# PHYTOCHEMICAL AND ANTIBACTERIAL ANALYSIS OF *XYLOPIA AETHIOPICA* FRUIT (NEGRO PEPPER)

**BY**


## ALAO BALIKIS AJOKE

## AST/2372070335

**A PROJECT WORK SUBMITTED TO THE DEPARTMENT OF PHYSICAL SCIENCE LABORATORY TECHNOLOGY, FEDERAL POLYTECHNIC, AUCHI EDO STATE.**

**IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF THE HIGHER NATIONAL DIPLOMA (HND) IN PHYSICAL SCIENCE LABORATORY TECHNOLOGY.**

**2022**

**CERTIFICATION**

This is to certify that the project titled: Phytochemical and Antibacterial Analysis of *Xylopia aethiopica* (Negro Pepper) was carried out by: **ALAO BALIKIS AJOKE** with **Mat. No: AST/2372070335** Of the Department of Physical Science Laboratory Technology, School of Applied Science and technology, Federal Polytechnic Auchi.

We also certify that the work is adequate in a scope and quality in partial fulfillment of the award of Higher National Diploma (HND) in CHEMISTRY/BIOCHEMISTRY.

_____

_____

**MRS ALIU MUNAYAH M.** **DATE**

(Project Supervisor)

_____

_____

**MR. BRAIMAH JAFARU** **DATE**

(Head of Department)

## DEDICATION

This project work is dedicated to Almighty Allah, Whom is able to do all things and also to my beloved family.

## ACKNOWLEDGMENT

I give thanks to Almighty Allah (S.W.T) and His Rasullahi (S.A.W) for His mercy and protection towards my life And also for making me to accomplish my goal in life.

Special thanks and appreciation goes to my project supervisor Mrs Aliu Munayah M. who despite her busy schedule took pains and time in the supervision of this project work. I wish to express my appreciation to my Head of Department, Mr Jafaru Braimah. I also wish to express my profound gratitude to all my lecturer's in the department of physical science and laboratory technology and other lecturers and non academic staff of the school of applied science and technology.

Appreciation goes to my parents Mr and Mrs Alao Agbaje Tajudeen for their support towards my education may God Almighty continue to bless and protect them.

I wish to express my appreciation to my brother's and to my late sister may God Almighty bless you all. Appreciation goes to my siblings and my daughter may God Almighty bless you all.

Finally, a big thanks to my course mates, friends, and all those who helped me in one way or the other in my academic pursuit God Almighty

bless you all.


## ABSTRACT

*The phytochemical analysis of Xylopia aethiopica was carried out using standard scientific procedures and it was revealed that the plant contain bioactive substances. Screening of this medicinal plant for bioactive compounds may lead to development of less expensive new antimicrobial agents with improved safety and efficacy. The antibacterial activity of different leaf extract against all tested bacteria at different concentrations. The result of this research has shown that only the N-Hexane extract of Xylopia aethiopica proved to have antibacterial effect more on Streptococcus sp with a mean zone of inhibition of 0.55mm and 0.25mm for N-Hexane and ethanol extract respectively. The use of plant extracts to treat diseases has stood the test of time, more than 75% pure compounds derived from higher plants are used in modern medicine and Xylopia aethiopica was well known in complementary medical practice in treatment of several ailments. It is recommended to isolate and separate the bioactive compounds responsible for this antibacterial activity using advanced scientific techniques.*

**TABLE OF CONTENT**

# CHAPTER ONE

## 1.0 Introduction

Herbal medicine or phytomedicine is acknowledged as the most common form of alternative medicine. Long in the creation of mankind, plants have been used medicinally World Health Organization (WHO) estimates that about 80% of the world's population relies on these unconventional‖ plant-based medicines as their primary medical intervention especially in the developing as well as in the developed countries where modern medicines are largely used. Scientific evaluation of ethnopharmacological information from medicinal plants is necessary for the development of accessible, affordable and high safety herbal therapies. One of such commonly used medicinal plants is *Xylopia aethiopica* (Annonaceae). Several of its documented traditional uses had been authenticated and published in several scientific journals. Thus, the need to assemble these numerous scientific findings had prompted for this present review which would draw the interest of natural product researchers throughout the world to focus on the explored potential of *Xylopia aethiopica* (Erhirhie and Moke, 2014).

*Xylopia aethiopica* is an evergreen, aromatic tree, of the Annonaceae family that can grow up to 20m high. It is a native to the lowland rainforest and moist fringe forests in the savanna zones of Africa. The dried fruits of *X. aethiopica* (grains of Selim) are used as a spice and an herbal medicine (United States Department of Agriculture USDA, 2008).

*Xylopia* is a compression from Greek (*xylon pikron*) meaning "bitter wood". The second part of the plant's binomial name, *aethiopica*, refers to the origin of the tree, in Ethiopia, though currently it grows most prominently as a crop in Ghana, Togo Nigeria and other parts of West Africa. It has its English name as **Negro pepper or grains of Selim.**

*Xylopia aethiopica* grows in tropical Africa. It is present in rain forests, especially near the coast. It also grows in riverine and fringing forest, and as a pioneer species in arid savanna regions. *Xylopia aethiopica* is used extensively in construction, African cuisine and traditional medicine (Fetse *et al.*, 2016).

The plant's bark is used to make doors and partitions. The wood is known to be resistant to termite attack and is used in hut construction: posts, scantlings, roof-ridges and joists. The wood is also used for boat construction: masts, oars, paddles and spars. In Togo and Gabon, wood was

traditionally used to make bows and crossbows for hunters and warriors (Harris *et al.*, 2013).

An infusion of the plant's bark or fruit has been useful in the treatment of bronchitis and dysenteric conditions, or as a mouthwash to treat toothaches. It has also been used as a medicine for biliousness and febrile pains. The bark, when steeped in palm wine, is used to treat asthma, stomach-aches and rheumatism.

In Senegal, the fruit is used to flavor café Touba, a coffee drink that is the country's spiritual beverage and the traditional drink of the Mouride brotherhood. In the Middle Ages the fruit was exported to Europe as a 'pepper.'(Erhirhie and Moke, 2014).

In the eastern part of Nigeria, the plant's fruit is an essential ingredient in preparation of local soups to aid new mothers in breastfeeding. It remains an important item of local trade throughout Africa as a spice, and flavouring for food and for medicine. The fruit is sometimes put into jars of water for purification purposes (Harris *et al.*, 2013).

## 1.1 Aims and objective

The aim of this research are:

- ✓ To evaluate the phytochemicals present in *Xylopia aethiopica*,
- ✓ To determine the antibacterial effectiveness of extracts of *Xylopia aethiopica*,

## 1.2 Significance of study

Plants are known to contain wide range of bioactive chemical substances that exhibits physiological and biochemical effects in human body. This study is significant as it is aimed at determining the effectiveness of extracts of *Xylopia aethiopica* used in the treatments of bacterial infections while assessing the effect on the ecosystem (the environment).

## 1.3 Limitation of study

Financial problems as well as equipment unavailability and power failure were the major limitation of this study.

# CHAPTER TWO

# LITERATURE REVIEW

## 2.1 Literature review

Dietary spices contain a wide variety of volatile and nonvolatile chemicals obtained from parts of plants such as the fruits, berries, roots, pods, and the barks, majority of which are used in herbal medicine for the treatment of diseases. The World Health Organization (WHO) gave a list of about 21,000 plants used for medicinal purposes around the world. Plants have the ability to synthesize a wide variety of chemical compounds that possess therapeutic properties and defend against attack from predators such as insects, fungi and herbivorous animals. About 12,000 of such compounds, representing less than 10 % of the total, have been extracted, isolated and characterized. These compounds have been found to exert their effects on the human body through mechanisms that are similar to those already established in conventional drugs revealing that herbal medicines may be as effective as the latter. Furthermore, the plant kingdom is believed to hold many new drug templates; hence, the continued investigation into ethno-medicinal plants (Ahamefula *et al*., 2020).

Several of its documented traditional uses had been authenticated and published in several scientific journals. Thus, the need to assemble these numerous scientific findings had prompted for this present review which would draw the interest of natural product researchers throughout the world to focus on the explored potential of *Xylopia aethiopica* (Erhirhie and Moke, 2014).

Owning to several criticisms on health risks of using antibiotics, and their ban in animal feed by European Union (EU) United States Department of Agriculture (USDA). The ban has encouraged the use of plant-based compounds called phytogenics or phytobiotics that are incorporated into diets of farm animals to improve their productivity; therefore, the search for natural feed additives as replacement for growth promoting antibiotics is gaining more research attention. Hence, African Negro pepper could be seen to have potentials to be among the league of these feed additives. The seeds and fruits are used as spices. It has pharmaceutical and medicinal properties (Ndelekwute and Enyenihi, 2018).

Negro Pepper **(***Xylopia aethiopica* **)** is an evergreen, aromatic tree, of the Annonaceae family that can grow up to 20m high. It is a native to the lowland rainforest and moist fringe forests in the savanna zones of Africa.

The dried fruits of *X. aethiopica* (grains of Selim) are used as a spice and an herbal medicine.

*Xylopia* is a compression from Greek (*xylon pikron*) meaning "bitter wood". The second part of the plant's binomial name, *aethiopica*, refers to the origin of the tree, in Ethiopia, though currently it grows most prominently as a crop in Ghana, Togo Nigeria and other parts of West Africa. It has its English name as Negro pepper or grains of Selim.

Negro Pepper **(***Xylopia aethiopica* **)** grows in tropical Africa. It is present in rain forests, especially near the coast. It also grows in riverine and fringing forest, and as a pioneer species in arid savanna regions. *Xylopia aethiopica* is used extensively in construction, African cuisine and traditional medicine (Fetse *et al.,* 2016).

The plant's bark is used to make doors and partitions. The wood is known to be resistant to termite attack and is used in hut construction: posts, scantlings, roof-ridges and joists. The wood is also used for boat construction: masts, oars, paddles and spars. In Togo and Gabon, wood was traditionally used to make bows and crossbows for hunters and warriors.

An infusion of the plant's bark or fruit has been useful in the treatment of bronchitis and dysenteric conditions, or as a mouthwash to treat toothaches. It has also been used as a medicine for biliousness and febrile pains. The bark, when steeped in palm wine, is used to treat asthma, stomach-aches and rheumatism.

In Senegal, the fruit is used to flavor café Touba, a coffee drink that is the country's spiritual beverage and the traditional drink of the Mouride brotherhood. In the Middle Ages the fruit was exported to Europe as a 'pepper.'(Erhirhie and Moke, 2014).

In the eastern part of Nigeria, the plant's fruit is an essential ingredient in preparation of local soups to aid new mothers in breastfeeding. It remains an important item of local trade throughout Africa as a spice, and flavouring for food and for medicine. The fruit is sometimes put into jars of water for purification purposes.

## 2.2 Medicinal Plants

Apart from serving as a source of food, plants invariably remain a major source of medicine in most parts of the world. This is particularly true about the people of Africa especially those in the tropical regions of

the continent. This may be due to the fact that the tropical and subtropical regions of Africa alone contain about 45,000 species of plants with potential medicinal value. Despite the overabundance, only about 5,000 of these plant species have been exploited for medicinal use so far and even much less have been investigated for corroboration of the said therapeutic use and safety (Fetse *et al.*, 2016). The emphasis on the use of medicinal plants had hitherto been placed on the treatment rather than prevention of diseases. However, there exists in the literature considerable report in recent times on research work on the use of medicinal plants and their constituents in disease prevention.

A World Health Organization (WHO) Expert Group defined Traditional Medicine as the sum total of all knowledge and practices, whether explicable or not, used in diagnosis, prevention and elimination of physical, mental, or social imbalance and relying exclusively on practical experience and observation handed down from generation to generation, whether verbally or in writing (WHO, 1976). For Africa, this may be extended further by including an expression, such as 'while bearing in mind the original concept of nature which includes the material world, the

sociological environment whether living or dead and the metaphysical forces of the universe'.

A medicinal plant is any plant which, in one or more of its organs, contains substances that can be used for therapeutic purposes or which are precursors for the synthesis of useful drugs. This description makes it possible to distinguish between medicinal plants whose therapeutic properties and constituents have been established scientifically, and plants that are regarded as medicinal but which have not yet been subjected to a thorough scientific study. A number of plants have been used in traditional medicine for many years. Some do seem to work although there may not be sufficient scientific data (double-blind trials, for example) to confirm their efficacy. Such plants should qualify as medicinal plants. The term 'crude drugs of natural or biological origin' is used by pharmacists and pharmacologists to describe whole plants or parts of plants which have medicinal properties (Obhakhan *et al*, 2014).

A definition of medicinal plants for the purpose of this presentation should include the following.

a. plants or plant parts used medicinally in galenical preparations (e.g. decoctions, infusions, etc.) e.g. Cascara bark;

b. plants used for extraction of pure substances either for direct medicinal use or for the hemi-synthesis of medicinal compounds (e.g. hemi-synthesis of sex hormones from diosgenin obtained from *Dioscorea* yams);
c. food, spice, and perfumery plants used medicinally, e.g. ginger;
d. microscopic plants, e.g. fungi, actinomycetes, used for isolation of drugs, especially antibiotics. Examples are ergot (*Claviceps purpurea* growing on rye) or *Streptomyces griseus;* and
e. fibre plants, e.g. cotton, flax, jute, used for the preparation of surgical dressings.

## 2.3 Botanic Description

Negro Pepper **(***Xylopia aethiopica* **)** is a slim, tall, evergreen, aromatic tree to 15–30 m high and about 60–70 cm in diameter with straight stem, many-branched crown and sometimes buttressed. Bark grey-brown, smooth or finely vertically fissured and peeling easily.

Leaves simple, alternate, oblong, elliptic to ovate, 8-16.5 by 2.8-6.5 cm, leathery, bluish-green and without hairs above, but with fine brownish hairs below, margin entire, and glabrous; petiole 0.3-0.6 cm, thickset and dark-coloured.

(Obhakhan *et al*, 2014).

## 2.4 Classification of *Xylopia aethiopica*

**Scientific classification of Ethiopian pepper**

**Kingdom: Plantae**

Phylum: Tracheophytes

Class: Magnoliopsida

Order: Magnolioles

Family: Annonaceae

Genus: Xylopia

Species: ***X. ethiopica***

**Binomial name**

***Xylopia aethiopica***

(Erhirhie, 2014).

## 2.5 Distribution of Xylopia aethiopica

*Xylopia aethiopica* commonly known as “African guinea pepper” or “Ethiopian pepper” is wide spread in tropical Africa, Ethiopia, Guinea, Zambia, Mozambique and Angola . In Nigeria, it is found all over the lowland rain forest and most fringe forest in the savannah zones of Nigeria. It is used as a pepper substitute in Europe and India (Obhakhan *et al.*, 2014) and highly valued in other countries because of its medicinal and pharmacological properties. The fruits are used as spices and its aqueous decoctions are used especially after child birth; probably due to its antiseptic properties to arrest bleeding. The seeds contain bitter principles, alkaloids, glycosides, saponnis, tannins, sterols, carbohydrate, protein and free fatty acid, mucilage’s and acidic compounds some of which might be responsible for the reported uses.

## 2.6 Chemical composition of *Xylopia aethiopica*

Although Herbal medicines tend to look primitive and unscientific when compared to synthetic (conventional) drugs, about 80% of the world population relies on traditional medicine for primary health care and more than 30% of plant species has been used medicinally. Though it is believed by many people that products labeled “natural” are always safe and good

for them, this is not necessarily true because some herbs such as comfrey and ephedra can cause serious harm (Chris-Ozoko *et al.,* 2015). Plants have the ability to synthesize a wide variety of chemical compounds (phytochemicals). Many of these compounds have beneficial effect on long term health when consumed by humans, and also can be used to effectively treat human diseases. Chemical compounds in plants mediate their effect on the human body in the same way like conventional drugs; thus they do not differ from conventional drugs in term of how they work. It enables them to be as effective as conventional medicine, and also gives them the same potential to cause harmful side effects.

Plants are known to contain wide range of bioactive chemical substances that exhibits physiological and biochemical effects in human body. Some spices are examples of plants with such effects (Iwu *et al*., 2022). Chemical constituents of plants reveal their medicinal values, prompting the evaluation of levels of the chemical constituents in many plants. Plants have been the source of modern drugs used in orthodox medicine. Medicinal plants play a vital role in drug discovery and are very useful for human to cure different ailments. Any plant part such as bark, leaves, flowers, roots, fruits, or seeds may contain active plant based

natural components (Imo *et al.*, 2018). Spices are commonly used for flavoring, as preservatives, and for seasoning of foods. They are rich in aromatic compounds. The use of spices in preparation of food enhances the taste of food, as well as beverage and drug. Different spices are used for different purposes. Some spices such as *Xylopia aethiopica* fruits are mostly used as condiments and ingredients in foods.

## 2.7 Phytochemical Composition of *Xylopia aethiopica*

Phytochemicals (from Greek phyto meaning : "plant") are chemical compounds produced by plants through primary or secondary metabolism. They are plant food, which may have health effects. Examples are flavonoids, phenolic acids, tannin etc. *Xylopia aethiopica* contains a number of bioactive compounds including β-pinene, 1,8-cineol, α-terpineol, terpinene-4-ol, paradol, bisabolene, linalool (E)-β-ocimene, α-farnesene, β-pinene, α-pinene, myrtenol and β-phellandrene. Therefore, its medicinal activities may be due to the presence of these bioactive compounds in it.

## 2.8 Nutritional values of *Xylopia aethiopica*

Some of the powerful properties found in *Xylopia aethiopica*– alkaloids, flavonoids, cineol, phytosterols, saponins, carbohydrates, trans-

pinocarveol, limonene, linalool, tannins, and β-caryophyllene (Abolaji *et. al.*, 2007).

Training on the phytochemical composition of Negro pepper discovered that it comprises paradol, α-terpineol, β-phellandrene, bisabolene, trans-pinocarveol, α-farnesene, linalool, myrtenol, spathulenol, verbenone, terpeness, and terpinene-4-ol.

## 2.9 Medicinal values of *Xylopia aethiopica*

*Xylopia aethiopica* possesses great nutritional and medicinal values and all the parts are very useful medicinally, although the fruits are most commonly used for therapeutic purposes.

- ✓ It can be taken as a decoction, concoction or even chewed and swallowed for the management of various aches and pains.
- ✓ *Xylopia aethiopica* is used in the treatment of a number of diseases including cough, malaria, constipation, uterine fibroid, and amenorrhea. It is also used locally as carminative, stimulant and adjunct to other remedies for the treatment of skin infection (Fetse *et al.*, 2016).

- ✓ The bark decoction is administered for the treatment of bronchitis, asthma, stomach-aches and dysenteric conditions and the infusion of the plant's bark is used in the treatment of biliousness and fever.
- ✓ The mixture of *Xylopia aethiopica* bark with palm wine is useful in the management of rheumatism, asthma, and stomach-ache. The bark is also used as a postpartum tonic and also taken to promote fertility and to ease childbirth (Obhakhan *et al*, 2014).. The dried root powder of *Xylopia aethiopica* dissolved in alcohol and administered orally as an anthelmintic and as mouth-wash to relive toothache.
- ✓ The powdered root is used as a dressing for skin sores and also rubbed on gums for gum diseases and in local treatment of cancer. The aqueous root concoction is administered after child birth as an anti-infective drug.
- ✓ The decoction is also administered as an antihemorrhagic agent.The leaves are used in traditional medicine to manage boils, sores, wounds and cuts and the decoction of the leaves and roots used as a tonic and also to treat fever and debility. Additionally, the decoction of the leaves is also used as an anti-emetic. The leaf-sap can be

administered to treat epileptic seizure. Powdered leaves are inhaled for the treatment of headaches and its decoction used to treat rheumatism (Obhakhan *et al*, 2014).

- ✓ A decoction of the fruit is useful in the treatment of asthma, bronchitis, stomach-aches and the dried fruit used to treat dysenteric conditions.
- ✓ The fruit of *X. aethiopica* is also used as a reliever of pain caused by rheumatic conditions. It is also used as a tonic to improve fertility in women and is an essential ingredient in preparation of local soups to aid new mothers in breastfeeding (Abolaji *et. al.*, 2007).
- ✓ The dry fruits are smoked like tobacco and the smoke inhaled to relieve respiratory ailments. Traditional medical practitioners and birth attendants use a decoction of the seeds to induce placental discharge postpartum due to its abortifacient effect. The crushed seeds are applied topically on the forehead to treat headache and neuralgia (Fetse *et al.*, 2016).
- ✓ The decoction of the seeds is also used as a vermifuge for roundworms.

Apart from the medicinal uses, the powdered fruits of *Xylopia aethiopica* can be mixed with shear butter and used as body creams.

- ✓ The powdered dry fruits and seeds can also be used as spice to flavour food.
- ✓ The bark of *Xylopia aethiopica* is resistant to attack by termites and as such used to make doors and partitions during construction of buildings (Abolaji *et. al.*, 2007).
- ✓ The wood is also traditionally used to make bows and crossbows for hunters.

## 2.10 Health Benefits Of *Xylopia Aethiopica*

**Has Anti-inflammatory Purposes**

Inflammation is the body's means of responding to injuries, infections, wounds, and any other tissue damage.

*Xylopia aethiopica* seed is filled with a lot of anti-inflammatory properties, including beta-caryophyllene.

**Packed with Antioxidants**

This means that this spice can be efficiently used for lessening the risk/effect of cancerous tumors.

**A Great remedy for Dermatological Problems**

If you are battling with any of the aforementioned dermatological problems, *Xylopia aethiopica* seed might be what you need.

**Treats Respiratory System Diseases**

Studies disclose that the bark of *Xylopia aethiopica* tree encompasses some potent properties, which are very active in treating respiratory system diseases, comprising the common cold, bacterial pneumonia, asthma, and bronchitis.

**Prevents/Treats malaria**

Malaria as we know is one of the life-threatening diseases globally and is mainly initiated by parasites spread to people through the bites of infected mosquitoes.

*Xylopia aethiopica* seed is filled with some effective antimalarial properties which can be used for making local remedies for reducing the effect of malaria in humans.

**Keeps Rheumatism at bay**

Rheumatism is a canopy term for diseases that cause chronic pain and inflammation in the joints, muscles, or connective tissue.

*Xylopia aethiopica* tree contains anti-arthritic, antipyretic, and anti-inflammatory properties used in treating a myriad of rheumatic disorders, as well as arthritic pain.

**Relieves Gastrointestinal Problems**

The leaves of *Xylopia aethiopica* have shown to be aseptic, which means that they are adept at relieving the aforementioned internal disorders.

**Might be used to treat Menstrual Problems**

*Xylopia aethiopica* seeds when dried are usually used in traditional medicine to improve menstrual blood flow, and for treating amenorrhea.

## 2.11 Side Effects of Negro Pepper (*Xylopia aethiopica* )

*Xylopia aethiopica* seed has been stated to induce miscarriages. Though, there is no medical evidence that connects *Xylopia aethiopica* seed and miscarriage.

Make sure to discuss with your physician before taking *Xylopia aethiopica* seeds (Obhakhan *et al*, 2014).

If you experience nausea or dizziness after consuming *Xylopia aethiopica* seed, you should stop taking it to avoid complications and seek medical help.

# CHAPTER THREE

## 3.0 Materials and Method

## 3.1 Materials

Weighing balance, Mortar and pestle**,** Beakers, Test-tubes, Flasks, Measuring Cylinders, conical flasks, pipette, rotary evaporator, autoclave, nutrient agar, round bottom flasks, petri dishes, cotton wool, forceps, Bunsen burner and glass spreader.

**Chemicals and Reagents**

N-hexane**,** Potassium hydroxide, ethanol, distilled water, chloroform, Ethyl acetate, Hydrochloric acid, Hydrogen tetraoxosulphate (VI) acid, Ethanol, Phenol all of analytical grade, Ferric chloride.

## 3.2 Collection preparation of sample:

The Negro pepper or grains of Selim *Xylopia aethiopica* were gotten from Uchi market in Auchi Metropolis. Sample was handpicked to remove dirt particles, after which it was properly washed and air-dried under room temperature. It was then measured and crushed to sizeable size using mortar and pestle, after which it was transferred immediately to the laboratory for further analysis.

### 3.2.1 Extraction

The *Xylopia aethiopica* was crushed using mortar and pestle to reduce the seed to a sizable size for easy accessibility of the solvent. After which 20g of the crushed fruits and then soaked with the appropriate solvent. After extraction the solvent was distilled off under vacuum in a rotary evaporator and kept in a beaker with an air-tight lid under cooling temperature until being used for further analysis.

## 3.3. Phytochemicals Analysis

The phytochemical analysis was evaluated with different reagents as described by Mohammed and Hamza, (2008), to determine the existence or nonexistence of saponins, alkaloids, flavonoids, glycosides, phenolic acids, phenol, steroids, terpenoid and tannins in *Xylopia aethiopica* extracts.

### 3.3.1 Test for Steroid

2 ml of the chloroform was mixed with 2ml of concentrated $H_2SO_4$, then 4ml of*Xylopia aethiopica* extract was added to the solution, the mixture was shaken for few seconds and a then a red colour was observed at the interphase at the chloroform layer, which confirm the presence of steroid in the *Xylopia aethiopica* extract.

### 3.3.2 Test for Terpenoids

2ml of *Xylopia aethiopica* extract was mixed with 3ml of chloroform. 1ml of concentrated $H_2SO_4$ was carefully added to the mixture to form a layer. A reddish-brown colouration was formed at the inter-phase, which indicates the presence of Terpeniods.

### 3.3.3 Test for Tannin

2ml of the *Xylopia aethiopica* extract was measured into a test tube and then 5% of ferric chloride was added, the mixture was allowed to react for a few seconds, and there was appearance of a violet colour, which indicates the presence of tannin.

### 3.3.4 Test For Phenolics Acids

2ml of the *Xylopia aethiopica* extract was mixed with 1% ferric chloride. The absence of the formation of deep blue or blue-black colouration is an indication of a negative result.

### 3.3.5 Test for Saponin (Froth test)

About 2ml of *Xylopia aethiopica* extract was diluted with 5ml of distilled water. The mixture was shaken vigorously; the formation of stable foam confirmed the presence the presence of saponin.

### 3.3.6 Test for Glycosides

2ml of acetic acid was added to 2ml of the extract. The mixture was cooled in cold water bath. The 2ml of concentrated $H_2SO_4$ was added. The absence of a blue to bluish-green colour indicates a negative result of glycosides.

### 3.3.7 Test for Arthraquinones

1g of the powder seed was placed in dry test tube and 20ml of chloroform was added. This was heated in steam bath for 5mins. The extract was filtered while hot and allowed to cool. To the filtrates, was added an equal volume of 10 % ammonia solution. This was shaken and the upper aqueous layer was not observed for bright pink colouration as indicative the absence of Arthraquinones.

### 3.3.8 Test for Phenol

2ml of oil extract was measured in a test-tube, few drops of 10% lead acetate was added to the *Xylopia aethiopica* extract. White precipitate was formed, which confirm the presence of phenol.

### 3.3.9 Test for Alkaloids

2ml of the *Xylopia aethiopica* extracts was added to 2ml of 10% Hydrochloric acid. To the acidic medium a drop of dragendroff's reagent

was added. There was appearance of orange red precipitate which indicated the presence of alkaloids.

### 3.3.10 Test for Flavanoids

Few drops of dilute Sodium Hydroxide were added to 2ml of the *Xylopia aethiopica* extract in a test tube, and then four drops of dilute HCl was added to the mixture. An intense yellow colour was formed which turned colorless on addition of few drops of dilute HCL acid, indicating the presence of flavonoids.

## 3.4. Antibacterial screening of *Xylopia aethiopica*

### 3.4.1 Sterilization of Materials

All glass wares were first washed with detergent and rinsed with distilled water, wrapped with aluminum foil after drying and sterilization by dry heat method in the oven at a temperature 0f $160^{O}$C for 2-3 hours.

### 3.4.2 Disinfection of Working Area

The working area were disinfected thoroughly before and after use with ethanol (75%V) cotton wool was soaked in ethanol and used to clean the working bench, a Bunsen burner was put on and the flame was allowed to, this helped in sterilizing the air in the laboratory.

### 3.4.3 Source of Test Organisms

Slants of clinically identified microorganisms that had already been characterized were collected from the Microbiology Laboratory of Auchi Polytechnic Cottage Hospital and taken to the Microbiology Laboratory of the Department of Biological Science Laboratory Technology; Auchi Polytechnic Auchi where they were treated with *Xylopia aethiopica*extracted using different solvents. The identified organisms were: *Escherichia coli streptococcus sp.* and *Staphylococcus aureus.*

## 3.5 Culture Media

The media used in this study is Nutrient Agar and the media was prepared according to manufacturer's specification.

### 3.5.1 Preparation of Nutrient Agar

28g of nutrient agar powder was weighed using a weighing balance and dispensed into a beaker; 1000mls of distilled water was measured using a measuring cylinder and dispensed into the beaker containing the agar powder; it was stirred to dissolve for 10mins. The mixture was transferred into a conical flask and the neck of the flask was corked with cotton wool wrapped in aluminum foil. It was autoclaved at a temperature of 121$^{o}$c and pressure of 15psi for 15-20minutes.the sterilized agar was

allowed to cool to about 45$^{o}$c and then aseptically poured into Petri dishes and allowed to set (Cheesbrough, 2008).

### 3.6 Antibacterial Sensitivity Testing

The disc diffusion method was used in the Antibacterial Sensitivity Testing. The antimicrobial sensitivity test was carried out using the methods modified by Isu and Onyeagba (2002). Molten sterile nutrient agars were poured into different petri dishes. After solidification cultures of bacteria were introduced into the surface of the sterile nutrient agar plate and a sterile glass spreader was used for even distribution. Sterile paper disc were cut and soaked in the different extracts and these were placed on top of the inoculated plates and the plates were incubated at 37°C for 24 hours. The plates containing the controls were incubated also. (The controls were inoculated but the extracts were not added i.e no paper disc soaked in *Xylopia aethiopica*extract).

The plates were examined after 24hours for zones of inhibition, which indicated the degree of susceptibility of the test organisms.

# CHAPTER FOUR

## 4.0 Results and Discussion

## 4.1 Result

The phytochemical compositions of X. aethiopica tested are summerised in table 1.

**Table 1: Phytochemical constituent/properties present in** *Xylopia aethiopica*

| **Properties** | **N-hexane** | **Ethanol** |
|---|---|---|
| Phenolic acids | - | - |
| Glycosides | - | - |
| Alkaloids | - | + |
| Tannin | + | + |
| Flavonoids | + | + |
| Saponin | + | + |
| Steriods | + | + |
| Phenol | + | + |
| Terpeniod | + | + |
| Phlobatannins | - | - |
| arthraquinones | - | - |

**Keys: (+) indicates presence**

**(-) indicates absence**

Table 2. Mean zone of inhibition (mm) of extracts of *Xylopia aethiopica* on some human pathogenic microorganisms (*Staphylococcus aureus*, *Streptococcus sp.* and *Escherichia coli).*

| | **Staph** | **Strep** | **E.coli** |
|---|---|---|---|
| Ethanol | - | 0.25mm | - |
| N-Hexane | 0.1mm | 0.55m | 0.2mm |

## 4.2 Discussion

The qualitative phytochemicals estimations of *Xylopia aethiopica* investigated as summarized above in Table 1, the results of analysis showed that *Xylopia aethiopica* contains most of the phytochemicals except phlobatannins and arthaquinones in both extracts. The results obtained agreed with *Aguoru et al.,* 2016. Phytochemicals as antioxidants, play a vital roles in human health. Flavoniods which are beneficial, have anti-inflammatory effect and they protect the cell from oxidative damage that can lead to disease, they

can prevent the development of cardiovascular disease, diabetes, cancer and cognitive disease like Alzheimer's and dementia. Saponins help to reduce cholesterol levels, kill disease causing bacteria, scavenge oxidative stress and inhibit tumor growth. According to recent research, they improve lipid metabolism and help prevent and treat obesity.

Phenol are known to be antioxidants, they can stop the reaction of free radicals, prevent damage of NDA as well as long-term health effects.

Alkaloids are important chemical compounds that serve as a rich reservoir for drug discovery. Several alkaloids exhibit antiproliferation, antibacterial, antiviral, insecticidal activities, antiparasitic, antiplasmodial, anticorrosive, antioxidative, anti HIV and antimetastatic effect on various types of cancer both in vitro and invivo.

Steriods are anti-inflammatory medicines used to treat a range of conditions. Terpeniods are widely acclaimed for their anticancer activity. Another study suggested that they act by suppressing the expression of breast tumor cyclin DI (Miller *et al.*, 2013). Terperniods are important because of their antidiabetic property. They also act as antidepressant. These phytochemical constituents are shown in the present study. Glycosides, Phlobatannins, anthraquinones and Phenolic acid were not present in the study.

Table 2 shows the susceptibility of *Xylopia aethiopica* extracts to some human pathogenic microorganisms (*Staphylococcus aureus, Streptococcus sp.* and *Escherichia coli)* explains their use in native medicine for the treatment of bacterial infections. The *Streptococcus sp.* were more sensitive to N-Hexane extracts of *Xylopia aethiopica* when compared to *Escherichia coli and Staphylococcus aureus.* This agrees with the observation made by some researchers that plant extracts show considerable activity against Gram positive bacteria than Gram negative bacteria (Nostro *et al.,* 2000).

The result of this research has shown that only the N-Hexane extract of *Xylopia aethiopica* proved to have antibacterial effect more on *Streptococcus sp* with a mean zone of inhibition of 0.55mm and 0.25mm for N-Hexane and ethanol extract respectively. The use of plant extracts to treat diseases has stood the test of time (Anwannil and Atta, 2006), more than 75% pure compounds derived from higher plants are used in modern medicine and *Xylopia aethiopica* ) was well known in complementary medical practice in treatment of several ailments.

The result of this analysis showed that the leaves of *Xylopia aethiopica* exhibited potential activity against *Streptococcus sp* with the zones of inhibition, while *Escherichia coli* and *Staphylococcus aureus* was resistant to ethanol extract of the plant; this completely disagrees with the work of (Nostro *et al.*, 2000) and it might be attributed to the fact that most people abuse the use of *Xylopia aethiopica* thus leading to antibiotic resistance of these organisms to extracts of the plant. Generally from the research on antibacterial activity of *Xylopia aethiopica* extract, the N-Hexane extract had more efficacy on all bacterial tested compared to the ethanol extract even when the same procedure was followed.

# CHAPTER FIVE

## 5.0 Conclusion and Recommendation

## 5.1 Conclusion

This study has shown that *Xylopia aethiopica* is a potential source of new drug for treating infections resulting from the test organisms and other clinical pathogens. The antibacterial activity of the extract could be enhanced if the components are purified. The study also indicates that extracts *Xylopia aethiopica* possesses antibacterial activity which can be explored further in the field of human medicine and that substance abuse should be avoided. Therefore further research should be carried out to exploit other solvents in extracting the bioactive constituent of *Xylopia aethiopica.*

## 5.2 Recommendation

This study highlighted the antimicrobial effects of *Xylopia aethiopica* on some known pathogens. Some antibiotics have been obsolete because of the problem of drug resistance. Thus improvement of health using herbs as raw materials should be reconsidered. *Xylopia aethiopica* is a known invasive weed in Nigeria and readily spreads with ease inhabiting any available space. The ability of *Xylopia aethiopica* exhibiting

antimicrobial activities in the current research work indicates a potential for alternative use of the weed as raw materials for the production of medicine that can be used in diseases caused by *Streptococcus sp*,. It is therefore recommended that extracts of this plant should be used in the treatment of infections like sore throat caused by *Streptococcus sp*.

## References


Abolaji, O.A., Adebayo, A.H. and Odesanmi, O.S. (2007). Nutritional Qualities of Three Medicinal Plant Parts (*Xylopia aethiopica, Blighia sapida and Parinari polyandra)* commonly used by Pregnant Women in the Western Part of Nigeria. *Pakistan Journal of Nutrition, 6: 665-668.*

Ahamefula. A. A., Ukaogo , P.O., Igara, C. E, and Obike, A. I. (2020) Chemical Constituents of Methanol Fruit Extract of Xylopia aethiopica by GC-MS and FT-IR Spectroscopy; *ARC Journal of Pharmaceutical Sciences Volume 6, Issue 1*, 2020, PP 14-24 ISSN No.: 2455-1538 DOI: http://dx.doi.org/10.20431/2455-1538.0601003.

Anwannil, H. G. and Atta, R. (2006). Trends in ethnopharmacology. J. Ethnopharmacol, 100: 43 – 49

Cheesbrough, M. (2008).District Laboratory practices in tropical Countries. Volume 2. Cambridge University Press, UK. Pp. 35-70

Chris-Ozoko, L. E., Ekundina, V. and Winiki, C. (2015) Histomorphological Effects of Xylopia aethiopica on the Liver and Kidney of Albino Wistar Rats, *Scholars Academic Journal of Biosciences (SAJB);* 2015; 3(2A):150-154. ISSN 2321-6883 (Online).

Erhirhie, E. O, and Moke, G. E. (2014) Xylopia Aethiopica: A Review of its Ethnomedicinal, Chemical and Pharmacological Properties. *American Journal of PharmTech Research* 2014. 4(6) ISSN: 2249-3387

Fetse, J. P., Kofie, W. and Adosraku, R. K. (2016). Ethnopharmacological Importance of Xylopia aethiopica (DUNAL) A. RICH (Annonaceae) - *A Review. British Journal of Pharmaceutical Research.* 11. 1-21. doi: 10.9734/BJPR/2016/24746.

Imo, C., Yakubu, O. E., Imo, N. G., Udegbunam, I. S. and Onukwugha, O. J. (2018) Chemical composition of *Xylopia aethiopica* fruits,

*American Journal of Physiology, Biochemistry and Pharmacology*, 2018 VOL 7, NO. 2, PAGE 48–53 10.5455/ajpbp.20180521064020.

Iwu, I. C., Ukaoma, A , Alisa, C., Ali, B., Obiagwu, I., Uchenna, O. L. and Anozie, R. C. (2022) Chemical And Medicinal Properties of Xylopia Aethiopica Harveste from The South Eastern Nigeria. Chem Pharm Res. 2022; 4(1): 1-9.

Mohammed M.I. and Hamza Z.U., (2008), *"Physicochemical Properties of Oil Extracts from Sesamum Indicum L. Seeds Grown in Jigawa State – Nigeria. Journal of Applied Science and Environmental Management"* 12 (2): 99 – 101

Ndelekwute, E.K. and Enyenihi, G.E. (2018), Negro Pepper (Xylopia ethiopica) in Feed is Antibacterial and Can Improve Broiler Chickens Productivity; *British Journal of Poultry Sciences* 7 (1): 10-15, 2018 ISSN 1995-901X.

Nostro, A., Germane, M. P., Angelo, V. and Marino, A. (2000). Extraction methods, and bioautography for evaluation of medicinal plant antimicrobial activity. Lett Appl. Microbiol, 30: 379-384.

Obhakhan, J.O., Ozor M.O. and Obodo B.N. (2014) Histological Effects of Xylopia Aethiopica on the Kidney of Adult Wistar Rats. *International Journal of Herbs and Pharmacological Research IJHPR*, 2014, 3(1): 2 – 7. ISSN: 2315-537X.

WHO, author. *The Promotion and Development of Traditional Medicine. Report of a WHO meeting.* Geneva: World Health Organization; 1978. Technical report series 622.