

**ANTIMICROBIAL ACTIVITIES OF *Sesamum indicum* AGAINST SOME
PATHOGENIC MICROORGANISM**

**BY
AGBO, ANTHONY IKECHUKWU
ST/BST/M/HND/21/049**

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DECLARATION

I (**Agbo, Anthony Ikechukwu**) with the registration number (**ST/BST/M/HND/21/049**) hereby declare that this work is the product of my own research effort, undertaken under the supervision of (**Mr. Usman Aliyu Maiha.**) and has not been presented elsewhere for the award of any certificate. All sources of information have been duly distinguished and appropriately acknowledged.

.....
Agbo, Anthony Ikechukwu
ST/BST/M/HND/21/049

.....
Date

CERTIFICATION

This is to certify that this project: (**Antimicrobial Activities of *sesamum indicum* against some Pathogenic Microorganism**) was done by (**Agbo, Anthony Ikechukwu**) with Registration Number **ST/BST/M/HND/21/049**) an defend during the 2022/2023 academic season in the department of Biological Science and Technology Federal Polytechnic Mubi. The work was examined and found to meet the requirement governing the award of Higher National Diploma (HND) of the Federal polytechnic Mubi and it's approved for its contribution to knowledge and literacy presentation.

.....
Mr. Usman Aliyu Maiha
(Supervisor)

.....
Date

.....
Dr. Okpara A. N
(Head of Department)

.....
Date

.....
(External Supervisor)

.....
Date

DEDICATION

This project is dedicated to Almighty God for given me the wisdom, strength, knowledge, zeal, courage, guidance, protection and aspiration to accomplish this research work. To God be the glory.

ACKNOWLEDGEMENT

I wish to express my profound gratitude to God almighty for given me the strength and the ability to make this great task possible.

My sincere gratitude goes to my project supervisor Mr. Usman Aliyu Maiha, the Head of Department Dr. Okpara A. N. and the project committees who tirelessly work to ensure the correct from and content of the research work, may the almighty God reward all in abundance.

I wish to express my profound gratitude to the entire staff of the department of biological science for their coordinating ability and working round the clock for a successful completion of our studies and this project work.

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Lastly my regards go to my colleagues and to friends in person of Solomon J. Ibrahim and Obinna Princewill Affamuefuna for their support during my studies with them. May the Almighty God grant them their heart desire. Amen.

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ABSTRACT

Concern about the rising prevalence of anti-biotics resistant stains of pathogenic micro-organisms has triggered an intensive study on the extracts from biologics/active compound isolated from natural plants aqueous extract of fresh and dried leave of sesamum indicum were studies for their in-vitro antimicrobial /activity against tested pathogenic micro-organism. Aqueous extracts of both the fresh and dried leaves of sesamum indicum were strongly effective against E-cole and staphylococcus aureus, while mildly effective against pseudomonas-aeruginosa at full concentration of fresh leave only. However, there was no inhibitory from the dried leaves against pseudomonas-aeruginosa.

CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

Plants contain diverse groups of phytochemicals such as tannins, terpenoids, alkaloids, and flavonoids that possess enormous antimicrobial potentials against bacteria, fungi and other microorganisms. These are much safer than synthetic drugs and show lesser side effects (Ravi, 2011). The search for components with antimicrobial activities has gained increasing importance in recent times, due to growing worldwide concern about the alarming increase in the rate of infection by antibiotic-resistant microorganisms (Davis, 1982; Shittu *et al.*, 2007). Many plants have the potentials as potent remedies for treating different diseases, especially those used by indigenous people. It is therefore pertinent to provide scientific ground for such medicinal plants regardless of their habit, distribution, economic input and the use for which they are employed.

Antimicrobial activity has formed basis of many applications, including pharmaceutical, raw and processed food preservations, alternative medicine and natural therapies. This aspect assumes a particular relevance due to an increased resistance of some bacteria strains to the most common antibiotics and antimicrobial agents for food preservation (Grainger, 2001; Bruneton, 2009).

Concern has been expressed about the rising prevalence of pathogenic microorganisms which are resistant to the newer or modern antibiotics that have been produced in the last three decades worldwide (Cohen, 1992; Nascimento *et al.*, 2000). Coincidentally, the last decade has also witnessed increasing intensive studies on extracts and biologically active compounds isolated from plant species used for natural therapies or herbal medicine (Nascimento *et al.*, 2000; Rios and Recio, 2005).

Sesamum indicum is a major constituent of an herbal preparation named Somina which has sedative, hypnotic and anxiolytic activities (Azmat *et al.*, 2008). Kumar *et al.*, (2011)

reported the anticonvulsant activity of *Sesamum indicum* using various animal models. Sesame oil contains carboxylic acids having a thioether, a sulfoxide or sulphon which function in dermatological and cosmetic compositions promoting skin exfoliation in stimulating epidermal regeneration. They are also useful for controlling intrinsic and extrinsic skin ageing (Maignan, 1998).

1.2 Statement of the Problem

Increase in resistance of these pathogenic organisms, high cost, adulteration and potential side effects of these common antimicrobial drugs coupled with their inadequacy in treating diseases further compound the challenges of multi-drug resistant strains of pathogenic organisms. The seeds and oil of *Sesamum indicum* and its related species have received a lot of attention from researchers owing to the economic values of its parts; but the leaves have attracted only the locals who use it mostly as vegetable and in treating some diseases. It is however of concern that the species is gradually being relegated as some other vegetables have since been used as substitute to this highly valued green leafy species.

1.3 Aim and Objectives

1.3.1 Aim

This research work is to investigate the antimicrobial activity of *Sesamum indicum* against some pathogenic microorganisms. The purpose is to prove the ethnic claim of the antimicrobial effectiveness of this plant against microorganisms.

1.3.2 Specific Objectives

- i. To determine the phytochemical constituent of *sesamum indicum* leave.
- ii. To investigate the antimicrobial activity of *Sesamum indicum* against some infectious bacteria and fungi (*E.coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*).

1.4 Significance of the Study

The study will help in the acquisition of the knowledge of knowledge on the antimicrobial activity of the leaves especially the synergistic activity of aqueous extract of *Sesamum indicum* against some common pathogenic microorganisms.

1.5 Scope and Limitation

The research will be conducted to ascertain the Antimicrobial activity of aqueous extract of *Sesamum indicum* against some pathogenic microorganisms, such as bacteria and fungi (*Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Candida albicans*).

CHAPTER TWO

LITERATURE REVIEW

2.0 Introduction

2.1 *Sesamum indicum*

Sesamum indicum is a major constituent of an herbal preparation named Somina which has sedative, hypnotic and anxiolytic activities (Azmat et al., 2008). Kumar et al., (2011) reported the anticonvulsant activity of *S. indicum* using various animal models. Sesame oil contains carboxylic acids having a thioether, a sulphoxide or sulphon which function in dermatological and cosmetic compositions promoting skin exfoliation in stimulating epidermal regeneration. They are also useful for controlling intrinsic and extrinsic skin ageing (Maignan, 1998). The seeds and oil of sesame and its related species have received a lot of attention from researchers owing to the economic values of its parts; but the leaves have attracted only the locals who use it mostly as vegetable and in treating some diseases. It is however of concern that the species is gradually being relegated as some other vegetables have since been used as substitute to this highly valued green leafy species.

Throughout history, natural products from plants have played major sustaining roles in the lives of humans, especially as food sources and for medicinal purposes. Indigenous peoples living on their traditional territory histories largely rely on ethno medicinal plants for healthcare and they are therefore rich in ethno pharmacological knowledge, Upreti et al. (2010) and Birendra (2017). The use of natural products with therapeutic properties is as ancient as human civilization because medicinal plants are capable of synthesizing an overwhelming variety of low molecular weight organic compounds called secondary metabolites, usually with unique and complex structures, Birendra et al (2017), Rajeev et al (2011), Sumit et al (2011), Singla (2012), Salim (2011) and Vidhu et al (2011). For thousands of years, plants have been known to be valuable sources of medicinal agents with proven potential of treating infectious diseases and

with lesser side effects compared to synthetic drugs Cohen (1992), Gills (1992) and Nascimento (2000). *Sesamum indicum* of Pedaliaceae family, is an indigenous Benue plant of Nigeria and its leaves are reported to have antimicrobial activity as reported by Ijigah *et al.* (2011).

2.2 Importance of *Sesamum indicum*

Infectious diseases are the leading cause of deaths in developing countries (Pamar & Rawat, 2012). The frequent use of antimicrobial agents led to the emergence of widespread resistant strains of pathogenic organisms. Increase in resistance of these pathogenic organisms (Cohen, 1992), high cost, adulteration and potential side effects of these common antimicrobial drugs coupled with their inadequacy in treating diseases further compound the challenges of multi-drug resistant strains of pathogenic organisms (Shariff, 2001). The World Health Organization (WHO) reported that 80% of African populations use traditional medicine to meet their primary healthcare needs, most of which involve the use of plants (Quave, 2016). There has been an increasing research on medicinal plants to validate their folkloric uses (Nascimeto *et al.*, 2000; Rios & Reico, 2005). These plants have been a valuable source of medicinal agents with proven potential of treating infections and minimal side effects when used cautiously (Iwe *et al.*, 1998). Plants are the treasure houses of potential drugs that could be the source to obtain variety of future drugs (Thite *et al.*, 2013). *Sesamum radiatum* (Schum and Thonn), commonly called Benniseed or Sesame seed (English), eweatura (Yoruba) and karkashi (Hausa) (Jimam *et al.*, 2015), is one of such plants with significant medicinal values. It is a plant of African origin belonging to the family Pedaliaceae (Purseglove, 1974). It occurs wild in West and Central Africa and is also cultivated there on a small scale. It does not occur in East and South Africa (except in Northern Angola), but it is sometimes cultivated and found naturalized in Tropical Asia (Bedigian, 2003). The decoction of the leaves is used for the treatment of catarrh, eye pains, bruises and erupted skins (Bankole *et al.*, 2007) and many forms of intestinal disorders especially

diarrhea and dysentery (Gills, 1992). Its warm water leaves infusion is used as gargle to treat inflamed oral membranes (Gills, 1992).

The decoction of both leaves and root has been found to be effective against chicken pox and measles and has a cosmetic use as a shampoo for *Taenia capitis* (Gills, 1992). Several literatures also indicate that *S. radiatum* is used by several communities because of its ability to improve fertility and ease childbirth (Ojekale et al., 2006; Konan et al., 2013). The hypotensive effects of aqueous extract of *Sesamum radiatum* was also reported by Konan et al. (2013). In studies carried out by Konan et al. (2013) and Hamzah et al. (2013), *Sesamum radiatum* extract was found to contain flavonoids, phenols, tannins and terpenoids. These phytochemical constituents have been linked with antibacterial activities (Mujeeb et al., 2014). Shittu et al. (2006), Bankole et al. (2007), Ahmed et al. (2009), Osibote et al. (2010) and Agbankpe et al. (2016) reported the antimicrobial activities of the leaf extracts whereas Seukep et al. (2013) reported the antimicrobial activity of the leaves and stem of the plant. However, the present study was aimed at investigating the antimicrobial activity of the aqueous and methanolic extracts of the whole plant of *S. radiatum* (Schum and Thonn.), with focus on antibacterial and antifungal activities.

2.3 Pharmacological Activity

2.3.1 Anti-inflammatory and Antipyretic Study

Sesame oil produced significant antipyretic effect comparable to paracetamol. In a study, the sesame oil administered as dietary supplement produced analgesic, antipyretic and antiinflammatory activities in animal models. The antiinflammatory activity was assessed on the basis of paw edema inhibition induced by the injection of carrageenan (an edematogenic agent) into the subplantar region of the right hind paw of the rat. Their results showed that the sesame oil and sesamin inhibited the formation of pleural exudate and the leucocyte migration

confirming the anti-inflammatory activity (Saleem *et al.*, 2011; Monteiro *et al.*, 2014; Patel *et al.*, 2012).

2.3.2 Anti-oxidant effect of *Sesamum indicum*

Sesame increases the recycling of vitamin E, improves liver functions and provides protection against alcohol-induced oxidative stress. Sesamin decreases cholesterol levels while increasing high-density lipoprotein levels. Sesame oil enhances hepatic detoxification of chemicals, reduces the incidence of chemically-induced mammary tumors, and protects against oxidative stress, which is involved in the pathogenesis of endotoxin intoxication. Oxidative stress may be caused by reactive oxygen intermediates (ROI). ROI, including singlet oxygen, nitric oxide (NO), hydrogen peroxide, and free radicals, all of which are important mediators of cellular injury and play a putative role in oxidative stress in endotoxin intoxication. The effects of ethanolic extract of sesame coat on oxidation of low-density lipoprotein (LDL) and production of nitric oxide in macrophages were investigated. The results showed that extract in the range of 0.01-0.8 mg/ml markedly inhibited copper-induced LDL oxidation and H₂O₂ induced cell damage that implies that ethanolic extract could exhibit a protective action on biomolecules and generation of inflammatory mediators *in vitro*. Clinically, it was found that sesame oil consumption helped in hypertensive patients remarkably reduced oxidative stress and simultaneously increases glutathione peroxidase (GPx), SOD and catalase activities (Sirato-Yasumoto *et al.*, 2001; Saleem *et al.*, 2011; Monteiro *et al.*, 2014; Patel *et al.*, 2012; Ide *et al.*, 2003; Wang *et al.*, 2007; Vishwanath *et al.*, 2012).

2.3.3 Antimicrobial Study

Sesame is naturally antibacterial for common skin pathogens such as *Staphylococcus* and *Streptococcus*, as well as common skin fungi such as athlete's foot fungus. As a throat gargle, it kills *Streptococcus* and other common cold bacteria. It helps sufferers of psoriasis and dry skin ailments. It is a useful natural ultraviolet protector. In a study, the results revealed that minimum

inhibitory concentration (MIC) of sesame oil against *Salmonella typhi* is 10 µl/ml. However, for other organism the MIC values were in the range of 350-500 µl/ml. The sesame oil shows best antimicrobial activity and also equal with standard Kanamycin and also it shows highest zone of inhibition against *S. typhi*. It reported that sesame oil is found to have the antibacterial activity against *Streptococcus mutans*, *Lactobacilli acidophilus* and total bacteria (Anand *et al.*,2008).

2.3.4 Anti-hypertensive Activities

In a study, it is revealed that the sesamin and its active metabolites can induce antihypertensive effects in experimental animal models. A study in hypertensive patients indicated that sesame oil consumption remarkably reduced oxidative stress and simultaneously increased superoxidase dismutase, and catalase activities. These results support the hypothesis that sesame oil consumption may help to enhance antioxidant defense system in human beings. The investigators suggested that sesamin is a useful prophylactic treatment in hypertension and cardiovascular hypertrophy. In another study, among the hypertensive patients using nifedipine (calcium channel blocker) was compared along with other edible oils. Among the groups, sesame appeared to be promising against the blood pressure rise (Nakano *et al.*,2006; Nakano *et al.*,2002; Sankar *et al.*,2005).

2.3.5 Lipid metabolism Study

Considering the chemical composition, the dietary intake of sesame oil is expected to improve the condition preventing any postprandial lipemia or lipid oxidation. Although many reports are available concerning the effect of sesamin on lipid metabolism, but only a few studies using the intact sesame oil as a diet are available. It seems it possess lipid peroxidation and also the lipid profile. It is apparent that sesame rich in lignans more profoundly affects hepatic fatty acid oxidation and serum triacylglycerol levels. Therefore, consumption of sesame rich in lignans results in physiological activity to alter lipid metabolism in a potentially beneficial manner. Sesamol has been shown to reduce lipopolysaccharide-induced oxidative stress and

upregulate phosphatidylinositol 3kinase/Akt/endothelial nitric oxide synthase pathways (Chavali *et al.*,1998; Ying *et al.* ,2011).

2.3.6 Wound Healing Study

Free radicals are generated at the site of injury, which are known to impair the healing process by causing damage to cellular membranes, nucleotides, proteins and lipids. In this context, several antioxidants, such as curcumin, vitamin E, have been reported to give protection against oxidative damage to tissues. The use of antioxidants has been shown to promote wound healing. Sesame oil extract has potential antioxidant activity which helps to prevent oxidative damage and promote the healing process. *Sesamum indicum* seeds and oil both promote wound healing in experimentally induced rats. Gel containing seeds or oil applied topically or administration of seeds or oil orally significantly promoted the breaking strength, wound contraction and period of epithelialization in incision, excision and burn wound models (Fukuda *et al.*,1986; Pascoe *et al.*,1987; Kiran *et al.*,2008).

2.3.7 Anti-atherosclerotic Study

Sesame oil could inhibit atherosclerosis lesion formation effectively, perhaps because of the synergistic actions of fatty acid and non saponifiable components. A modified form of sesamol (INV-403) to enhance its properties and assessed its effects on atherosclerosis. INV-403 is a novel modified lignan derivative that potently inhibits atherosclerosis progression via its effects on IKK2 and nuclear factor- B signaling (Bhaskaran *et al.*, 2006; Ying *et al.*, 2011).

2.3.8 Anti-cancerous Study

Sesame oil has been found to inhibit the growth of malignant melanoma in vitro and the proliferation of human colon cancer cells. Sesame seed consumption increases plasma γtocopherol and enhances vitamin E activity, which is reported to prevent cancer and heart diseases. Cephalin from sesame seed has hemostatic activity. Historically, fiber is used as an antidiabetic, antitumor, antiulcer, cancer preventive, cardioprotective and laxative. Myristic acid has

cancer preventive capability and is found in sesame seed ranging from 328 to 1,728 ppm (Chakraborty *et al.*, 2008).

2.3.9 Other Medicinal Uses

In recent experiments in Holland by Ayurvedic physicians, the oil has been used in the treatment of several chronic diseases including hepatitis, diabetes and migraine. These effects are supported by main pathophysiological theories of migraine such as neural and sensitization theories. Sesame flower extract possessed tumor arresting property (Bhaskaran, Santanam, Penumetcha & Parthasarathy, 2006). Sesame oil is used as a solvent for intramuscular and has nutritive, demulcent, and emollient properties and has been used as a laxatives. The leaves are rich in a gummy matter and when mixed with water from rich bland mucilage that is used in the treatment of infant cholera, diarrhea, dysentery, cataract, boils, carbuncle, menstrual irregularities, poly-urea, stomach- trouble, serious burns skin diseases, alopecia and used as a tonic (Stern, 2015).

2.4 Mineral Constituents

2.4.1 Protein

Approximately 20% protein is present in the seasam seed, lignan like sesamolin, sesamin is the main constituent of protein. Amino acids are also found in the protein described as follows; Isoleucine, Leucine, Lysine, Methionine, Cystine, Met + Cys, Phenylalanine, Tyrosine, Phe + Tyr, Threonine, Tryptophan, Valine, Histidine, Arginine, Alanine, Aspartic acid, Glutamic acid, Glycine, Proline, Serine etc (Fail, 1993).

2.4.2 Carbohydrate

Carbohydrate is present about 18-20% in composition of the seasam seed. The glucose and fructose are present in low amounts, an oligo sugar planteose [0-a-D-galactopyranosyl-(1, 6)-/3-D-fructofuranosyl-a-D-glucopyranoside] has also been reported in small amount (Wankhede and Tharanathan, 1976).

2.4.3 Vitamin

A significant amount of the vitamin B complex are also present in the sesam seed. Thiamin (Vitamin. B1) is 0.95 mg %, Riboflavin (vitamin B2) 0.25 mg %), and niacin 5.1 mg % present in per100g of sesam seeds. The complete loss of vitamin B. Complex is shown in hulled seeds (Slover HT, 1983). Sesam seeds also have a high amount of tocopherol (vitamin E), the tocopherol present in sesame is γ -tocopherol in more amount, while α -tocopherol content is found in very small amount (Fukuda, 1986), (Kamal-Eldin, 1994), (Speek, 1985).

2.4.4 Minerals

The sesam seeds are the rich sources of minerals such as Ca (1200 mg) P (540 mg) Fe (9.6 mg) Na 2mg) K (400 mg) in per 100g of seed. Magnesium, zinc, and selenium are also present in very low amount while iron and calcium are present in high amount in sesam seeds (Brito, 1982).

2.5 Role of Fungi in Medicine

Some fungi produce substances which help to cure diseases caused by the pathogenic microorganisms. These substances are called the anti-biotics. The term antibiotic, therefore, denotes an organic substance, produced by a microorganism, which inhibit the growth of certain other microorganism (Musa *et al.*, 2011). The most important antibiotics are produced by the moulds, actinomycetes or bacteria they are used to combat the evil effect of pathogenic bacteria and viruses. The use of antibiotics is not limited to disease treatment (Vafa *et al.*, 2010). The addition to certain antibiotics in small amounts to the feed of slaughter animals promotes rapid growth and improve the quality of the meat product. Application of an antibiotic to surface of freshly killed poultry preserves the freshly killed taste during long periods of refrigeration (Talvas *et al.*, 2009). The discovery of antibiotic agents as drugs is comparatively a recent history. The role of fungi in producing antibiotic substances was first established by sir (Alexander, 1929).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area

Mubi is located in the Northern part of Adamawa state between latitudes 9° 26'' and 10° 10'' N and longitude 13° 10'' and 13° 10'' E. It is bordered by the Mountain ranges of the Mandara in the republic of Cameroon to the East, Michika Local Government area to the North, Hong to the South and Askira-Uba to the West and occupies a landmass of about 506,440 square kilometers (Nwagboso & Uyanga, 2019). The climate of the area is characterized by a typical wet and dry season. The dry season span for 5 months (November to March), while the wet season lasts between April and October each year. The annual rainfall ranges from 700-1,050 mm (Adebayo, 2014).

Mubi North Local Government is located in the North-Eastern part of Adamawa state. The geographical location is between latitude 9° 33' 95" North of the equator and between Longitude 13° 09' 14" 19" East. The local Government Area is bounded with Cameroon republic to the East Mubi South Headquarters is located in central Mubi town district the population size as revealed by the 2006 census the local government population 156,393.00 the major tribes in the local government area are Fali, Fulani and Hausa, the people are endowed with rich traditional culture. The vegetation in the Local Government is Sudan Savannah; this maintains an annual rainfall ranging from 700-900mm and rainy season lasts for about 5-6 months in the local government area the farming activities in the local government are food crops and cash crops. Food crops comprises of cereals, legumes and root crops while cash crops are mainly rice, groundnut, millet and sugarcane.

3.2 Materials

Glass ware

Ethanol

Petroleum spirit

Chloroform

Ethylacetate

Methanol

Sparfloxacin

Flucozole

Soxhlet extractor

3.3 Samples Collection

Some samples of *Sesamum indicum* were purchased from Mubi main market, Mubi, Adamawa State, Nigeria. They were transported to the microbiology laboratory of Federal Polytechnic, Mubi. They were properly identified at the herbarium, Department of Biological Sciences Technology, Federal Polytechnic, Mubi, Adamawa Nigeria. The voucher specimen number was indicated. Thereafter, the leaves were dried for two weeks in the laboratory in readiness for the experiment.

3.4 Preparation of the dry plant Extracts.

The classical procedure for obtaining organic constituents from dried plant tissue by Harbone (1997) and Sofowora (2008) will be used. This method involved the use of a Soxhlet apparatus and a range of solvents. Each extraction process were exhaustive using petroleum spirit (60-80°C), followed by chloroform, ethyl acetate and methanol and finally concentrated under reduced pressure. 856 g portion of the coarse powdered leaf material will be packed into a soxhlet extractor thimble and were operated at a temperature of 80°C. The extracts were evaporated using a rotary evaporator under reduced pressure and kept in a desiccator.

3.4.2 Phytochemical analysis

The different crude solvent extracts obtained by the successive extractions from the soxhlet extractor were all be subjected to phytochemical screening using standard techniques of

plant secondary metabolites by Harborne (1997), Sofowora (2008) and Trease and Evans (2009). The crude plant extract was tested for alkaloids, saponins, phlobatannins, tannins, flavonoids, steroids, glycosides and cardenolides.

3.4.2.1 Test for flavonoids

Shinoda's test: The extract (0.5 g) to be tested was dissolved in ethanol, warmed and then filtered. Three pieces of magnesium chips were then added to the filtrate followed by a few drops of conc. HCl. A pink colouration indicated the presence of flavonoids (Markham, 1982).

3.4.2.2 Test for saponins

One gram of the extract were boiled with 5 ml of distilled water, filtered and the filtrate were divided into two portions.

To the first portion, 3 ml of distilled water was added and then shaken for about 5 minutes. Frothing which persisted on warming was an evidence of the presence of saponins (Sofowora, 2008).

To the second portion, 2.5 ml of a mixture of equal volumes of Fehling's solutions was added. A brick red precipitate indicated the presence of saponin glycosides (Vishnoi, 1979).

3.4.2.3 Test for phlobatannins

A small amount of each extract was boiled with distilled water and filtered. The filtrate was further boiled with 1 % aqueous HCL. The appearance of a red precipitate showed the presence of phlobatannins (Evans, 2009).

3.4.2.4 Test for tannins

The extract (0.5 g) to be tested was stirred with about 10 ml of distilled water. The filtrate was used for the following test; To 2 ml of the filtrate, a few drops of 1 % ferric chloride solution was added and the occurrence of a blue-black precipitate showed the presence of tannins. Two millilitre of 10% lead ethanoate was added to an equal volume of the filtrate. Formation of a white precipitate indicated the presence of tannins. The filtrate of the extract was boiled with 3

drops of 10 % HCl and 1 drop of methanol and a red precipitate indicated the presence of tannins (Sofowora, 2008; Evans, 2009).

3.4.2.5 Test for alkaloids

Preliminary test for alkaloids: The extract (0.5 g) will be stirred with 5 ml of 1 % aqueous HCl on water bath and then filtered. Of the filtrate, 3 ml was taken and divided equally into 2 portions in test tubes. To the first portion, a few drops of Dragendoff's reagent were added. The occurrence of an orange-red precipitate was taken as a positive.

To the second portion, 1 ml Mayer's reagent was added and the appearance of a buff-coloured precipitate indicated the presence of alkaloids and to the last 1 ml, a few drops of Wagner's reagent was added and a dark-brown precipitate indicated the presence of alkaloids (Brian & Turner, 1975).

3.4.2.6 Test for cardenolides

Keller-Killiani's test: The plant extract (0.5 g) was dissolved in 2 ml glacial acetic acid containing a drop of ferric chloride solution, and 1 ml of concentrated tetraoxosulphate (VI) acid will be added. The appearance of a brown ring at the interphase indicated the presence of digitoxose sugar characteristic of cardenolide. A violet ring would appear just below the brown ring, while in the acetic acid layer a greenish ring would form just above the brown ring and gradually spread throughout this layer (Evans, 2009).

3.4.2.7 Tests for Glycosides

Liebermann's Test. Added 2.0 ml of acetic acid and 2 ml of chloroform with whole aqueous plant crude extract. The mixture was then be cooled and we added H₂SO₄ concentrated. Green color showed the entity of aglycone, steroidal part of glycosides.

Keller-Kiliani Test. A solution of glacial acetic acid (4.0 ml) with 1 drop of 2.0% FeCl₃ mixture will be mixed with the 10 ml aqueous plant extract and 1 ml H₂SO₄ concentrated. A brown ring formed between the layers which showed the entity of cardiac steroidal glycosides.

Salkowski's Test. Added 2 ml H₂SO₄ concentrated to the whole aqueous plant crude extract. A reddish-brown color formed which indicated the presence of steroidal aglycone part of the glycoside.

3.4.2.8 Test for Steroids

2 ml of chloroform and concentrated H₂SO₄ was added with the 5 ml aqueous plant crude extract. In the lower chloroform layer red color appeared that indicated the presence of steroids.

3.5 Antimicrobial activity

3.5.1 Zone of Inhibition

The antimicrobial activities of the various extracts from the plant *Sesamum indicum* will be determined using some pathogenic microorganisms. The test microorganisms such as *Staphylococcus aureus*, *Streptococcus pneumonia*, *Salmonella Typhi*, *Candida albicans*, *Pseudomonas aeruginosa* and *Candida tropicalis* will be obtained from the Baffa Clinic, Mubi, Adamawa State. All the isolates will be checked for purity and will be maintained in slants of nutrient agar for the bacteria and slant of Sabouraud dextrose agar for the fungi. Well diffusion method was the method used to determine the antimicrobial activities of the extracts from the plant. 0.1g of the extract was dissolved in 10 ml of absolute DMSO to obtain a concentration of the extracts. The active positive controls used were sparfloxacin 2mg/ml for the bacteria and flucozole 5 mg/ml for the fungi. This will be the initial concentrations used to check the antimicrobial activities of the extracts from the plant. Mueller Hinton agar and Sabouraud dextrose agar were the media used as growth media and were prepared according to the manufacturer's instructions (Oxoids of England) Cushine, (2005) and Farraro *et al.* (2000).

3.5.2 Minimum Inhibitory Concentration

Minimum inhibitory concentration of the extract will be carried out on the microorganisms that were sensitive to the extract and was done using broth dilution method. Nutrient broth was prepared according to the manufacturer's instructions as recommended by

NCCLS (National Committee for Clinical Laboratory Standards) Farraro *et al.* (2000). Minimum inhibition McFarland turbidity standard scale number 0.5 was prepared to give turbid solution. Normal saline was prepared and dispensed into test tube. The test microorganisms were inoculated into the normal saline and incubated at 37°C for 6 hrs. Dilution of the microorganism in the normal saline continued until the turbidity matched that of the McFarland by visual comparison.

3.5.3 Minimum Bactericidal and Fungicidal Concentration

Minimum bactericidal and fungicidal concentrations of the extracts will be carried out to check whether the test microbes were killed or only their growth was inhibited. Mueller Hinton and Sabouraud dextrose agars were prepared according to the manufacturer's instruction as recommended by NCCLS (National Committee for Clinical Laboratory Standards) Farraro *et al.* (2000). The contents of the MIC in the serial dilution was inoculated on to the media, the media were incubated at 37°C for 24 hours for the bacteria and at 30°C for 1-7 days for fungi, after which the plate were observed for colonies growth. The MBC/MFC were the plate with lowest concentrations of the extract without colony growth.

CHAPTER FOUR

4.0 Result

Table 4.1 Phytochemical/screening of *sesamum indicum* (Ethonilic extract) of fresh and dried leaves.

Test	Parameters							
Fresh extract	Alk	Sap	Phl	tan	earo	qty	ste	fla
	-	+	-	+	-	-	+	+
Dried extract	-	+	-	-	+	+	+	+

The result the obtained in table 1 showed the *sesamum indicum* contain almost all the phytochemical constituent of both the fresh and dried extract, but the dried extract had most positive test than the fresh extract.

Table 2

Sensitivity to micro-organism ton aqueous textiles of the fresh sesamum indicum leaves.

Microorganism	Sensitivity to different concentration	
	200%	100%
E-cole	10mm	8mm
Staphylococcus aureus	12mm	5mm
Pseudomonas	5mm	-

The result obtained in table 2 shared that aqueous extract from fresh leaves had strong antimicrobial effect against E-cole at 200 (full concentration) and at 100 dilution and had strong antimicrobial effect against staphylococcus aureus at 200 and mild antimicrobial effect at 100 dilution, while had a mild antimicrobial effect against pseudomonas aeruginosa at 200 and had no effect at 100 dilution.

Table 3 sensitivity of microorganism to aqueous extract of the dried *sesamum indicum* leaves.

Microorganism	Sensitivity to different concentration	
	200%	100%
E-cole	15mm	10mm
Staphylococcus	11.0mm	5mm
Pseudomonas	-	-

The result obtained in table 3 showed that aqueous extract from dried leaves had a very strong antimicrobial effect against E-cole at 200 and at 100 dilution, had strong antimicrobial effect against staphylococcus at 200 and mild at 100 dilution, while no any effect against pseudomonas aecisuginosa at all dilution

CHAPTER FIVE

DISCUSSION, CONCLUSION AND RECOMMENDATIONS

The aqueous extract of fresh *sesamum indicum* leaves had mildly effect against *Pseudomonas aeruginosa* at all (table 3)

This may reflect the significance of the preservation of some of the active ingredient sesame lignans such as sesaminol and its glucosides which are water soluble in nature and were extracted effectively during extraction processes of the *sesamum indicum* leaves (Rios & Recio, 2005). This finding confirmed the folkloric claim of the antimicrobial effectiveness of locally consumed sesame leaves extract in many areas of the state (Adamawa). However, for solution/extract with a low antimicrobial activity one will need a large concentration or volume made possible Trull holes rather than the disk method with limited applications (Ahamed et al; 2000). It is very effective against bacteria.

5.1 Discussion

In this study, the phytochemical constituent of *sesamum indicum* leaves extract of both extract of the test screening gave positive result but the dried extract analysis had more positive test than the fresh, the fresh extract had a positive test on saponin, tannin, steroid, and flav. While negative on some parameters ALK, Phlo, curo, gly, ster and flave. But have negative test on Alk, phy, and tannin. (Table 1).

The finding showed that the aqueous extract of both fresh and dried *sesamum indicum* leaves had strong antimicrobial effect against *E-coli* and *staphylococcus aureus* (Table 2&3)

5.2 Conclusion

In conclusion, the aims and objective of this research was achieved. This finding confirmed the folkloric claims of antimicrobial effectiveness of the locally consumed *indicum* (*sesamum*) leaves extract in many areas of the state (Adamawa). It is very effective against bacteria. The

mean inhibitory effect of the dried extract irrespective of the diluents/solvents used were higher than that of the fresh extract. For solution/extract with a low antimicrobial activity, one will need a large concentration or volume to increase its effectiveness.

5.3 Recommendations

There has been a rising interest in the discovery of new antimicrobial and antioxidant agent, the study on the medicinal plant will allow for the demonstration of their physiological activity leading to the development of a more potent drugs with nil or minimal toxicity and high sensitivity especially towards the emerging microbial agents, from the result of this research work, the following are recommended.

- i. *Sesamum indicum* leaves had strong antimicrobial effect against bacterial and can be used as natural therapist against some infection caused by bacteria.
- ii. In natural therapy against bacterial infection, (*pseudomonas aeruginosa*) one should use highly concentrated extract from leaves as it is midly effective against the tested sample.
- iii. Government, Non-governmental organization and pharmaceutical companies should encourage the large scale farming of *sesamum indicum* plant.

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