**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, row 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](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2816499/#R5); [Nascimento *et al.*, 2000](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2816499/#R18)). 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](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2816499/#R18); [Rios and Recio, 2005](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2816499/#R22)).

*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 sulphoxide or sulphon which function in dermatogical 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 been 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 proof the ethnic claim of the antimicrobial effectiveness of this plant against microorganisms.

**1.3.2 Specific Objectives**

1. To investigate the antimicrobial activity of *Sesamum indicum* against some infectious bacteria and fungi (*Staphylococcus aureus, Pseudomonas aeruginosa and Candida albincans)*.
2. To determine the minimum inhibitory concentration for the selected microorganisms.
3. To examine the differences in the effectiveness of the aqueous extract obtained from the dried plant material and the extract from the fresh plant material.

**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 albincans)*.

**CHAPTER THREE**

**MATERIALS AND METHODS**

**3.1 The Study Area**

Mubi is located in the Northern part of Adamawa state between latitudes 9º 26’’ and 10º 10’’ N and longitude 13º 10’’ and 13º 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 900 33 950 North of the equator and between Longitude 30 0914019 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 int eh Local Government is Sudan Savannah; this maintains an annual rainfall ranging from 700-900m 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

Ethylacete

Methanol

Sparfloxacin

Flucozole

Soxhlet extractor

**3.3 Samples Collection**

Some samples of *Sesamum indicum* will be purchased from Mubi main market, Mubi, Adamawa State, Nigeria. They will be transported to the microbiology laboratory of Federal Polytechnic, Mubi. They will be properly identified at the herbarium, Department of Biological Sciences Technology, Federal Polytechnic, Mubi, Adamawa Nigeria. The voucher specimen number will be indicated. Thereafter, the leaves will be 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 will involve the use of a Soxhlet apparatus and a range of solvents. Each extraction process will be exhaustive using petroleum spirit (60-80oC), 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 will be operated at a temperature of 80oC. The extracts will be 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 will 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 will be tested for alkaloids, saponins, phlobotannins, tannins, flavonoids, steroids, glycosides and cardenolides.

## 3.4.2.1 Test for flavonoids

Shinoda’s test: The extract (0.5 g) to be tested will be 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 will be boiled with 5 ml of distilled water, filtered and the filtrate will be divided into two portions.

To the first portion, 3 ml of distilled water will be 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 will be 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 will be 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 will be 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 Draggendoff’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 will be 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 will be 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) will be 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 will 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 will be 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,* P*seudomonas 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 37oC for 6 hrs. Dilution of the microorganism in the normal saline continued until the turbidity marched 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 Hunton and Sabouraud dextrose agars were prepared according to the manufacturer’s instruction as recommended by NCCLS (National Committee for Clinical Laboratory Standards) Farraro *et a*l. (2000). The contents of the MIC in the serial dilution was inoculated on to the media, the media were incubated at 37oC for 24 hours for the bacteria and at 30oC 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.

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