy products are offered to consumers for complete sale and retail and whole sale marketing. The mixing, pooling, and homogenising of raw milk is a routine common practice and the mixing of raw milk increases the risk factors of cross-contamination. ESBL are enzymes produced by *E.coli* that mediate resistances to Extended-spectrum cephalosporin's and aztreonan. Studies to determine the proportion and antimicrobial susceptibility of *E. coli* in the feces and meat of sheep, goats and cattle have been conducted in several cities in Pakistan, e.g., Islamabad, Lahore, Peshawar (Mohammadi, et al., 2013; Salah, et al., 2009). However, single research was conducted to detect the various shiga toxin-producing *E. coli* strains. Therefore, the current study was designed to determine the prevalence of Shiga toxin-producing *E. coli* serotypes recovered from raw milk sampled collected from various milk selling points, street vendors and dairy farms. To some extent, an attempt was also made to characterize the antibiotic resistance and ESBL production pattern.

MATERIALS AND METHODS

Sample collection, isolation of *E. coli*

The sampling period was from June 2020 to January 2021. Raw milk samples (n=800) were collected from milk shops, dairy farms, milk collection centers' and street vendors of various locations on the base of the dairy animal population of the province. The samples were placed in the icebox and transported immediately to the laboratory for bacteriological analysis.

Bacterial Culture

The raw milk samples were 10ml mixed with buffered peptone water (BPW) (90ml) broth, which was modified with 0.1 mg/ml Ceftrixime and incubated at 37 °C for 24 hr of biomass enriched. After 24 hr, the culture growth and turbidity were checked. A loopful from buffered peptone water broth was streaked on MacConkey agar and incubated for 24 hr at 37 °C growth appeared in pink colonies. The single pinkish colonies were chosen for selective isolation and identification by further streaking on the media (Eosin methylene blue agar, EMB) (Oxide, England) plates and incubated for 24 hr at 37 °C. *E. coli* growth appeared and a "metallic sheen" on EMB agar. Purified assumed as an *E. coli* colonies for further morphological study by method of Gram staining and biochemical classification with tests as Indol, Catalase, Oxidase, methyl red, vogues prosker, Citrate utilization, and nitrate reduction. All *E. coli* positive isolates of were further sub-cultured on sorbitole MacConkey agar to identify the STEC serotype, which does not ferment sorbitole and produced colorless or pale colonies be STEC. While pinkish-coloured, colonies were assumed to be non-STEC. Non-sorbitole fermenter isolates were subjected to a latex agglutination test for further confirmation. STEC O157:H7 have preserved a mixture of glycerol in nutrient broth and stored at -20C.

Latex Agglutination Test for Confirmation of STEC

All the STEC isolates non-sorbitole fermenting from sorbitole MacConkey agar were confirmed by Rapid Latex agglutination test containing latex suspension particles specific for *E. coli* O157 and isolated culture as antigen; these were mixed and spread out on the card. The result based on the agglutination of the test template within 1 minute was measured as a positive result as described by the manufacturer (Pro-lex, UK, Jufare., 2018)

Antibiotic susceptibility test of STEC

The antimicrobial susceptibility test of STEC isolates was performed on Mueller Hinton (MHI) agar media was used (Oxoid,

Ltd., Hampshire, England) according to the Kirby-Bauer disc diffusion method and followed the protocol of Clinical Laboratory Standard Institute guidelines (Melvin, P. Weinstein, et al., 2020). The inoculums of isolated *E. coli* were prepared using normal saline and turbidity compared with 0.05 McFarland standard and inoculums were spread on the Mueller Hinton agar plate and waited for a few minutes to apply the antibiotics discs to maintain the sterility. The different antibiotics disks were applied on the plates in the standard quantities and with specific zone diameter breakpoints for identifying into three groups, sensitive, intermediate and resistant isolates. Antibiotics of different classes' penicillin p- 10 Amoxicillin AMc, Augmentin Ag, Gentamicin CN-10, Streptomycin STEM, Oxytetracycline TET, Sulphamethoxazole, Sxt, Ceftriaxone Ctx, Norfloxacin Nrf, Enrofloxacin ENR, Florefenical, FRN, Cefotaxime & Clavulnic acid CtxCL.

Phenotypic Identification of ESBL

The *E. coli* was confirmed phenotypically as a ESBL producer using Kirby-Bauer disc diffusion testing method, both cefotaxime 30mg and ceftazidime disks 30mg with and without clavulanate 10mg as per the Clinical Laboratory Standard Institute method by Patel, et al., 2017). A standard parameter of difference of >5mm between the zone diameters of each disk and their clavulanate disk is calculated to phenotypically confirm the ESBL production by the *E. coli* isolates under the study.

DNA Extraction

E. coli DNA was extracted using a standardized simple boiling method as described by (Irshad et al., 2012). Briefly, 1.5 ml of brain heart infusion broth (BHI media) inoculated and saturated with biochemically confirmed *E. coli* isolates was transferred to Eppendorf tubes (1.5 ml) and centrifuged 1300rpm for 5 min. The supernatant was discarded and the pellet was re-suspended in 200 µl nuclease free water and then heated on a hot plate at 98°C* for 10 minutes followed by chilled or ice treatment; each tube was again centrifuged at 13000rpm eight minutes. The supernatant used as DNA template purity and concentration were checked through Nanodrop and gel electrophoreses for DNA confirmation and stored at -20°C for use as DNA template in PCR.

Detection of Shiga toxin-producing and ESBL virulence genes through polymerase chain reaction (PCR)

Amplification of *E. coli* DNA was performed in a thermal cycler PCR machine (Thermo Scientific, Waltham, USA) using 25µl total volume with sterile water containing 3ml of the DNA template, .05ml each primer, 0.15mM MgCl₂ of buffer, 0.1mM of each dNTP and one unit of Taq DNA polymerase (Macrogen, South Korea). The amplified product was run on 2% Gel containing ethidium bromide (8 µl) for 45-60 minutes at 90 volts and 120 current followed by a gel electrophoresis tank and was visualized and result observed by a gel documentation system. The PCR amplification was done in the following cycle condition with an initial denaturation at 94°C for 7 minutes followed by 25 cycles of denaturation at 95°C for 45sec, annealing temperature at 60°C for 45sec and elongation period at 72°C for 45 seconds and the mixture was held at 72°C for 7 min after the final cycle before cooling at 4°C. For confirmation and recognition of antibiotic resistance genes, primers' magnification conditions were already standardized and thermocycler was run at various temperature annealing different from 55°C to 62°C annealing temperature for was optimized at 58°C for blaCTXm, blaTEM and 58°C for bla SHV in a total volume of reaction of 25µl. The amplified product was run on 2% Gel containing ethidium bromide (8 µl) for 45 minutes at 90 volts and 120 current, followed by a gel electrophoresis tank and was amplified by a UV gel documentation system. The positive control for ESBL was departmental isolates provided by NARC Islamabad's animal science institute, and the negative control was DNA-free water.

Statistical Analysis

The statistical analysis used the Microsoft Excel program and applied the t-Test test to evaluate any significant association among the antibiotics resistance frequencies. The p-value of 0.05 was measured as a significant statistical point in the analysis. Results

Prevalence of STEC in Uncooked or Raw Milk Sample

Among the 800 raw milk samples, 321(40.5%) were positive for *E. coli* and out of 321 *E. coli* isolates positive for STEC 40 (5.0%) strains were found in this study.

Virulence Genes

Multiplex PCR showed that isolates carried different strains of virulence genes, stx1, stx2, eae, and hlyA, with different sizes of 100bp, 150bp, 200bp and 534bp, respectively.

Antimicrobial Resistance among STEC

Antibiotic sensitivity test was performed for all the *Escherichia coli* (STEC) positive samples and used antibiotics, penicillin, amoxicillin, Augmentin, sulphamethoxazole, Gentamicin, streptomycin, oxytetracycline, ceftriaxone, Norfloxacin, Enrofloxacin, Florefenical, cefotaxime & clavulnic acid antibiotics that exhibited high antimicrobial activities all isolates

with good effect for Norfloxacin (54%) followed by Enrofloxacin (50%), Florefenical (40%) and most minor sensitivity was recorded for sulphamethoxazole (32.45%). The shiga toxigenic positive samples are highly resistant to penicillin, amoxicillin, and cefotaxime clavulnic acid, cefotaxime, oxytetracycline, streptomycin and spiramycin. The STEC isolates were resistant to more than six different antibiotics tested.

Detection of ESBL Virulence Genes of STEC

During ESBL detection, a total of 11 (1.37%) STEC isolates were phenotypically positive as an ESBL producer by the double disc method in this study. All phenotypically ESBL positive STEC isolates were detected to have bla ctxM (540 bp) and no positive bla Tem 1086bp) virulence genes and blaSHV by PCR (Fig 1). The ESBL-producing STEC belonging to two districts was positive compared to other regions (Table 3). Antibiotics susceptibility test of the STEC-positive ESBL isolates showed a high rank of resistance to cefotaxime, ceftazidime, amoxicillin, penicillin, Oxytetracyclin and Gentamicin with the range of 72- 40%. The drugs Enrofloxacin and Norfloxacin were found to be sensitive to these pathogens.

DISCUSSION

Raw milk samples were collected to analysis Shiga toxin-producing *E. coli* (STEC) from different locations of the Khyber Pakhtunkhwa province, Pakistan. Approximately 800/321 (40.52%) of the total raw milk were found to yield *E. coli* and 40 (5.0%) of these samples contained STEC. Although the STEC O157:H7 has been considered an important Sero-part of STEC with human diseases and outbreaks, case studies have been reported. The occurrence of *E. coli* in uncooked milk is not shocking, but the detection of the multidrug pathogenic strains of STEC in raw milk samples is alarming to public health. In the current study, both strains of pathogenic and non-pathogenic *E. coli* were isolated. However, non-pathogenic *E. coli* was used only as a marker of milk contamination with this pathogen. So, for the known pathogenic *E. coli*, only the prevalence was stated concerning the associated risk factors and categories. However, STEC was discussed in detail as follows since it was the focus of the current study due to its great public health importance. In the current study, The studies report published on occurrences of the pathogenic strains of STEC in raw dairy milk and other food in Pakistan is limited. Out of 100 raw milk samples, the prevalence of STEC *E. coli* O157:H7 was reported from two cities of Peshawar and Quetta regions of Pakistan at 21% and 12%, respectively, and these isolates were tested further positive for ESBL virulence genes (Irshad, et al., 2020; and Tahira, et al., 2017). In our research study, the *E. coli* isolates positive for four virulence genes of STEC stx1, stx2, hlyx and eae, in a different ratio, respectively. Our results are supported by other research data in which a higher prevalence of virulence genes stx1, stx2, hlyx and eae, STEC strains and especially O157:H7 strain isolates has been reported (Samad, et al., 2018; irshad, et al.,2020). Another study reported the prevalence of STEC and *E. coli* O157:H7 14 (8.5%) from commercial branded milk samples in the Peshawar region (Abid et al., 2009). A study was conducted by (Disassa, et al., 2017), who found 380 raw milk samples examined 129 (33.9%) and 11(2.9) for STEC strain O157:H7. Similarly, A study conducted in 2016 by (Azhar et al., 2016) showed 53 strains of STEC O157:H7 in a total of 137 *E. coli* positive in stool samples. Another study conducted by (Worley, et al.,) found 90% isolates for *E. coli* strain O157:H7 in animal farms. The difference in results may be due to different sample sources. This high isolation rate of STEC in milk samples poses a threat to milk consumers. Although the isolation rate of STEC showed, many significant reports from various regions of the world provided different prevalence rates of STEC from raw milk samples. Some of the reports of the world on the prevalence of these pathogenic strains were in line with the current study results whereas others showed smaller or higher expected prevalence. The current study finding from raw milk (5.0%) was in agreement with the research work of different countries reported as 2.6% from Egypt (Meshref, 2013), 2.0% again from Egypt (Abdul-Raouf, Ammar, and Beuchat, 1996), and 2.0% from Ochigun State, Nigeria (Ivbade, Ojo, and Dipeolu, 2014) from raw milk. These could be due to having somewhat similar milking and milk handling practices along the dairy value chain. These related findings could be due to somewhat associated milk production, handling, storage, distribution and hygienic practices irrespective of the regions. However, lower isolation of STEC was reported as 1% (Omore, et al., 2001) isolated in milk samples from a marketing survey in the Kenyan highlands and 1% again in Egypt (Amer, and Soliman, 2004) from raw milk. In contrast to the current finding, higher isolation of STEC O157:H7 from raw milk was reported as 12% in Bishoftu (Bedasa, et al., 2018), 33.5% in Malaysia (Chye, Abdulla, and Ayob, 2004) in raw milk samples, 27.08% from Basrah City-Iraq (Abbas, Ghadban, and Alghanim, 2017) from raw milk. These higher isolation rates may be because of a higher risk of contamination during production, a greater chance of contact with fecal materials, unhygienic activities, the use of contaminated water and improperly cleaned milk containers, transporting and storing milk at a temperature that is suitable for bacterial recovery and improper handling of milk after milking. Generally, there is great variation in STEC prevalence in the

dairy value chain, across the regions, and in different studies. These differences may arise from various reasons. The variation in the prevalence of STEC in the dairy value chain might be due to the time taken and mishandling during transportation. However, the variation in animal production and management, hygienic activities, milking system and milk handling practices might be the sources of the difference in frequency of isolates across the regions. The variation reported in prevalence in different studies may be due to differences in sample size, farming system, farm size, milking equipment, milking technique, ecology, geography, duration of milk transportation, and hygienic conditions, as stated by (Soomro, et al., 2002).

In our study, an antibiotic susceptibility test was performed for all STEC-positive samples using 12 selected antibiotics, including Penicillin (P), Augmentin, Ag, streptomycin (S), ceftriaxone (CRO), sulfa-methazole (SXT), Florefenical (F), Amoxicillin (AMC) Oxytetracycline, Gentamicin, Ceftriaxime showed MDR 72.5% *E. coli*. Norfloxacin and Enrofloxacin were among the antibiotics that exhibited high antimicrobial activities against all isolates with good effect. In a study by (Disassa, et al., 2017) was reported that STEC is incredibly resistant to tetracycline, streptomycin and kanamycin, respectively.

Furthermore, a study by (Elmonir, et al., 2021) proved that Shiga Toxin-producing *E. coli* was particularly resistant to Amoxicillin, Chloramphenicol, Ampicillin, Doxycycline, Ciprofloxacin, and Tetracycline antibiotics. The difference in the result may be due to regions where antibiotic resistance varies from low to high in different parts of the world. The reason is the misuse of antibiotics by health personnel in agriculture, poultry and animal forms.

A high quantity of multidrug resistance amongst Shiga Toxin-producing *Escherichia coli* strains might be connected to their capability to produce ESBL strains. In the study, 11 out of 40 (2.5%) isolates were phenotypically and genotypically documented as ESBL producers. The STEC showed resistance to broad-spectrum antibiotics amoxicillin, cefotaxime, and ceftriaxone. All STEC 2.5% isolates of ESBL were less sensitive to antibiotics, a similar report was presented by Ali, et al., 2016 found an incidence of 23.5% from bovine uncooked milk samples. It is a severe issue that infectious organisms may be entered into human consumers or young calves, resulting in the distribution of antibiotics resistances.

For molecular ESBL confirmation, three genes were selected bla CTXM, bla TEM and bla SHV for all 11 samples using Polymerase chain reaction and gel electrophoresis. As a result, a band of 550bp was observed on Gel electrophoresis for bla CTXM, and no bla TEM, bla SHV for all samples. A study showed that more than 91% of the isolates were resistant to multiple antibiotics, of which 71.10% were resistant to colistin. Of these 275 isolates, mcr-1 was identified as the most predominant gene carried by 72.63% (197/275) of isolates, 40.59% (78/197) of the isolates were hiding both mcr-1 and ESBL genes (bla CTX-M, bla SHV and bla TEM). ESBL genotyping showed that bla CTX-M was the most predominant ESBL (68.49%), followed by blaSHV (16.4%) and blaTEM (15%).

(Khan, et al., 2018, and Rahman, Ahmad) In our study, 11 ESBL-positive STEC isolates were tested against 12 antibiotics, with the highest susceptible results being (Norfloxacin, Enrofloxacin and florephephicole). While all STEC isolates contained the bla CTXM resistance gene, the three isolates from the Peshawar region also contained the bla ctxM1 resistance genes. The STEC were potentially multi-drug resistant. Indeed, six STEC isolates were multi-drug resistant to the different antibiotics. In addition, a research study on antimicrobial susceptibility reported that many of the STEC isolated from milk were resistant. Therefore, raw milk, apart from the potential source of food-borne bacterial pathogens, can also reason an extreme health hazard to consumers due to antimicrobial residues.

CONCLUSION

This study has confirmed 5% of shiga toxin-producing *E. coli* strains in 800 raw milk samples collected from milk shops, street vendors and dairy farms in Khyber Pakhtunkhwa province of Pakistan. The drug-resistant ESBL virulence genes are significantly present in 11 of the 800(1.37%). Raw milk and its byproducts are consumed by all age groups and sold in the market, which may threaten human health. A comprehensive epidemiological study is required on STEC in dairy farms and different food animal species, especially in the province and throughout the country. In our study, most of the STEC showed resistance to aminoglycoside and cephalosporins and less sensitivity to the antimicrobial drugs. Thus, antibiotic resistance in milk-borne pathogens, such as STEC, should be monitored regularly. Proper training should be given to dairy Industry workers to stop these zoonotic pathogens. Appropriate measures are needed to control STEC contamination in milk production and value chain system. Strict hygienic measures are needed for milk production in dairy farms, milk collection and processing in the dairy market of Khyber Pakhtunkhwa province.

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Annexures

Table 1: Primers (Oligonucleotide) for PCR amplifying the genes stx1, stx2, eae, and ehxA and E. coli Sero-group STEC and ESBL virulence genes isolated from raw milk in Khyber Pakhtunkhwa. PCR reactions were carried out in the mPCR system vertical (Applied Biosystems, Waltham, USA)

Genes	PCR conditions	PCR reaction volume
Stx1, Stx2, eae, ehxA	1cycle 96 °C-10 min 35cycles 95°C-45 Sec 60°C-45 Sec 72°C-45 Sec	2.5ml of 10x PCR buffer 0.15mM MgCl ₂ 0.1m M of each dNTP (Thermoscientific, Waltham, USA) 0.2mM of each primer one unit of Taq DNA polymerase 2mL of DNA
		1 Cycle final volume of 25mL with sterile water 72°C -8 min
bla CTxm, bla TEM, bla SHV)	1cycle 2.5ml of 96 °C-5 min 25 cycles 95°C-1min 56°C*, 58°C*-1min 72°C-1 min 1 Cycle	2.5ml of 10x PCR buffer 10x PCR 0.15m MMgCl ₂ buffer 0.1mM of each dNTP (Thermoscientific, Waltham, USA) 200 mM of bla CTxm, TEM, SHV primers one unit of Taq DNA polymerase 5mL of DNA 72°C -10 min held 4°C* forever.
		the final volume of 20mL with sterile water

Table 2: Cross tabulation of region with *E. coli* N=800:

Region	<i>Escherichia Coli</i> (<i>E. coli</i>)		Total
	Positive	Not Detected	
Hazara	26	74	100
Kohat & DI Khan	83	117	200
Malakand	71	129	200
Peshawar	141	159	300
Total	321	479	800
Chi-Square=16.145		P-Value= 0.001	

Table 3: Cross tabulations of milk source with (E. coli, STEC, ESBL) contaminations N=800.

Source detail	E.Coli		STEC_Coli		ESBL		Total
	Positive	Not Detected	Positive	Not Detected	Positive	Not Detected	
Dairy Farms and Individual Farmer	073	127	08	192	02	198	200
Milk Collection Centre	013	013	01	025	00	026	026
Milk Shops	207	293	30	470	08	492	500
Milk Vendors	028	046	01	073	01	073	074
Total	321	479	40	760	11	789	800
Probability Statistics	p-Value=0.449		p-Value=0.305		p-Value=0.860		

Table 4: Chi-square and Fisher's exact test (statistics) of E. coli with STEC E. coli N=800:

Escherichia Coli (E.Coli)	Shiga Toxin producing Escherichia Coli (STEC)		Total
	Positive	Not Detected	
Positive	40	281	321
Not Detected	00	479	479
Total	40	760	800
Chi-square=62.830	P-Value=0.000	Fisher's Exact Test =0.000	
a. 0 cells (.0%) have expected count less than 5. The minimum expected count is 16.05.			
b. Computed only for a 2x2 table			

Table 5: Antimicrobial Susceptibility Testing of Shiga Toxin-Producing E. coli (STEC) Isolated from Raw Milk in Khyber Pakhtunkhwa (CLSI 2013) N=18

Antibiotics	Discs	Resistance N (%)	Intermediate N (%)	Sensitive N (%)
Penicillin	p-10	8(44.4)	6(33.3)	4(22.2)
Amoxicillin	AMC	11(61.1)	3(16.7)	4(22.2)
Augmentin	Ag	7(38.9)	5(27.8)	6(33.3)
Sulphamathoxole	SXT	5(27.8)	7(38.9)	6 (33.3)
Gentamicin	CN-10	6(33.3)	5(27.8)	7(38.9)
Streptomycin	STM	8(44.4)	3(16.7)	5(27.8)
Oxytetracycline	TET	6(33.3)	8(44.4)	4(22.2)
Ceftriaxone	CTX	10(55.6)	6(33.3)	2(11.1)
Norfloxacin	NRF	3(16.7)	4(22.2)	11(61.1)
Enrofloxacin	ENR	3(16.7)	3(16.7)	12(66.7)
Florefenical	FRN	3(16.7)	5(27.8)	10(55.6)
Cefotaxime & clavulanic acid	CTXCL	10(55.6)	5(27.8)	3(16.7)
Chi-Square=37.01		P-Value= 0.023		

*RXC Method by using EPI-info software.

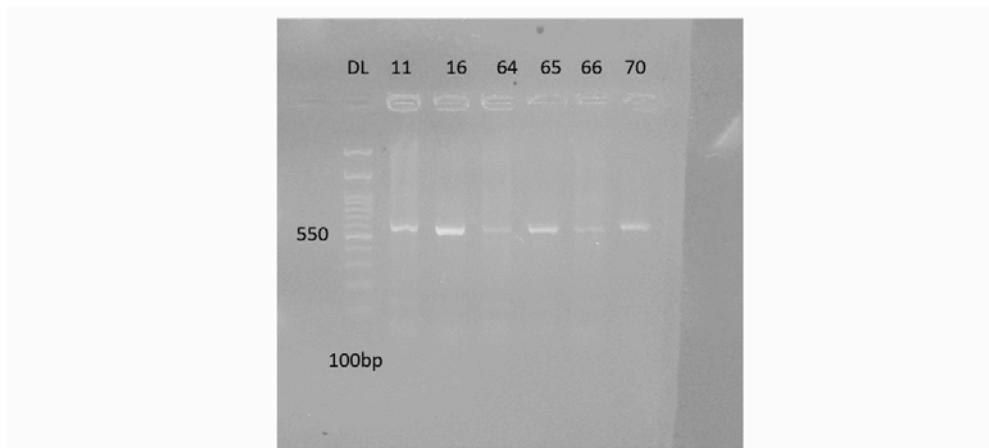


Figure 1: blaCTXM gene amplified by PCR. Marker 100bp with 2% Gel added ethidium bromide, showing a positive result of 550bp for bla CTXM1 from raw milk samples

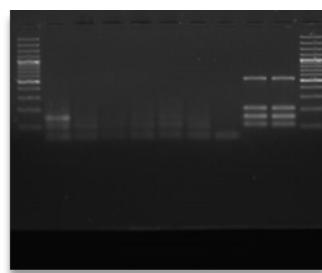


Figure 2: STEC virulence's (Stx1 200bp, stx2 150bp, eae100bp, ehyA534bp) gene amplified by PCR, Marker is 100 bp with 2% Gel added ethidium bromide, showing positive results from raw milk samples

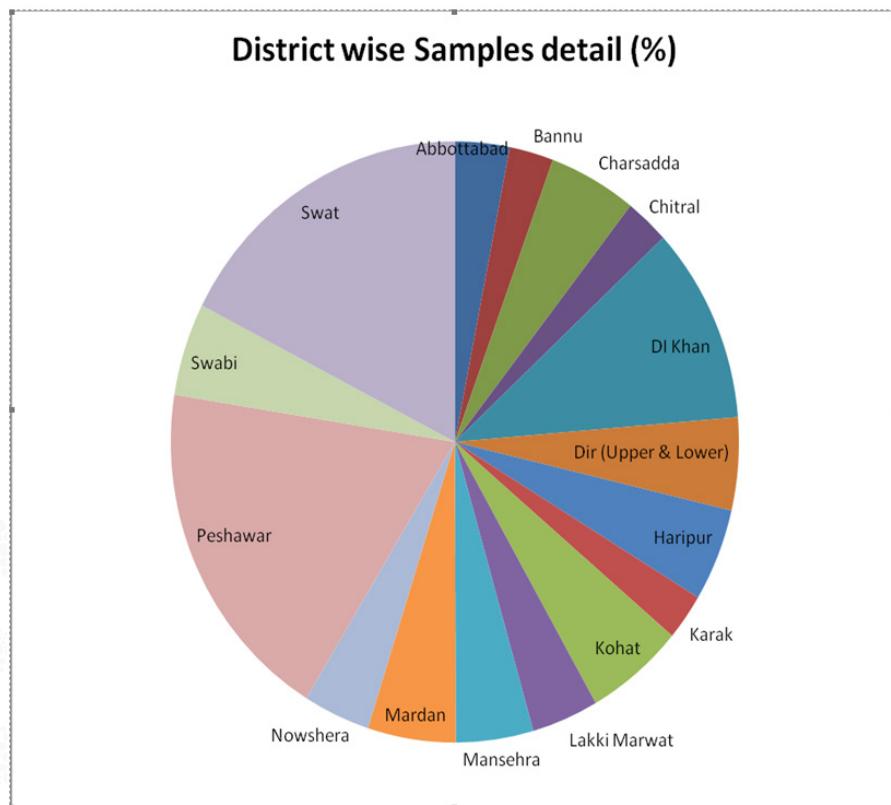


Figure 3: Prevalence of Sample Source N=800.



BANGLADESHI MEDICINAL PLANTS CLASSIFICATION USING CNN

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ABSTRACT

Medicinal vegetation classification is an important issue in our human life. There are many types of medicinal plants in nature which fulfill medicinal properties. We are using a Convolutional Neural Network(CNN) for medicinal vegetation identification. We collected the plants in the medicinal garden, 5 species of plants were used in the dataset and a total of 1500 images are taken of these medicinal plants, 300 images were taken per species. These 5 medicinal plants are Bohera, Haritaki, Nayantara, Pathorkuchi, and Lemongrass. We used leaf structure, color, and form characteristics. We displayed our medicinal vegetation classification containing a total of one thousand fifty images are train, three hundred images for testing, and one hundred fifty images are validation. We applied 14 models among them Xception model accuracy is 100%.

Keywords: CNN, Medicinal plants classification, Image classification.

INTRODUCTION

Medicinal vegetation is an extremely significant part of our existence. In the 1980s, the natural remedy groups (Herbal and Yunani) of the country assembled eighty percent of their requirements from the herbal forests inside the country, and the final 20 percent were fulfilled by import. The importance of medicinal vegetation moreover derivatives is increasing quickly with human being progress in pharmaceutical farmland. The call for medicinal herbs is growing thanks in aspect to the recognition of fewer issue effects. They will be also considered to be a fee-effective manner of developing new and soar-forward tablets. Herbal plants represent an important essential wealth of a nation. As a tropical state Bangladesh is pretty rich in clearly available medicinal plant life. The medicinal vegetation drastically range in nature, for example, Pathorkuchi, Horitoki, Bohera, Nayantara, and Lemongrass. The dataset's picture represents the most valuable statistics than the herbal description in lots of applications like plant identification, face detection, and many others. To acquire the best accuracy the method has to train properly with the benefits of training datasets. Also, it offers the best quantity of accuracy in the identification process. Inside the programs like face reputation, this Parameter offers the permission to simplest authorized customers Whereas, within the packages like medicinal plant Popularity system, it identifies the wanted Medicinal plant to store the existence of an affected person. Commonly normal human beings are assigned the activity of gathering plants from the forests. Now and again they Could not apprehend the rare and critical plants due to the fact Of human errors. These uncommon forms of plant life are very Vital to shop the survival of a patient.

Additionally, to the equivalent time. Those people could choose-up the wrong species possibly Harmful plants. In these facts, it is far more important to apply the Automatic plant identification method. This method helps normal humans to identify unique Plant species.

LITERATURE REVIEW

Raisa et al., the dataset of 10 medicinal plants and the whole wide variety of raw photos of plant training are 1671.CNN modelAccuracyis71.3%. In destiny, we can recognize by exploring the CNN version for higher performance in each individual and composite picture in addition we can enlarge our dataset.

Gopal et al., raised a dataset of ten medicinal flora taken, amongst them, skilled with a hundred (ten digits of every plant category) leaves and tested including fifty (various plant species). With the use of optical strategies, various steps are done consisting of type, characteristics extraction, and sample reputation. Accuracy92%. Future work is to improve recognition

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accuracy.

Kalananthni et al., used ten thousand sixteen pictures of three special species to the equal order Classifier using support-vector machines, Personal Digital Assistant, and Radiofrequency. Accuracy 92.6%. The future trends for leaf identification systems determination facilitate users to recognize leaves at any time and everywhere the usage of their cellular telephones.

Ashish et al., proposed a Convolutional Neural Network for medicinal flora leaves image classification, and the medicinal utilization of the vegetation is displayed. Gathered leaf pics of fifty medicinal flora from Google photos. Applying the SVM classifier displayed 98% accuracy. Destiny's work would be to make bigger datasets with medicinal vegetation leaf pictures.

Gunjan et al., This research conferred a pc imaginative and prescient-primary based approach for the concurrent category of medicinal leaves classes and consequent maturity stages. Applied three famous species of medicinal flora particularly, Kalmegh, Tulsi, and Neem. They completed 99% category accuracy with the use of this cited technique.

Zaidah et al., used three types of medicinal flora leaves. The cited model is convolutional Neural networks (CNN) and famous pre-trained CNN specifically, AlexNet and GoogleNet, and the consequences produced have been 0.91, 0.99, and 0.90 individually.

Lawankorn et al., on this paper, construct a CNN-based total version to discover the herb with its medicinal characteristics from unidentified pictures. Used 2,700 medicinal pictures through 11 medicinal classes. The characteristic extraction construction and model structure had been done by using (Fast R-CNN) and (VGGNet) which create the repeal as better than 0.75 and the accuracy as better than 0.80.

C.Amuthalingeswaran et al., manufactured a version (Deep Neural Networks) for the classification of medicinal vegetation. To qualify the model they used around 8,000 images belonging to 4 distinctive species. Correspond to the investigation of correctness values created by way of the two various models. The MNN model accuracy is 85% and the Mobile Net model accuracy is 72%. The destiny work is to raise the shape of the databases.

Amrutha et al. specimen the pictures of ten classes of medicinal vegetation. The training database consists of approximately 650 pictures and the testing set involve 260 photos. Identified using SVM and KNN identifiers. So, the accuracies for KNN were achieved as 100% and SVM was regarding 93.23%.

Kan et al., used 12 species of medicinal vegetation. Identification accuracy of various capabilities and identifiers is SF and TF 86.6667%, KNN model accuracy is 85%, and PNN model accuracy is 90.8333% with them SVM identifier get at an identification rate of 93.3% and it is superior to the several 3 identifiers.

Trung et al., The vegetation two hundred databases include a total of 20,000 photographs of medicinal vegetation that includes 2 hundred classes. Divided into 50: 10: 40 equivalent to the training, validation, and testing images. Most five models accuracy are Resnet50 88.00%, InceptionV3 82.50%, Densenet121 88.00%, Xception 88.26%, VGG16 76.00%, and MobileNetV2 87.92%.

Dileep et al., the propound database incorporate leaf specimens of 40 medicinal flora. The identification is executed using SVM and Softmax identifiers. Alexie 40 with a CNN accuracy of 94.87%, DLeaf 43 with a CNN accuracy of 93.16%, AyurLeaf 40 with a CNN accuracy of 95.06%, and Ayurveda 40 with an SVM accuracy of 96.76%.

Architha et al., This work identifies 7 species of medicinal flora basis on their leaves applying 3 various models -SVM, VGG16 Model, and their accuracy VGG16 with 98%, SVM model accuracy with 97%, and GridSearchCV with 84% accuracy.

Anchitaalagammai et al. The 5 medicinal vegetation database consists of 58,280 pictures together with nearly 10,000 pictures per class. We use the structure CNN for excessive accuracy. Due to this version, a 96.67% achievement rate in locating the consequent medicinal vegetation.

Malabe et al., proposed ten medicinal vegetation with 5000 pictures of leaves are gather in the datasets. They proposed a strong approach to the usage of CNN for the classification of uncommon medicinal vegetation. The correctness of 90% end up acquired with the aid of the use of TensorFlow in the dataset which we built.

DATASET PREPARATION

A strong data set provides much information about the data. The plants are collected from a medicinal garden and 5 species of medicinal vegetation are used in our dataset. Images of 5 medicinal vegetation classes are captured and separated for training, testing, and validation. The medicinal plant written below haritaki(*Terminalia chebula*) is very useful in curing various diseases of the human body. This plant is found in most parts of Bangladesh. Haritaki fruit powder is used for fever, indigestion, dysentery, and many diseases. It is especially effective in curing constipation and nervous debility. It acts as a preventative and

antidote. It is a very useful herb used by doctors to treat respiratory diseases such as cough and shortness of breath. Haritaki fruit contains fructose and amino acids. It simultaneously cleanses the body's weapons and increases the body's strength. Haritaki dispels a multiplicity of diseases for this cause archaic saint name it haritaki. Eating haritaki fruit daily purifies the blood as well as keeps our body free from pollution. Nayantara (*Catharanthus roseus*) contains about 70 alkaloids that act against several diseases. Nayantara flora comprises a few components that preserve the blood sugar stages in taking a look at thereby preserving diabetes. Every day drinking tea made from Nayantara flower petals besides eating the leaves juice helps to increase the level of a substance called vincamine in the body that improves brain power. Nayantara flower contains a lot of anti-cancer properties that keep away cancer. Prevents various eye diseases such as premature vision loss. It contains anti-inflammatory ingredients that reduce eye irritation. By growing the extent of oxygen within the blood, the body's immune system is more potent. Nayantara flowers contain reserpine that helps to keep the heart healthy. Blood stress is controlled by using consuming the juice of this plant. Bohera (*Terminalia bellerica*) trees are especially found in the various districts of Bangladesh like Chittagong, Tangail, and Mymensingh. Bohera plays an effective role in curing dysentery and diarrhea. Regular consumption of bohera fruit can cure heart and liver diseases. Bohera oil is very effective in curing white disease. It works to cure a common cold, and cough. Starting from the bohera tree its leaves, fruits, and even the bark of the tree are full of medicinal properties. If there is an injury, the bark of the tree is applied to the affected area to reduce the swelling. Many people suffer from insomnia nowadays, bohera works first-rate for excellent sleep. Constipation and shortness of include are cured via ingesting bohera often. Triphala is also one of the Ayurvedic breaths that are made up of three fruits Amalaki, haritaki, and bohera. Bohera fruit peel improves digestion and helps reduce appetite. lemon grass (*Cymbopogon*) is used for a variety of stomach problems. Drinking a lemongrass-rich drink after a meal is very beneficial because the primary ingredient in lemongrass is citral, which aids in digestion. Lack of hemoglobin in the blood reduces the supply of oxygen in the blood due to which anemia occurs. Lemongrass contains folic acid, thiamine, zinc, copper, etc. Which helps in increasing red blood cells. It also contains antioxidants and pharmacological agents. Lemongrass has no pairs for weight loss which is known as a detox tree all over the world. Nutritionists call lemongrass a superfood for its many health benefits. Because it contains potassium that reduces lower blood pressure and stimulates blood circulation. Not only this, but it also keeps the liver healthy and absorbs cholesterol from the intestines. Pathorkuchi (*Kalanchoe pinnata*) is known as one of the medicinal plants in medical science. The juice of crushed leaves helps in removing kidney stones. Pathorkuchi leaf juice is also used to prevent flatulence. In an Incident of bleeding pain, the sap of this leaf is helpful. The sap of leaves could be very useful in alleviating life-lengthy colds. The juice of crushed leaves is very useful in relieving chronic colds and coughs. If the juice of pathorkuchi leaves is massaged on the belly to alleviate any ache in a little one it'll heal. The sap of crushed leaves is used to treat many skin problems. As it carries much water. Applying the juice of sparkling leaves to the affected region for damage in any part of the body gives release. The juice of pathorkuchi leaves is very useful for any liver problem. Applying the sap of this leaf is beneficial if bitten by a poisonous insect anywhere on the body problem. The Contribution of pathorkuchi in fixing bladder troubles and controlling high blood strain is simple. Both above and below leaf surfaces are taken in this paper, we can talk about the identification and class of medicinal flora and growth of the attention of human beings about the significance and useful uses of medicinal vegetation.

The mentioned dataset comprises 1500 images and 300 images per species were taken. We used leaf structure, color, and form characteristics. Images have amassed the usage of digital cameras with a high resolution of 48 MP. Most effectively the leaf region is cropped and stored each photo in jpg format. A commonplace identification conference is used to mark every picture, and vegetation classes name are accompanied by a unique sequence digit. It is very difficult for a common man to find topically obtainable medicinal flora



Kalanchoe pinnata



Terminalia chebula



Terminalia bellerica



Catharanthus roseus



Cymbopogon

Figure 1: Five medicinal plants of our datasets.

not including adequate information.

Convolutional Neural community(CNN) and leaf pics in this presentation arrived at a brand new technique for classifying leaves of medicinal flora. There are 722 species of medicinal plants in Bangladesh among of these 255 plants are used by Ayurvedic and Unani medicine practitioners.

The chart below consists of the listing of excerpt papers

Table 1: scientific name of medicinal plants

S.No	Local name	Scientific name
1	Pathorkuchi	<i>Kalanchoe pinnata</i>
2	Haritaki	<i>Terminalia chebula</i>
3	Bohera	<i>Terminalia belerica</i>
4	Nayontara	<i>Catharanthus roseus</i>
5	Lemongrass	<i>Cymbopogon</i>

Applied models

- **ResNet 50:** ResNet full form is a Residual Neural Network and it is a CNN model. ResNet is used as the spine for computer vision work and it permits very profound neural network training with 150+ layers. A solution is to build deep models through the individuality mapping layer and copied from other learned not deep models. The built solution explains that the profound model provides less training error. Following the plane network, we put shortcut links in the remaining network to its equivalent version. Flat networks mostly have 3x3 convolutional layers relying on the size of the output characteristic map and the alike digit of filters, and if the map length is halved the dimensions of filters are doubled to hold the time complexity in keeping with the layer.
- **Xception:** It has an equal variety of parameters as Inception V3. The overall performance profits aren't due to elevated capability but alternatively to extra efficient use of version parameters. It is a convolutional neural network architecture and this structure is the basis of completely depth-wise dividable convolution layers with more potent hypotheses, expected structure is referred to as Xception, which stays for severe Inception. It has attempted to clear out in a 3d area with a width-top and a channel measurement. Xception has 36 convolutional layers per layer promoted within 14 modules, that have linear other connections. Which builds the networks simple to identify by excessive-stage frameworks including Keras or TensorFlow.
- **InceptionV3:** It is also a convolutional neural network architecture that performs various computer vision solutions and computes 7x7 coefficients by label blooming. InceptionV3 is a version of the network that trained a lot of pictures from the ImageNet dataset. It is the third version of the CNN model that was induced during the ImageNet reacquaint challenge. Here we pursue a path to build up networks that can be focused to use Addition calculations accomplished as it should be as correct as possible to resolve Convolution and invasive regulation. Even though accelerated version length and computational cost generally Tend to translate to on-the-spot first-rate profits for maximum tasks (So long as sufficient categorized facts are furnished for training), Computational performance and occasional parameter rely on are still allowing factors for numerous use instances which includes Cellular vision and massive-facts scenarios.
- **MobileNet:** We currently have a category of proficient models referred to as MobileNets for cellular and embedded imaginative and prescient programs. MobileNets are foundation completely on a

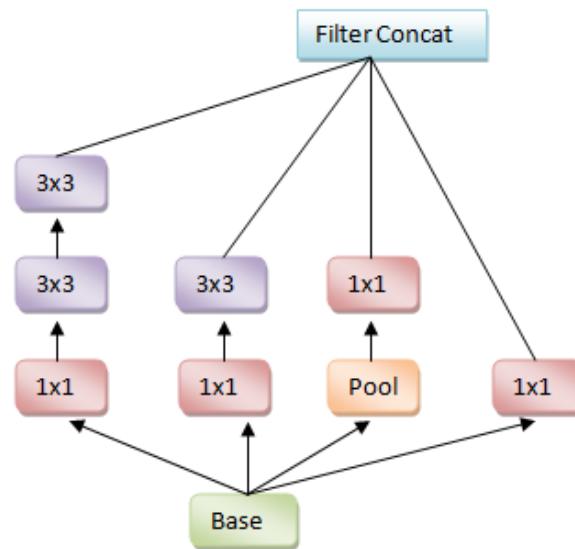


Figure 2: Every 5x5 convolution is changed by 3x3 convolutions, as counseled via precept 3 of segment 2.

streamlined structure that makes use of depth-dividable convolutions to construct mild-weight networks. Two simple worldwide high parameters are introduced that efficiently exchange accuracy. These high -parameters permit the model builder to select the properly sized model for their petition primarily based definitely On the restrictions of the trouble. We currently have massive experiments on useful structure and accuracy tradeoffs and display Strong overall performance as compared to different famous models on ImageNet kind.

- **MobileNetV2:** It elevates the nation of the artwork performance of cell models on more than one responsibility and benchmarks in addition to across a spectrum of various version Sizes. We additionally describe proficient methods of making use of these Cellular models for item detection in a novel framework We referred to as SSDLite. The midway continuation layer makes use of Mild-weight depth smart convolutions to clean out functions as a supply of non-linearity. Moreover, we discover that it's far more important to take away non-linearities in the slender layers as a good way to hold representational energy. Eventually, our technique permits the decoupling of the domains to the imaginative of the conversion, which offers a handy framework for further evaluation.
- **NASNetLarge:** In our research, we look for the notable convolutional layer at the CIFAR-10 dataset and then examine this cellular to the ImageNet dataset, every with their parameters to layout a convolutional structure, which we call a NASNet structure. We additionally introduce an ultra-modern regularization technique referred to as the Scheduled Drop way that drastically improves normalization inside the NASNet models. Finally, we display that the photo functions found out by NASNets are generically beneficial and switch to different pc vision troubles. In our investigation, the capabilities Found with the aid of NASNets from ImageNet categorization can be Mixed with the quicker RCNN framework. NASNet built from satisfactory mobile achieves diverse published Works.
- **VGG16:** In this artwork, we inspect the impact of the convolutional community depth on its validity in big-scale photo acknowledgment placing. Our predominant contribution is a radical assessment of networks of growing depth through the use of a structure with too many short convolution filters, which displays that a big development at the previous-art configurations may be finished by pushing the intensity. We moreover display our representations nicely to different datasets, in which they benefit contemporary consequences. VGG16 means it has 16 layers with weights. This community is quite a massive community and includes approximately 138 million measurements. The input of this network is the (224, 224, 3) dimensions image. The first two levels contain a 3×3 filter which has 64 channels and the highest level of stride (2, 2).
- **Inception-ResNetV2:** Currently, the creation Of remaining connections at the side of a more conventional structure has yielded the latest overall performance.

Its overall performance become the same as the modern-day technology Inception-v3 network. We additionally current numerous recent streamlined structures for each Residual initiative network. Its transformation beautifies the individual-frame reputation common overall performance. Inception structure that has been proven to obtain superb performance at especially few computational costs. Using residual connections no longer only eliminates the degradation trouble caused by deep structures

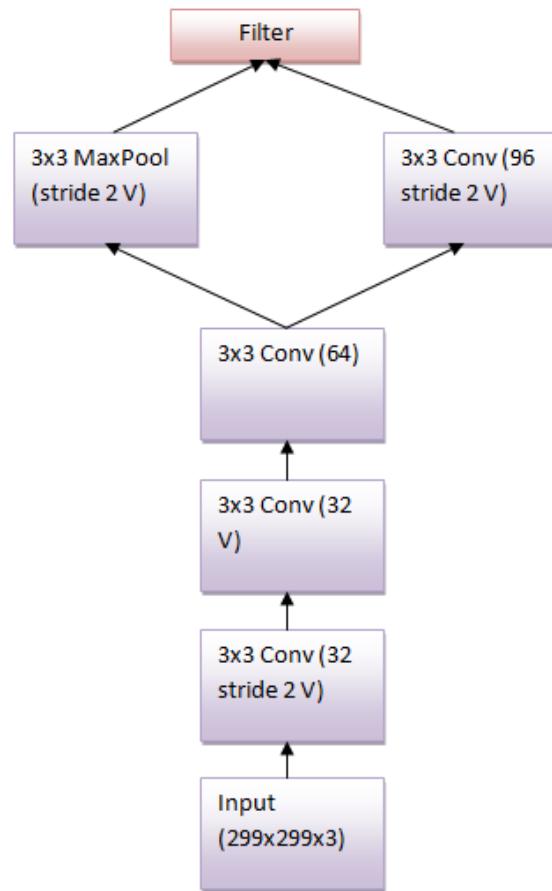


Figure 3: The design for the stem of the InceptionResNetv2 networks.

but additionally reduces the schooling time. The Inception-ResNetV2 has a photo enter size of 299-by-299 and the output is a chart of approximated elegance possibilities and 164 deep layers that categorize photos within 100 objects.

RESULT AND ANALYSIS DISCUSSION

In our research, we have created a dataset of medicinal plants of 5 species. The trees are – Pathorkuchi, Haritaki, Bohera, Nayantara, Lemongrass. Dataset has been divided in train(70%), test(20%) and validation(10%).The background of the images was removed with Adobe Photoshop. Our datasets are implemented with 14 deep CNN model(Resnet 50, Resnet 101, Resnet 152, Resnet 50V2, Resnet 101V2, Resnet 152V2,InceptionV3, MobileNet, MobileNetV2, NASNetLarge , NASNetMobile, Xception,VGG16, Inception-ResNetV2) he highest accuracy(100%) is achieve on Xception model.

- **ResNet50:** The performance of the medicinal plant's classification dataset result is satisfied. The classification report for bohera, haritaki, lemon grass, nayon tara, and pathor kuchi.The precision for bohera is 91%,haritaki 100%, lemon grass 100%,pathor kuchi 100%,nayon tara 100%. Whereas recall for bohera 100%,haritaki 95%, lemon grass 100%, nayon tara 100%, pathor kuchi 95%.ResNet50 has f1-score for bohera 95%, haritaki 97%, lemon grass 100%,nayon tara 100%,pathor kuchi 97%. Our dataset is predicted for bohera 60,haritaki 57 and 3 numbers for false prediction, lemon grass 60, nayon tara 60, and pathor kuchi 57 were 3 number false predictions. Learning rate0.0001 and a total number of layers 117.
- **ResNet152:** We gained classification reports of the dataset that are precision for bohera 100%,haritaki 100%, lemon grass 97%, nayon tara 95%, pathor kuchi 100%.The recall value for bohera is 100%,haritaki 100%, lemon grass 100%, nayantara 100%, pathorkuchi 92%. The f1-score for bohera is 100%,haritaki 100%, lemon grass 98%, nayon tara 98%, pathor kuchi 96%. Our model correctly predict bohera 60 ,haritaki 60, lemon grass 60, nayon tara 60, pathorkuchi 55, and 5 digits were wrong identified. A whole number of layers 517.
- **ResNet50V2:** The classification report gained these models. The precision for bohera 92%,haritaki 95%, lemon grass 97%, nayontara 98%, pathorkuchi 100%.The recall value for classes bohera 100%,haritaki 97%, lemon grass 100%, nayon tara 100%, pathorkuchi 85%. The f1-score for bohera 96%,haritaki 96%,lemon grass 98%, nayon tara 99%, pathorkuchi 92%. ResNet50V2 model correctly predict for bohera 60 ,haritaki 58 and 2 digits false identified, lemon grass 60, nayontara 60, pathorkuchi 51 and 9 digits wrong identified. The entire number of layers is 192.
- **ResNet101V2:** Our model obtained the precision value for bohera 100%,haritaki 98%,lemon grass 95%, nayon tara 86%, pathor kuchi 100%.The recall value for bohera is 93%,haritaki is 93%, lemon grass is 100%, nayontara is 100%, pathor kuchi is 95%. The f1-score gained for bohera is 97%,haritaki 94%, lemon grass 98%, nayon tara 97%, pathorkuchi 97%. The prediction for bohera 56 and 4 number false identified,haritaki 56 and 4 digit false identified, lemon grass 60, nayontara 60, pathorkuchi 57 and 3 number wrong prediction. The complete number of layers is 379.
- **ResNet152V2:** The consideration of the dataset is very satisfying. The precision of species for bohera 100%,haritaki 95%, lemon grass 95%, nayontara 94%, pathorkuchi 98%.Wheres species recall value for bohera is 98%,haritaki 93%, lemon grass 100%, nayontara 100%, pathorkuchi 85%. The f1-score obtained for bohera is 99%,haritaki 96%, lemon grass 98%, nayontara 92%, pathorkuchi 92%. The model correctly predicts for bohera 59, haritaki 56 and 4 digits false identified, lemon grass 60, nayontara 60, pathorkuchi 51, and 9 number wrong prediction. The full number of layers is 566.
- **InceptionV3:** This model acquire the precision for bohera 97%,haritaki 100%,lemon grass 92%, nayon tara 100%, pathor kuchi 100%.The classes recall value for bohera 100%,haritaki 97%, lemon grass 100%, nayontara 100%, pathor kuchi 92%. This model f1-score for bohera 98%,haritaki 98%, lemon grass 96%, nayon tara 100%, pathor kuchi 96%. The prediction for bohera 60 ,haritaki 58 and 2 digits false prediction, lemon grass 60, nayon tara 60, pathor kuchi 55, and 5 number wrong prediction. The total number of layers is 313.
- **MobileNet:** The classification report of precision for bohera100%,haritaki 100%, lemon grass 94%, nayon tara 97%, pathorkuchi 100%.Its recall value for bohera is 100%,haritaki 98%, lemon grass 100%, nayontara 100%, pathorkuchi 92%. And its f1-score for bohera 100%,haritaki 99%,lemon grass 97%, nayon tara 98%, pathor kuchi 96%. Our model gained prediction for bohera 60, haritaki 59, lemon grass 60, nayon tara 60, pathorkuchi 55, and 5 digits wrong count. The total number of layers is 313.
- **MobileNetV2:** The accuracy of these models is 97%. The precision for bohera is 94%,haritaki 100%, lemon grass 92%, nayon tara 100%, pathor kuchi 98%. The value of recall for bohera 98%,haritaki 93%, lemon grass 100%, nayon tara 98%, pathor kuchi 93%.MobileNetV2 has f1-score for bohera 96%,haritaki 97%, lemon grass 96%, nayon tara 99%, pathor

kuchi 96%.The prediction for bohera 59, haritaki 56 and 4 digit false count, lemon grass 60, nayon tara 59, pathor kuchi 56 and 4 number wrong count. The full number of layers is 156.

- **Xception:** Satisfying results are gained from this model and every species' accuracy are 100%.This model correctly identified 1500 images with 5 species. Our model has the value of precision for bohera 100%,haritaki 100%, lemon grass 100%, nayon tara 100%, pathorkuchi 100%. The recall for bohera 100%,haritaki 100%, lemon grass 100%, nayon tara 100%, pathor kuchi 100%. Xception has an f1-score of bohera 100%,haritaki 100%, lemon grass 100%, nayon tara 100%, pathor kuchi 100%. The prediction is very good for every species that are 60 digits counted among 60 digits. The number of layers is 134.

Here is the classification result of the medicinal plant's dataset.

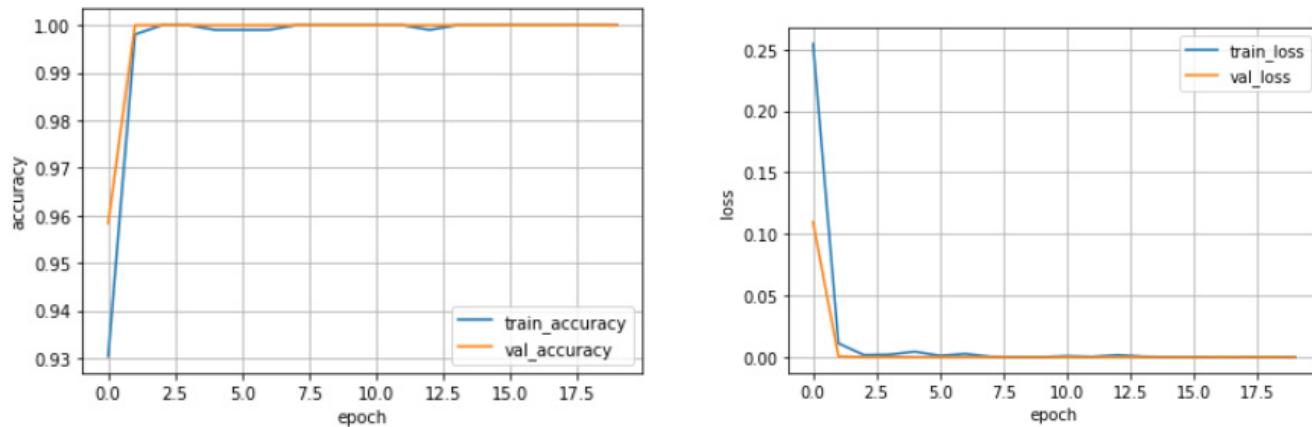


Figure 4: The top diagram is train and Validation accuracy and the next diagram is train loss and validation loss with the Xception model.

- **NASNetMobile:** Our developed dataset has a classification report. The value of precision for bohera is 98%,haritaki is 100%, lemon grass is 94%, nayon tara is 98%, pathor kuchi is 100%. This model recall value for bohera 100%,haritaki 98%, lemon grass 100%, nayon tara 100%, pathor kuchi 92%.The f1-score for bohera is 99%,haritaki is 99%, lemon grass is 97%, nayon tara 99%, pathor kuchi 96%. This model predicts bohera 60 ,haritaki 59, lemon grass 60, nayon tara 60, pathor kuchi 55, and a 5-digit false count. A whole number of layers 771.

Classification Report					
	precision	recall	f1-score	support	
Bohera	1.00	1.00	1.00	60	
Horitoki	1.00	1.00	1.00	60	
Lemon grass	1.00	1.00	1.00	60	
Nayon tara	1.00	1.00	1.00	60	
Pathor kuchi	1.00	1.00	1.00	60	
accuracy			1.00	300	
macro avg	1.00	1.00	1.00	300	
weighted avg	1.00	1.00	1.00	300	

Figure 5: Classification report with Xception model

- **NASNetLarge:** We applied the NASNetLarge model and achieved the precision for bohera 95%,haritaki 87%, lemon grass 98%, nayon tara 100%, pathor kuchi 100%. The recall value for bohera 100%,haritaki 100%, lemon grass 100%, nayon tara 95%, pathor kuchi 83%. The model has f1-score for bohera 98%,haritaki 93%, lemon grass 99%, nayon tara 97%, pathor kuchi 91%. The prediction of this model for bohera 60 ,haritaki 60, lemon grass 60, nayon tara 57, pathor kuchi 50. The full number of layers is 1041.
- **InceptionResNetV2:** our overall classification result of the dataset is obtained. Whereas the precision for bohera is 100%,haritaki 97%, lemon grass 97%, nayon tara 95%, pathor kuchi 100%. The recall value of our dataset for bohera is 98%,haritaki 100%, lemon grass 100%, nayon tara 100%, pathor kuchi 90%. The f1-score for the classes bohera is 99%,haritaki 98%, lemon grass 98%, nayon tara 98%, pathor kuchi 95%. Our models are predicted for bohera 59 , haritaki 60, lemon grass 60, nayon tara 60, pathor kuchi 54, and 6-digit false identified. The total layers of this model are 782.

Table 2: Whole Models test accuracy and test loss.

Model Name	Test Accuracy	Test Loss
VGG16	90%	0.3762
InceptionResNetV2	97%	0.0808
NASNetLarge	95%	0.2393
NASNetMobile	97%	0.124
Xception	100%	0.0026
MobileNetV2	96%	0.0885
MobileNet	97%	0.0397
InceptionV3	97%	0.0585
ResNet152V2	96%	0.0934
ResNet101V2	95%	0.1402
ResNet50V2	96%	0.1784
ResNet152	98%	0.0624
ResNet50	97%	0.0416
ResNet101	97%	0.1768

- **VGG16:** The complete dataset we achieved was precision, recall, and f1-score. The precision value for bohera is 96%, haritaki 84%, lemon grass 100%, nayon tara 80%, pathor kuchi 100%. This model is recall value for bohera 88%, haritaki 88%, lemon grass 100%, nayon tara 100%, pathor kuchi 78%. The value of the f1-score for bohera is 92%, haritaki 86%, lemon grass 100%, nayon tara 89%, pathor kuchi 88%. Our model has a prediction for bohera 53 , haritaki 53 and 7-digit false predict, lemon grass 60, nayon tara 60, pathor kuchi 47, and 3digit false prediction. The entire layers are 21.
- **ResNet101:** Whole classification results are achieve applied this model. Our precision value for bohera is 100%, haritaki 100%, lemon grass 95%, nayon tara 94%, pathor kuchi 100%. The recall for bohera 100%, haritaki 98%, lemon grass 100%, nayon tara 100%, pathor kuchi 90%. The f1-score value for bohera 100%, haritaki is 99%, lemon grass is 98%, nayon tara is 97%, pathorkuchi is 95%. The prediction for bohera 60 , haritaki 59, lemon grass 60, nayon tara 60, pathorkuchi 54, and 6 digits false identified. The complete layers are 347.

CONCLUSION AND FUTURE WORK

In our paper, we delivered a concentration structure for the venture of extracting capabilities for plant identification and recognized medicinal vegetation from leaf pictures. The multiple characteristic datasets retain five kinds of medicinal leaves. We are using CNN(Convolutional Neural Network) to explore 14 CNN models for medicinal plant classification. Among these models, the Xception model has been identified accurately, which accuracy is 100%. The convolutional neural network consists in a convolutional layer that decreases the excessive measurement of snapshots except reducing its information. The method plays an important role to classify medicinal plant leaf identification.

In future work, we are making a large dataset with more species of medicinal plants, and using mobile devices normal people can identify medicinal plants in any place.

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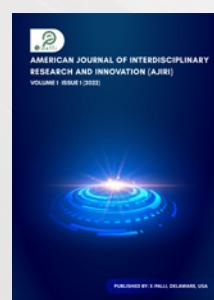
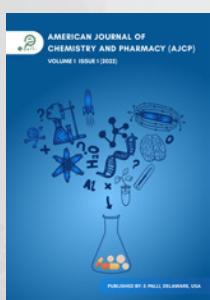
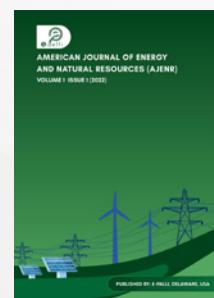
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