**CHAPTER ONE**

**INTRODUCTION**

**1.1** **Background of the study**

*Citrus limon* (L) or what is commonly called as lemon, belong to *Rutaceae* family, and its distribution is widespread from South East Asia, India to southern China. Lemon is a pale yellow, elliptically shaped berry fruit. Like all other citrus fruits, it too generally contains sugar, other polysaccharides, organic acids, lipids, vitamins, minerals and volatile compounds. Juice of lemon has been reported to exhibit antimicrobial activity against Vibrio cholera. The peel of this fruit is rich source of flavonoids, glycosides, coumarins, and volatile oils (Shahnah *et al.*, 2007). Many polyethoxylated flavones have also been detected in the peel, having several important bioactivities, which are very rare in other plants (Ahmed, *et al.,* 2008). Antimicrobial activity of the peel extract is directly concerned with the components that they contain. Certain essential oils, protopine, corydaline, alkaloids, lactones, polyacetylene, hypeicinand compound are reported to be effective toward various bacteria (Hammer *et al.,* 2007), and (Kumar *et al.,* 2011). Nevertheless, other active terpenes, as well as alcohols, aldehydes and esters contribute to the overall antimicrobial effects of the lemon peels (Cushnie and Lamb. 2007) and (Keles 2012). The antibacterial assay of lemon peel extract in different solvent such as ethanol, methanol and acetone were previously studied using different Microorganisms (Dormans and Deans, 2009). Discussed the possibility of using this peel extract in various applications including food preservation.

Lemon peel is an agric-horticultural waste produced in large quantities from various fruit processing industries. It is normally discarded and dumped in the environment that can create environmental concerns (Rafiq, *et al.,* 2016). Citrus by-products, if utilized fully, could be major sources of phenolic compounds. The peels, in particular, are an abundant source of natural flavonoids, and contain higher amount of phenolics compared to the edible portions. It has been reported that the contents of total phenolics in peels of lemons, oranges, and grapefruit were 15% higher than those in the peeled fruits (Sawalha *et al.,*2009). Flavonoids in citrus are a major class of secondary metabolites. The peel contains the highest amount of flavonoids than other parts and those flavonoids present in citrus fruits belong to six peculiar classes according to their structure. They are: flavones; flavanones; flavanols; is of Lavone; anthocyanidins and flavanols (Sawalha, *et al*., 2009).

Lemon is an important medicinal plant of the family *Rutaceae,* which have anticancer activities and the antimicrobial potential in crude extracts of different parts (leaves, peels, seeds and flower). Citrus fruits are mainly used by juice processing industries while the peels are generally wasted. During the processing of citrus fruit for juice, peels are the primary byproduct, the highest amount of flavonoids (a major group of citrus secondary metabolites) occurs in the peel which are very rare in other plants.

Lemon is a good source of potassium, calcium & vitamin C. limon or juice of lemon have been reported to exhibit antimicrobial activity against Vibrio cholera. The antimicrobial activity of plants had been received attention many years ago as one of the most effective mechanism for the control of microorganisms (Mathur, *et al.,* 2011). Commonly citrus fruit products are known to potent antimicrobial agents like, bacteria, fungus.

Different parts of plants are good source of potent and powerful drugs and are used in medicine in different countries. Their extracts are used as raw medicine and believed to be the important source of new chemical substances with potential therapeutic properties. Phyto-chemicals produced by plants could be used directly for the production of new drugs. The Phyto-medicines are believed to be more acceptable by the human body, as compared to modern synthetic drugs.

Medicinal plants are essential curative agents for different types of ailments. The experiment carried out by scientists has shown the antimicrobial ability and capacity of plant components which was discovered first in the 19th century (Abalaka, and Bello, 2016).

Medicinal plants have been used for therapeutic goal and around 80% of the population in this world uses herbal medicines to treat disease especially for infectious diseases. This herbal medicine widely used in developing country than developed country.

For a long period of time, there are many naturally occurring materials which are having biologically active substance and show biological activity for the health of human beings and they have a great potential for producing new drugs. In plant chemotherapy, the use of naturally occurring antimicrobial substances is gaining more importance and have higher significant values, (Nada and Zainab, 2008).

According to WHO, medicinal plants are used in order to the therapeutic purpose and be used as a pioneering the synthesis, semi-synthetic chemical drugs. About 80 % of the world population use herbal medicine to treat the ailment. From the statistics, it is under in developed countries and higher in less developed countries (Velus, *et al*., 2015).

Medicinal plants have an important role for the health of individuals and communities. These plants have a great medicinal value that lies various chemical substances which produce physiological action on the human body. Medicinal plants contains many chemical compounds such as alkaloids, flavonoids, glycosides, saponins, resins, oleoresins, sesquiterpene, phenolic compounds, fats and oils (Maruti, *et al.*,2011).

**1.2 Statement of the problems**

Fungi are the most common cause of plant disease and they are widespread and very destructive to both plants and humans (Persley, 1993). This fungal pathogen enter the harvested fruit and vegetables through cracks and wounds during the harvesting process (Adekunle and Ikumapayi, 2006). In fact, during storage, fungi can make food crops unfit for consumption, by changing the nutritional value of the seeds or producing mycotoxins that are harmful for human and animal health (Adekunle and Ikumapayi, 2006). Postharvest disease which play a major role in reducing the quantity and quality of fruit include anthracnose and powdery mildew of various tropical fruit cause by *Fusarium, Aspergillus, lasiodiplodia*, *penicillium*. (plaza *et al*., 2004). For example *Penicillium digitatum* causes green rot while *Penicillium digitatum* causes blue rot on sweet orange fruits. *Fusarium, solani* cause dry rot in potatoes. (Plaza *et al*., 2004)

**1.3** **Aim and Objectives**

**1.3.1 *Aim***

The aim of this study is to determine the antimicrobial potency of lemon peels extract against selected organisms

**1.3.2** ***Objectives***

**The objectives of this research work are to:**

I. Identify the phytochemical constituent of lemon peels (citrus lemon)

II. To accertain the antimicrobial activites of lemon peels against some selected organism

**1.4** **Scope and Limitation of the Study**

This research is limited to:

Determine the antimicrobial potency of lemon peels extract against some selected organism in a study area.

**CHAPTER TWO**

**LITERATURE REVIEW**

**2.1 Clinical Significance**

Lemon is an important medicinal plant of the family *Rutaceae.* It is cultivated mainly for its alkaloids, which are having anticancer activities and the antibacterial potential in crude extracts of different parts (viz., leaves, stem, root and flower) of Lemon against clinically significant bacterial strains has been reported. Citrus flavonoids have a large spectrum of biological activity including antibacterial, antifungal, antidiabetic, anticancer and antiviral activities. Flavonoids can function as direct antioxidants and free radical scavengers, and have the capacity to modulate enzymatic activities and inhibit cell proliferation. In plants, they appear to play a defensive role against invading pathogens, including bacteria, fungi and viruses. Flavonoids are generally present in glycosylated forms in plants, and the sugar moiety is an important factor determining their bioavailability. Preparation from peel, flowers and leaves of bitter orange (*Citrusaurantium* L.) are popularly used in order to minimize central nervous system disorders.The peel of Citrus fruits is a rich source of flavonoid glycosides, coumarins, and volatile oils (Shahnah *et al*., 2007). Many polymethoxylated flavones have several important bioactivities, which are very rare in other plants (Ahmed *et al*., 2008). In addition the fiber of citrus fruit also contains bioactive compounds, such as polyphenols, the most important being vitamin C (or ascorbic acid), and they certainly prevent and cure vitamin C deficiency-the cause of scurvy.

(Rutaceae). For citrus lemon, the most common name is lemon and it is an edible fruit. After orange, lemon is the third most signiﬁcant specie of citrus fruits which is cultivated more than 4.4 million ton in each year. At present, the biggest producer of lemons in the world is Argentina with 1.2 million tones. In lemon, very important natural compounds containing ascorbic acid, minerals, citric acid, essential oils and ﬂavonoids are present in high quantity (Makni M *et al.,* 2018). Lemon shows anticancer and antibacterial activity due to the presence of alkaloid constituent in different parts of lemon leaves, stem, root and ﬂower (Sonawane *et al*., 2011).

**2.2 Graphical distribution**

It is native in north Indian but cultivated in Turkey, Mexico, United States, Argentina, Iran, Brazil, Italy, Spain and People Republic of China. The fruit of lemon can be used for non-culinary and culinary purposes all over the world. Predominantly, lemon is used for its juice from side to side the pulp and taste is as well used mostly in baking and cooking (Mohanapriya *et al.,* 2013).

Toxicological Classiﬁcation:

Kingdom: Plantae

Subkingdom: Angiosperms

Phylum: Eudicots

Class: Rosids

Order: Sapindales

Family: Rutaceae

Genus: Citrus

Species: C. Limon

**2.3 Botanical description**

Citrus trees produce fruits of different shapes and sizes (from round to oblong), these trees are evergreen and full of ﬂavor, fragrance, and juice (Okwu 2008). The color of leaves of lemon is dark green; these are 6.5 to 10mm and are arranged on stem in alternate manners. There are ﬁve petals in lemon with white fragrant color. The pulp of lemon is highly acidic and its ﬂower is bisexual and male. ‘Pink Lemonade’ is lemon cultivar from which the ﬂower of lemon originates. The fruit of this cultivaris striped and leaves are variegated. Commonly lemons are oval in shape with smooth and spongy surface (Zhao 2007). Lemons are varied in their shapes and sizes, some are like grape fruits and some are little bigger in size. Lemons are mostly greenish yellow in color after gaining its full size it shows bright yellow color instead of greenish yellow. The small seeds are found with-in the fruit called ﬂoopies (Gamarra *et al*., 2006).

**2.4 Medicinal uses**

Lemon was cure the plague and a syrup known as acridine citri is prepared from its juice which is used for quenched the thirst of someone and also fought over at the same time. Honey is boiled with lemon and used as digests. It is also used for the removal of pimples and dark spots on face (Uduak *et al*., 2014).

To kill the intestinal worms, to remove the kidney stone and gravel, lemon juice was used (Arias *et al.,* 2005). It is suggested by the various reports that, reduction in oxidative stress, prevention in atherogenic modiﬁcations of LDL cholesterol, improve in the blood lipid proﬁle, platelet aggregation and improves HDL-cholesterol concentrations have been done by drinking substantial volumes of a mixture of citrus juices. It is studied that its extract has anti-thyroidal properties, which suggest its potential to ameliorate hyperthyroidism (Uduak *et al.,* 2014).

Due to their similar phytonutrient contents, all citrus fruits contain nutritional and therapeutic properties. The main source of primates’ device vitamin C1 is citrus. Absorption of iron from small intestine in the body is enhanced by ascorbic acid which is compulsory for connective metabolism particularly the scar tissue, teeth and bones. It is essential as an anti-stress and defender against chills damp and cold. It quenches and scavenge the free radical created by ultra violet radiation stabilization, It performances also as antioxidants in the skin (Okwu 2008).

The collagens production is also reliant on vitamin C. Collagens supports in the advancement and renewal of skin and ﬁne wrinkles are improved by it. Total number of protein in the body and its energetic constituent are increased by its extracts which are responsible for the raise of protein serum such as d- limonene. Citrus fruits and their peels are found to help in improving various metabolic and inﬂammatory disorders attributing to the abundance in its bioactive compounds (Ganesh *et al*., 2016)

**2.5** **Pharmacological activities**

Due to their physiological, medicinal and pharmacological activities, many Citrus species are recognized which includes antioxidant, anti-inﬂammatory, hypoglycemic, anticancer and antimicrobial activities. Studies have also shown that the main compound of C. limon essential oil D-limonene in concentrations of 0.5% and 1.0%, administered to mice by inhalation, has a signiﬁcant calming and anxiolytic effect by activating serotonin and dopamine receptors (Umashanker and Shruti 2011). In addition, D-limonene has an inhibitory effect on pain receptors, similar to that of indomethacin and hyoscine. The pharmacological potential of C. Limon is determined by its rich chemical composition. The most important group of secondary metabolites in the fruit includes ﬂavonoids and also other compounds, such as phenolic acids, coumarins, carboxylic acids, amino acids and vitamins. The main compounds of essential oil are monoterpenoids, especially D-limonene. These valuable chemical components are the reason for the important position of C. Limon in the food and cosmetics industries. Another pharmacopoeia raw material obtained from C. Limon is the outer part of the mesocarp the ﬂavedo. A monograph entitled ‘Citrus Limon ﬂavedo’ can be found in older editions of the French Pharmacopoeia, for example, in its 10th edition from 1998. C. Limon also has a positive recommendation in the European Commission’s Cosmetics Ingredients Database (Cosing Database) as a valuable plant for cosmetics’ production (Ekiert *et al.,* 2020).

**2.6** **Anticancer**

An irregular growth of cells or tissues of body is called cancer. If abnormal cells divide without control and invade nearby tissues is called malignant tumor and also known as malignancy. If the abnormal cell growth does not invade nearby tissues is called benign tumor or non-cancerous growth. A number of chemo protective activities and chemicals are found in plants in which some of these are taking as clinical experiments. A novel procedure for cancer therapy is Inhibition of angiogenesis. The selected plants may be carefully used as anti-angiogenic therapy and in cancer controlling (Umashanker and Shruti 2011). Citrus Limon contain vitamin C, folate, dietary ﬁbre and other bioactive components, such as carotenoids and ﬂavonoids, which are suggested to be responsible for the prevention of cancer and degenerative disease. Citrus limonoids shows anticancer property in aqueous extract of fruit, containing those compounds which shield the cells from damage which is the cause of cancer (Tripoli *et al.,* 2007).

**2.7** **Antioxidant activity**

The important sources of antioxidants are citrus juices and fruits and these antioxidants are ascorbic acid, ﬂavonoids and phenolic compounds (Moosavy *et* *al.,* 2017). The source of glycosides and phenolic compounds are citrus peels. The bioactive compounds are phenolic acids which are responsible for the antioxidant and many other biological activities (Jing et al., 2010). The highest antioxidant activity can be found in citrus fruits. Ascorbic acid (vitamin C) interpretations for a great proportion in some fruits can act as antioxidant. Moreover, other phytoconstituents like carotenoids, ﬂavonoids, glutathione, and various enzyme systems may also contribute to the antioxidant activity (Oriakhi *et al.,* 2016).

**2.8** **Anti-ulcer activity**

The pathophysiology of peptic ulcer dieses involves an imbalance between offensive (acid, pepsin, and H. pylori) as well as suspicious aspects (Umashanker and Shruti 2011). Indian Medicinal plants and their derivatives have been a valuable source of therapeutic agents to treat various disorders including Antiulcer diseases. Fruit aqueous excerpt also shows the antiulcer activity against gastric ulcer (Ahmed *et al*., 2012).

**2.9 Anti-diabetic activity**

A metabolic disorder is called Diabetes. A system, through which our body utilizes the digested food energy and growth, is known as metabolism. The eaten food is converted into aform of blood sugar and the basic component of blood sugar is called glucose. The main source of fuel for our body is glucose. In absence of insulin, glucose cannot enter our cells. A class of organic substance known as polyphenols which is present in lemon and a high amount in lemon peel (Ganesh et al., 2016). It is investigated that polyphenols considerably inhibited the fat accumulation, high blood glucose levels and insulin resistance, development of hyper lipidemia, weight gain which are different aspects of obesity. A range of essential oils is present in citrus peel. The pathogenic bacteria are killed or their growth is inhibited by suing these essential oils. The lowering of diabetes and antiper oxidative effects are possessed by citrus peel due to the high content of total polyphenols (Umashanker and Shruti 2011).

**2.10 Pollination**

A lemon flower’s stigma must receive pollen which contains the flower’s sperm. More specifically, the pollen grains sperm must be transferred to the stigma, which found the top and the longer column in the middle of the flower. Lemon trees can be growing in open area in warmer climate. Lemon trees can be growing in pots in cooler region. Pollen was characterized by a very different emission profile with respect to other part of flower. The trans-nerolidol (30.7%) is the volatile detected in pollen, (Flamini, *et al.,*2007).

**2.11 Phytochemicals:**

Phytochemicals are a large group of plant – derived compounds hypothesized to be responsible for much of the disease protection conferred from diets high in fruits, vegetables, beans, cereals, and plant -based barrages such as tea and wine (Sina, *et al;* 2002). More than 4,000 phytochemicals were catalogued, and are classified by protective function, physical characteristics and chemical characteristics (Adaramola, *et al.,* 2012) and about 150 phytochemical studies in detail (cotonou, 2012). Phytochemical accumulate in different parts of the plant such as in the roots, stems, leaves. Flowers fruits or seed (costa, *et al*; 1999). It is well known that plant produces these chemicals to protected themselves, but recent researches demonstrate that many phytochemicals can also protect both human and livestock against diseases (Builders, 2012).

**2.12 Classification of phytochemicals:**

In recent year’s phytochemical are classified as primary or secondary constituents, depending on their role in plant metabolism primary constituent include the common sugar amino acids, proteins. Purines secondary constituents are the remaining plant Alkabloid and terpenes (Sparg, 2004).

**2.12.1 Flavonoids:**

Flavonoids are important group of polyphenols widely distributed amongst plant. The compounds are derived from parent compound flavonoid are known to exist and some of them are pigment in higher plant (Samanta, 2011). structurally, they made of more than benzene ring in its structure ( a range of CIS aromatic compounds) and numerous reports supports their use as antioxidants or free radical scavengers, antiviral, anti-allergic, anti-platelet, anti-inflammatory and antitumor (Heyman, 2009) flavonoid constitute a wide range of substances that play important role in protecting biological systems against the harmful effect of oxidative processes on macromolecules, such as carbohydrates, protein lipid and DNA (Koura, 2011)

**2.12.2 Steroids:**

Plant steroids (or steroid glycosides) also referred to as cardiac glycoside’ are one of the most naturally occurring plant phytoconstituents that have found The rapeutae application as arrow poisons or cardiac drugs (Sparg *et al*,. 2004). The cardiac glycosides are basically steroid within an inherent ability to afford a very specific and powerful action mainly on the cardiac muscle when administered through injection into man or animal. Steroids (anabolic steroids) have been observed to promoter nitrogen retention in osteoporosis and in animal with wasting illness (Ivanisova *et al*,. 2008).

**2.12.3 Saponins**

The term saponin is derived from Saponaria vaccinia (Quillaja Saponaria), a plant, which abounds in saponins and was once used as soap saponins therefore possess soap like behavior in Water, I.e., they produce form (Nord, 2000) on hydrolysis, aglycone is producer, which is called Sapogenin. There are two types of sapogenin. Steroids and treterpoenoidal. saponins are soluble in water and insoluble in ether, and like glycosides on hydrolysis, they give aglycones (Augustin, 2011). Saponins are extremely poisoning, as they cause hemolysis of blood and are known to cause cattle poisoning (Turkman and velioglu, 2007). The Possess a bitter and acrid taste besides causing irritation to mucous membranes. They are mostly amorphous in non-polar organic solvent like hexane and non-hexane. (Sparg, 2004).

Saponins are also important therapeutically as they shown to have hypolipidemic and anticancer activity. They also have antibacterial activity and may increase the digestion rate of the livestock. Saponins are also necessary for activity of cardiac glycosides (Kauffmann, 2004).

**2.12.4 Alkaloids:**

This are the largest group of secondary chemical constituents made largely of ammonia compounds comprising basically of nitrogen bases synthesized from amino acids building blocks with various radical replacing one or more of the hydrogen atoms in the peptide ring, most containing oxygen (Petterson, *et al.,* 1999). These nitrogenous compounds function in the defense of plant against herbivores and pathogens, and are widely used as thecal ones thetic and CNS stimulant, pharmaceuticals, narcotics and poisons due to their potent Biological activities (Adams and Jimoh, 2011).

**2.12.5 Terpenes**

Terpenes are among the most widespread and chemicals diverse groups of natural products. They are flammable unsaturated hydrocarbons, existing in liguid from commonly found in essential oil, resin or oleoresin. Terpenoid include hydrocarbons of plant or gain of general (C548) II and are classified as mono-distir and sesquiterpenoid depending on the number of carbon atoms example of commonly important monoterpenes include terpinene -4-ol, thujone, camphor, Eugenio and menthol (Dawis, 2000). Terpenoids are classify according to the number of isoprene Unite involved in the formation of these compounds (Ashour, 2010). The sesquiterpene being one of the classes of terpenoid acts as irritants when applied externally and when consumed internally their actions resembles that of gastrointestinal tract irritants (Ludwiczuk, 2017). A number of as quetiapine lactones have been isolated and broadly they have antimicrobial (particularity antiprotozoal) and neurotoxic action. The sesquiterpene lactone, palasonin, isolated from bites monospermic has anthelmintic activity, inhibits glucose uptake and depletes the glycogen content in Ascaridi Galli. (sparg *et al*; 2004).

**CHAPTER THREE**

**MATERIAL AND METHOD**

**3.1 Study Area**

This study will be carry out in laboratory of the Department of Microbiology Federal polytechnic Mubi. Mubi is a town comprising of both Mubi -North and Mubi-south local government Area (L. G. A) Of Adamawa State. The town is located in the north eastern region of Nigeria between latitude 10°14N' and 18'N of the equator and longitude 13°14'E and 13°19'E.it occupies a land of about 725.85km². The area has a tropical climate with an average temperature of 32°c and lies within the Sudan Savannah vegetation zone 4) of Nigeria. The area has an average relative humidity ranging from the 28%-45% and annual rain fall of about 1056mm (Adebayo, 2004).

**3.2 Cleaning and Sterilization of Equipment**

All glass will be wash in water and detergent, rinse in clean water, air dry and will be wrap for sterilization using standard method. The culture media will be prepared according to manufacturer’s instructions and was sterilizing by autoclaving for 15minutes at 121C°.

**3.3 Collection and Preparation of plant materials.**

Ripened *citrus* was purchased from Mubi Area of Adamawa State Nigeria. *Citrus* will be Peel off and then wash with distilled water to remove dirt and soil particles. Peeled will be allow to dry under shade for a period of 14 days then it will be grind into powder using electric blender to avoid contamination and kept in air tight bottle until require for use. (Junab *et al.,* 2017).

**3.4 Collection of test organism.**

A stored culture organism will be obtain from Microbiology lab*.*

**3.5** **Preparation of Plant Extracts Concentrations by Crude Method**

Three different method of extraction was used which are: ethanolic, cold water and hot water extraction

**3.5.1 Ethanolic Extraction of Medicinal Plants:** The plant part viz fruits were dried in the newspaper under the sunlight and will be pound pulverized to powder form and sieved. The plant extract will be obtain with a crude method. The plant extract will be obtain by mixing10g of the lemon peels powder with 100ml of ethanol and will be allow to soak properly for 24hrs. the mixture will be filter with filter paper Whatman No.1 .the filtrate will be distilled in distillation apparatus to get rid of the ethanol at the same time distilled water was added so that the ethanol will evaporate and live the lemon filtrate.

**3.5.2 Cold Water Extraction:** For the purpose of extraction, a 10g amount of the pulverized peel was separately soaked in 100ml cold sterile distilled water for 24hrs and was filtered with Whatman paper No.1

**3.5.3 Hot Water Extraction:** also, the same amount (i.e.10g) of pulverized peel will be immersed in 100mL of hot sterile distilled water (100°C) and will be allow to stand for 30min on a water bath with occasional shaking and kept undisturbed for 24h. The preparation was filtered through a sterilized Whatman No.1 filter paper

**3.6 Preparation of Media:**

28g of nutrient agar powder (NA) will be dissolve in 1000ml of distilled water in a conical flask. It will then be sterilized by autoclaving at 121°C for 15 minutes and allow to cool, and dispensed in a sterile petri- dishes and allow to solidify.

**3.7** **Determination of the Activity of the Phyto-Chemical Extracts On the Test Organisms**

The ethanol extracts will reconstituted in sterile distilled water into different concentration (75mg/ml, 50mg/ml and 25mg/ml) by doubling diffusion. The antifungal activities of the concentration of the extract was carry out using agar well diffusion assay technique of (Bankole *et al.*, 2012).

Aliquot (20µ1) of the fungi in physiological saline will be spread on already solidify nutrient agar (NA) surface using a sterile swab. The surface of the medium will allowed to dry and sterile 6mm cork borer will be use to bore holes on agar plate (one each), the base of each hole will seal with a drop of molten agar to avoid diffusion of extract under agar. Each extract concentrations was dispensed into each hole, the plate will allow to undisturbed for about 15min before they will incubate at 37°C for 72hrs. The diameter of zones of inhibition will measure using a transparent ruler and the zone of inhibition will measure in millimeters. A modify (Shahzad *et al*., 2020) was adopted.

**3.8 phytochemical screening**

The ethanol extract of *Citrus peel*will be subjected to phytochemical test for plant metabolite using standard method as described by (Sofowara, 2003) to determine the following; Alkaloids, Saponins, Tannins, Steroids, Phenol and flavonoids.

**3.8.1** **Test for Alkaloids.**

1.0g of *Citrus peel* powder will be added to a glass test tube and was shaken with 5.oml of 2% HCL on a steam bath and filter. To 1ml of filtrate. Mayer’s reagent was added. The formation of orange red precipitate confirms the presence alkaloids.

**3.8.2** **Test for Saponin.**

Frosting test: 1g of the extract will diluted in 1ml of distilled water in a test tube and shaken vigorously. Persistence foam indicates the presence of saponin.

**3.8.3 Test for Tannins**

Ferric Chloride test: 1g of extract will be added at 2ml of 1% HCL. Deposition of precipitate shows the presence of tannins

**3.8.4 Test for Steroid.**

The extract 1g will be dissolved in 2ml of chloroform in a test tube and the 1ml of Concentrate sulfuric acid will be added. Formations of reddish-brown cooler at the trephine indicate the presence of steroid.

**3.8.5 Test for phenol.**

(Ferric chloride test) the extract 1g will be added with 1ml of 10% ferric chloride. The formation of a greenish brown indicates the presence of phenols.

**3.8.6 Test for Flavonoid**

The extract 1g will be diluted in 1ml of diluted NaOH. Formation of yellow precipitate indicate the presence of flavonoid

**3.9 Experimental Design**

The experimental design is a completely randomized design (CRD) of one plant extract on some fungi isolated and three concentrations (25%, 50%, 75%). Each concentration will be used against fungi cultures and a control will be included.

**REFERENCE**

Abalaka, M. E., Adeyemo, S.O. & Daniyan, S.Y. (2011). Evaluation of The Antimicrobial Potentials of Leaf Extracts of Khayasenegalensis. *Journal of Pharmaceutical Research and Opinion,* 1(2), 48-51

Adama, A.Y. & Jimoh, Y.A. (2011). Production and classification of locust beans pod ash (LBPA) as a pozzolan. Ministry of works and infrastructural development, Minna, Nigeria and Department of Civil Engineering, University of Ilorin, Nigeria. *Journal of Civil Engineering,* 5, 91-110.

Adaramola, T.F., Ariwaodo, J.O. & Adeniji K.A. (2012). Distribution of Phytochemistry and Antioxidant properties of the Genus Anonna (Mimosaceae) in Nigeria. *International Journal Pharmacogn Phytochemical Research, 4, 172-178.*

Adekunle, A. & Ikumapayi, A. (2006). Antifungal property and phytochemical  
 screening of the crude extracts of Funtumia elastic and Mallotus  
 oppositifolius. *West Indian Medical Journal,* 55, 205-2I0.

Ahmed, A., Hakan, O. & Munevver, S. (2008). Antimicrobial and antioxidant of the essential oil and methanol extract of Napata cutaria. *Journal of Microbiology,* 58,69-79.

Arias, B. A. & Ramón-Laca, L. (2005). Pharmacological properties of citrus and their ancient and medieval uses in the Mediterranean region. *Journal of Ethnopharmacology,* 97, 89-95.

Ashour, M., Wink, M. & Gershenzon, J. (2010). Biochemistry of Terpenoid monoterpenes. *Biochemistry of plant secondary metabolism* pp.258-303.

Augustin, J. M., Kuzina V., Andersen, S.B. & Bark, S. (2011). Molecular activities, biosynthesis and evolution of triter period saponin. *Phytochemistry*, 72, 435-457

Buildere, M.I., Tarfa F., & Aquiyi, J.C. (2012). The Potency of Africa Locust-beans Tree. *International Journal Pharmacogn Phytochemical Research, 4, 172-178.*

ChunYan, H., Hong, P., ZhenYu, Z. & Jing, S. (2010). Evaluation of antioxidant and antitumour activities of lemon essential oil. *Journal of Medicinal Plants Research*, 4, 1910-1915.

Cotonou, B. (2012). Medicinal plants used Some Rural District in Senegal (West Africa). *American- Eurasian Journal of sustainable Agricutlture*, 6(4), 325-332.

Cushnie, T.A. & Lamb, J.A. (2007). Antimicrobial Activity of flavonoids. *International Journal Antimicrobial,* 26, 343-356.

Dawis, E. & M. & Croteau, R. (2000). Cyclization Enzymes in the Biosynthesis of monoterpenes, Sesquiterpenes, and diterpenes*. Topics in current chemistry*, 209, 53-95.

Dhanavade, M. J., Jalkute, C. B., Ghosh, J. S. & Sonawane, K. D. (2011). Study antimicrobial activity of lemon (Citrus lemon L.) peel extract. Br J Pharmacol Toxicol 2: 119122.

Dormans, H. J. & Deans, S.G. (2009). Antimicrobial Agent from plant: Antibacterial activity of plant volatile oils. *Journal Applied Microbial*, 88, 308-316.

Flamini, E. A., John. P. O. & Rose, F. G. (2008). Enumeration of Bacterial Count for Spoil Food. *Journal of food science,* 45, 785-4563

Gamarra, F., Sakanaka, L., Tambourgi, E. & Cabral, F. (2006). Inﬂuence on the quality of essential lemon (Citrus aurantifolia) oil by distillation process. *Brazilian Journal of Chemical Engineering,* 23, 147-151.

Hammer, K. A., Carson, C.F. & Riley, T.V. (2007). Antimicrobial activity of essential oils and other plant extracts. *Journal App Microbiol*, 86, 985-900.

Heyman, H. M., Hussein, A. A., Meyer, J. J. M. & Lau, N. (2009). Antibacterial Activities of South African Medicine Plants against methicillin resistant*. Staphylococcus aureus Pharmacy and Biology*, 47, 67-71.

Invanisova, E. M., Tokar, M., Mocko, K. J., Bonjnanska, T., Marecent, J. & Mendelova A. (2013). Antioxidan activity of selectedplants products. *Journal of Microbiology Biotechnology and food science*, 2, 1692-1703.

Kaura, K., Ganglo, J.C., Assogbadjo, A.E. & Agbanla, C. (2011). Ethnic difference in use value and usepattern of Ananas Comosus in Northern Benin. *Ethnommedical,* 7, 42.

Keles, O. S., Bakirel, A.T. & Alpinar, K. (2012). Screening of some Turkish plants for antibacterial activity. *Turk. J. Vet. Anim. Sci,* 25, 559-565.

Klimek-Szczykutowicz, M., Szopa, A. & Ekiert, H. (2020). Citrus limon (Lemon) Phenomenon. *A Review of the Chemistry, Pharmacological Properties, Applications in the Modern Pharmaceutical, Food, and Cosmetics Industries, and Biotechnological Studies,* 9, 119.

Kumar, A., Narayani, M., Subanthini, A. & Jayakumar, M. (2011). Antimicrobial activity &phytochemical analysis of citrus fruit peels-utilization of fruit waste. *International Journal of Engineering Science Technology*, 3, 5414-5421.

Ludwiczuk, A., Skalicka-Woznaik, K. & Geogiev, M. I. (2017). *Terpenoids pharmacognosy,* 233-266.

Maruti, J., Dhanavade, C. B., Jalkute, J. S. & Ghosh, D. S. (2011). Study antimicrobial activity of lemon. *Br J PharmacolToxicol*, 2, 119-22.

Moosavy, M., Hassanzadeh, P., Mohammadzadeh, E., Mahmoudi, R. & Khatibi, S. (2017). Antioxidant and antimicrobial activities of essential oil of Lemon (Citrus limon) peel in vitro and in a food model. *Journal of food quality and hazards control,* 4, 42-48.

Nada, K. & Zainab, A. (2013). Antimicrobial activity of different aqueous lemon extracts. *Journal of Applied Pharm Sci.,* 33, 74-81.

Nagaraju, B., Anand, S., Ahmed, N., Chandra, J. & Ahmed, F. (2012). Antiulcer activity of aqueous extract of Citrus medica Linn. fruit against ethanol-induced ulcer in rats. *Adv Biol Res.,* 6, 24-29.

Nord, L. J. & Kenne, L. (2000). Novel acetylated triterenoid saponins in a chromatographic fraction from Quilla sa ponariamolina. Carbohydr. *Journal of Research,* 329, 817-829.

Oikeh, E. I, Omoregie, E. S., Oviasogie, F. E. & Oriakhi, K. (2016). Phytochemical, antimicrobial, and antioxidant activities of different citrus juice concentrates. *Food Sci Nutr,* 4, 103-109.

Okwu, D. E. (2008). Citrus fruits: A rich source of phytochemicals and their roles in human health. *Int J Chem Sci.,* 6, 451-471.

Persley, D. (1993). Diseases of fruit crops, Department of Primary Industries,  
 Brisbane. *International Journal Pharmacogn Phytochemical Research, 4, 172-178.*

Plaza, P., Sanbruno, A., Usall, J., Lamarca, N. & Torres, R. (2004). Integration of curig treatments with decreeing to control the main postharvcst diseases of Calementine mandarins. *Postharvest Biology Technology,* 34, 29-37.

Rafiq, S., Kaul, R., Sofi, S. A., Bashir, N., Nazir, F. & Nayik, G. (2016). Citrus peel as a Source of Functional ingredient*. A review.Journal of the Saudi Society of Agricultural Sciences,* 4, 29-37.

Samanta, A., Das, G. & Das, S. (2011). roles of flavonoids in plant. *International Journal of Pharm Sci Tech,* 6, 12-35.

Sawalha, S., Arráez-Román, D., Segura-Carretero, A. &Fernández- Gutiérrez, A. (2009). Quantification of Main Phenolic Compoundsin Sweet and Bitter Orange Peel using CE–MS/MS. *Food Chemistry,* 116(2), 567-574.

Shahnah, S. M., Ali, S. & Bagri, P. (2007). New sequitur Penetrative Derivative from Fruit Peel of Citruslemon (Linn) Burn. *F. Sci.Pharmaceutical,* 75,165-170.

Sofowara, A. (2003). *Medicinal plants and traditional medicine in Africa*. Spectrum Books Ltd, Ibadan, Nigeria.

Sparg, S., Light, M. E., Van, E. & Standen, J. (2004). Biological activities and distribution of plant Saponins*. Journal of Ethnopharmocology,* 94, 219-243.

Sridharan, B., Michael, S. T., Arya, R., Mohana, S., Ganesh, R. (2016). Beneﬁcial effect of Citrus limon peel aqueous methanol extract on experimentally induced urolithic rats. *Pharm Biol* 54, 759-769.

Tripoli, E., Guardia, M., Giammanco, S., Majo, D. & Giammanco, M. (2007). Citrus ﬂ avonoids: Molecular structure, biological activity and nutritional properties: *A review. Food chemistry,* 104, 466-479.

Turkmen, N., Velioglu, Y. S. (2007). Determination of alkaloids and phenolic compound in Black tea processed by two different methods in different plunking season. *Journal science and food Agriculture,* 87(7), 1408-1416.

Uduak, O. A., Ani, E. J., Etoh, E. & Macstephen, A. (2014). Comparative effect of Citrus sinensis and carbimazole on serum T4, T3 and TSH levels. *Niger Medicinal* *Journal*, 55, 230-234.

Umashanker, M. & Shruti, S. (2011). Traditional Indian herbal medicine used as antipyretic, antiulcer, anti-diabetic and anticancer: A review. *International Journal of Research Pharm Chem,* 1, 1152-1159.

Velu, S., Abubakar, F., Mahyudin, N. A., Saari, N. & Zaman, M. (2014). In-vitro antimicrobial activity of musk lime. *International Food Research Journal,* 12, 379-86.

Zhao, J. (2007). Nutraceuticals, nutritional therapy, phytonutrients, and phytotherapy for improvement of human health: a perspective on plant biotechnology application. *Recent Pat Biotechnol,* 1, 75-97.