A COMPARATIVE ANTIMICROBIAL ASSESSMENT OF THE AQUEOUS AND METHANOL EXTRACTS OF *Pentaclethra macrophylla* BENTH. BARK ON SELECTED MICROORGANISMS

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(BIOLOGICAL SCIENCE TECHNIQUES)

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A PROJECT WORK SUBMITTED TO THE DEPARTMENT OF SCIENCE LABORATORY TECHNOLOGY (BIOLOGICAL SCIENCE TECHNIQUES), FACULTY OF LIFE SCIENCES, UNIVERSITY OF BENIN, BENIN CITY IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF BACHELOR OF SCIENCE DEGREE (B.Sc.) IN SCIENCE LABORATORY TECHNOLOGY

OCTOBER, 2025

CERTIFICATION

This is to certify that this project work was carried out by **Uyi Friday ENEKPOA** with Matriculation Number **"LSC2007291"**, of the Department of Science Laboratory Technology (Biological Science Techniques), Faculty of Life Sciences, University of Benin, Benin City under my supervision.

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DEDICATION

I dedicate this project work to God Almighty "whose love for me is the strongest of all love", for His Grace, directions and guardians in the completion of this project.

ACKNOWLEDGEMENTS

With great gratitude, I am grateful to Almighty God for His love, favour and provisions throughout the period of this project work.

To my project supervisor Dr. Peter Onezeyi Obaro and his wife Dr. Mrs. Obaro-Onezeyi for their parental disposition towards me and constructive criticisms made this project a reality.

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ABSTRACT

Pentaclethra macrophylla belongs to the Mimosaceae family, the different parts have been used traditionally for a host of different diseases and ailments over the years including dysentery, influenza, diabetes and jaundice. The antibacterial assessment of the bark part of *Pentaclethra macrophylla* was carried out against gram-positive (*Staphylococcus aureus* and *Bacillus cereus*) as well as gram-negative (*Pseudomonas aeruginosa and Escherichia coli*). The tree bark of *Pentaclethra macrophylla* was extracted with methanol and water (aqueous), at concentrationns of 500 mg/ml 250 mg/ml, 125 mg/ml, 62.5 mg/ml and 31.25 mg/ml. Antibacterial assay was carried out using Agar Well Diffusion method (A.W.D.). Statistical significant difference ($P<0.05$) was observed in the antimicrobial activities between the methanol and aqueous extracts and the standard antimicrobial drugs. The results of this study revealed that the methanol exhibited more significant antimicrobial activities in both gram positive and negative microorganisms. The results obtained revealed that both extracts could contain active antimicrobial compounds which may inhibit the growth of microorganisms associated with infectious diseases in *in-vitro* conditions. This study has provided the basis for the use of *Pentaclethra macrophylla* bark in the treatment of diarrhoeal, dysentery, and wound infection. This study does not only show the scientific basis for some of the therapeutic uses of this plant in traditional medicine, but also confirms the fact that ethno-botanical approach should be considered when investigating antimicrobial properties of plants. There is also need for the incorporation of its methanol and aqueous extracts of *P. macrophylla* bark in the manufacture of new drugs against common infections caused by the organisms whose growth was inhibited. Thorough works still needs to be done to harness the full antibacterial potentials of this plant for an improved health status of man as well as its nutritive potentials. These findings therefore justify its high acceptability in traditional medicine and the therapeutic uses.

CHAPTER ONE

1.1 BACKGROUND OF STUDY

The medicinal uses of poly herbal formulations were idealized by Ayurvedic medicine. Poly herbal formulation involves the use of three or more plant mixtures with various herbal products to eradicate diseases and their causes, restore balance and create a healthy lifestyle that is helpful in the prevention of recurrence of imbalance (Parasuraman *et al.,* 2014).

Species survival has always been dependent on the species relationship with other organism and their immediate environment. Since the beginning of mankind, man has always depended on animals for food and labour, while also depending on plants not only as a source of food and oxygen but also as source of medicine (Abel and Busia, 2024; Kumar *et al.*, 2021). In many countries of the world, extracts of different parts (leaves, stem, flower, root and seeds) of local plants have been used as remedy diseases (Schneider, 2022). Plants parts have been known to serve as reservoir of several complex and simple chemicals of high usefulness to man (Fachrurozi *et al.*, 2021). These chemicals have several yet untapped potentials including giving plants protective abilities against insects, rodent pest and even microbes (Cometa *et al.,* 2022; Fachrurozi *et al.*, 2021).

Phytochemical screening is one of the necessary steps to find out the chemical constituents which lead the isolation of bioactive compounds. Since the 19th century different bioactive constituents of plants have been isolated and characterized to show the medicinal activity as well as physiological activity (Ganguly *et al.,* 2023). These phytochemicals hold the key to the alternative control of even resistant species of bacteria and human pathogens and their uses have been shown to have a scientific basis. Many of these are used as the active ingredients of the modern medicine or as the lead compound for new drug discovery (Mathias *et al.,* 2024).

Bacterial and fungal infections are a serious problem in the world today. Micro-organism related diseases, as at 2016 were ranked amongst the top 10 causes of death in man (WHO, 2018). Third world countries with inadequate sanitation and primary health care programs are worse hit by microbial infections (Anwana *et al.,* 2022) As a consequence, there has been a sharp increase in the research of anti-microbial effects of different plants which can serve as cure to micro-

organism related illness (Anyanwu and Okoye, 2021). Already, at least 40% of pharmaceuticals are gotten from plants, animals, bacteria and fungi (Anwana *et al.,* 2022).

In Nigeria, a lot of plants have been studied for their anti-microbial properties using various methods to carry out this experiment. Examples of such plants include, *Mallotus oppositifolius, Pterocarpus osun, Vitex doniana, Eucalyptus camaldulensis,Cassia alata, Trichophyton rubrum, Gossypium arboretum, Baphia nitida, Garcinia kola, Carica papaya, Parkia globosa, Lawsonia inermis, Ageratum conyzoides, Acalypha wilkesiana, Butyrospermum paradoxum, Trichilia heudelotti, Aspilia africana, Maytenus senegalensis, Bridellia ferruginea, Cnestis ferruginea, Trichosanthes cucumerina, Luffa cylindrical,Momordica charantia, Xylopia aethiopica, Colocasia esculenta, Amaranthus hybridus, Ocimum gratissimum, Piper guineense, Basella alba, Hoslundia opposita, Maerua angolensis, Tridax procumbens, Trichilia roka, Anthocleista djalonen, Senna alata, Combretum tarquense, Dillenia indica, Phychitria brenani. Parkia biglobosa, Allium cepa, Juglan nigra, Moringa oleifera,* and *Anacardium occidentale* (Iwu *et. al.,* 2023). It is important to note that in the above named plants study, different parts of the plants were used for the study including, seeds, leaves, stems, barks and roots.

In this study, the comparative antimicrobial assessment of the aqueous and methanol extracts of *Pentaclethra macrophylla* bark on selected microorganisms using Agar Well Diffusion (AWD) method at different concentrations.

AIM AND OBJECTIVES

AIM

To study the comparative antimicrobial assessment of the aqueous and methanol extracts of *Pentaclethra macrophylla* bark on selected microorganisms

OBJECTIVES

The aim was achieved by the following objectives:

- Carry out the extraction of aqueous and methanol extracts of *Pentaclethra macrophylla* bark
- Determine the antibacterial effect using methanol and aqueous (water) extracts.
- Determine the Minimum Inhibitory Concentration (MIC) and zone of inhibition of the *Pentaclethra macrophylla* bark using methanol and aqueous extracts

CHAPTER TWO

2.1 LITERATURE REVIEW

The use of Plants for the treatment of diseases has been widely done in different parts of the world.Certain plants contain therapeutic properties and traditional medicine utilizes these plants in the treatments of ailments and procurement of cures for diseases. The medicinal properties of plants are as a result of the various array of bioactive compounds, such as alkaloids, flavonoids, tannins, phenolics, and terpenoids present in them.These compounds are sought for their antimicrobial, anti-inflammatory, antioxidant, and anticancer effects (Atanasov *et al*., 2022). These compounds have been used in both traditional medicine and modern pharmaceutical developments to treat and prevent diseases.

The use of plants for medicinal purposes dates back thousands of years, with evidence from ancient civilizations such as the Egyptians, Chinese, and Indians who documented the use of herbs in treating ailments. An example is, Artemisia annua, used in traditional Chinese medicine, led to the development of artemisinin, a highly effective antimalarial drug (Tu, 2021). The ongoing use and reference to plants as a source of healing continues to inspire modern drug discovery.

2.1 *Pentaclethra macrophylla* Benth

The *Pentaclethra macrophylla* Benth of the genus *Pentaclethra macrophylla* belongs to the Mimosaceae family. The genus *Pentaclethra* contains only three species; *Pentaclethra eetveldean*, *Pentaclethra macroloba* and *Pentaclethra macrophylla* (Lee *et al,* 2023)

Plants of the genus *Pentaclethra* have an ethnopharmacological background in the African traditional medicine where they are used for the treatment of several diseases such as itch, gonorrhoea, diarrhoea, small pox and infertility (Leonti, 2024))

Photochemical studies previously carried out revealed that the genus *Pentaclethra* plants are a rich source of metabolites such as alkaloids, saponins, tannin, Flavonoids and monoglycerides (Aiello, 2023).

Chemical investigation of the seeds of P. macrophylla led to the isolation of two diterpernoids, secopentaclethrolide and secopentaclethroside; one alkaloid, caffeoylputrescine and one glycerol derivative, glyceryl monotetracosanoate (folefoc *et al*., 2004) . Recent pharmacological studies on stem bark, and leaves of this plant revealed its antinociceptive , antidiarrheal , antimicrobial Nneoma et al. (2016) and hepatoprotective activities (Bomning *et.al.,* 2021)

2.2 Common names: African Oil bean, Congo acacia (English name), Ugba ,Ogba (Igbo name), Okphaga ,Akphaga (Edo name)

2.3 Taxonomy

The taxonomy of Pentaclethra macrophylla Benth., commonly known as African oil bean, can be structured as follows:

- Kingdom: Plantae (Plants)
- Sub kingdom: Viridiplantae (Green plants)
- Infra kingdom: Streptophyta (Land plants)
- Super division: Embryophyta

- Division: Tracheophyta (Vascular plants)
- Subdivision: Spermatophytina (Seed plants)
- Class: Magnoliopsida (Dicotyledons)
- Order: Fabales
- Family: Fabaceae (Legume family)
- Subfamily: Caesalpinioideae
- Genus: Pentaclethra
- Species: Pentaclethra macrophylla Benth.

This classification categorizes *Pentaclethra macrophylla* within the Fabaceae family, a group which is known for its leguminous plants and nitrogen-fixing properties, that improves soil fertility and support agroforestry practices in tropical regions (Orwa *et al*., 2022).

2.3.1 Fruits or Seeds: Dark brown or blackish in colour,Oblong pod,Rounded at the apex and tapering at the base, enclosing 5-8 large, flat, smooth seeds. The fruit is up to 50 cm long, 10cm wide and 2cm in thickness.

2.3.2 Tree and Plant: Large , grows to about 36 meters tall. Mature trees have brown barks, are scaly and low branching. The tree's base has thick buttressed roots and the trunks are twisted (Voorhoeve, 2021).

2.3.3 Flowers: Flowers are fragrant and pale yellow in colour. The flowering period is usually between February and April.

2.3.4 Leaves: The leaves of *P. macrophylla* are long and measure up to 45cm in length. They are alternate, bippinately compound in arrangement, 9-13 pairs of opposite pinnae with 8-14 pairs of

leaflets per pinnae.

The leaflets are shaped rhombic like, stipule is 3-5mm long and petiole can be up to 8cm long. Leaflets are oblong in outline, 1.2-2.5cm long with a width of 5-8mm.The apex of the leaflets is round and the base unequal, either cube are or articulate.

Calyx tube is 0.7mm long, calyx love are 5 and up to 0.5mm long, peaks are also 5 and up to 3mm long (Herbert *et.al.,* 2023)

The leaves of *Pentaclethra macrophylla* Benth have the following features:

- Type: Compound leaves (pinnate)
- Arrangement: Alternate
- Leaflets: Usually large, elliptical to ovate in shape, with entire or slightly lobed margins
- Size: Can be quite large, often reaching lengths of 25-30 cm
- Color: Dark green, with a glossy surface
- Venation: Prominent venation with a palmate pattern

Plate 2.1: Leaves of *Pentaclethra macrophylla* Benth. plant

2.4 Traditional uses of *Pentaclethra macrophylla* Benth.

A concoction of fermented extract of seeds of *P. macrophylla* has been known to be a potent treatment in the management of malnutrition, gastrointestinal diseases and dental problems.

The bark, fruits, seeds and the leaves are used as anthelmintics, they are used in the treatment of gonorrhea, convulsion, and used as an analgesic . The oil extracted from the seeds is used as remedy against pruritus, worms, and dysentery (Kamanzi *et.al*., 2002). In Nigeria traditional medicine, the seeds are used for the treatment of diabetes (Neosu *et.al*., 2017) In addition, it is used for the treatment of itching and pain in animals and in man (Okorie *et.al*., 2024). A decoration of the bark is used as topical treatments for sores and wounds .Oil extracts from the seeds are used to make butter "Owala", an ingredient used in making lubricants, candies and ointments in central Africa (Olotu *et.al*., 2024)

2.5 Phytochemical Properties of *Pentaclethra macrophylla*

Pentaclethra macrophylla is a plant rich in bioactive compounds that are of important use in traditional medicine. Phytochemical screenings of various parts of the plant, including seeds, bark, and leave, has revealed several important compounds with potential medicinal benefits.

2.5.1 Alkaloids

Alkaloids are defined as chemically organic compounds that contain nitrogen and are of natural origin. However, these compounds are mainly found in plants but also in certain bacteria, fungi and animals. They are consumed in plants, fruits, seeds, leaves and stems that produce or contain

alkaloids. This type of compounds may be characterized according to the way they are structured, their biological and ethnological origins. They are derived from achieved using some bio synthetic pathways including that of lysine, tyrosine, tryptophan, Ornithine and others.

Pentaclethra macrophylla contains alkaloids, which play a wide array of biological functions, with antimicrobial, anti-inflammatory, and analgesic activities included. Some works have documented their contribution towards the antimicrobial efficacy of plants (Ogbu *et al*., 2021).

2.5.2 Flavonoids

Flavonoids are known to be polyphenol compounds that are soluble in fruits, grains, vegetables and in the barks and roots.

Latest research on flavonoids indicates a rise in inhibition of disease conditions among individuals; this is mainly reducing the levels of inflammation, levels of oxidative stress and regulation of dysfunctions in metabolism. For instance, it was found that cyanidin -3-O-glucoside (C3G), which is isolated from the fruit such as bayberry, was capable of curing diarrhea after antibiotics by adjusting intestinal bacteria and inhibiting the nuclear factor-kappa B (NF-kB) (Wang *et al*., 2023).

Flavonoids are primarily found in *Pentaclethra macrophylla* and have been known for their antioxidant activity of scavenging free radicals extending the life span of the cells against factors associated with oxidative stress.

It has also been said that flavonoids possess antimicrobial and anti-inflammatory activities, which aids the use of the plant for therapeutic purposes (Adeoye *et al*., 2020).

2.5.3 Tannins

Tannins are polyphenol biomolecules having various subunits and are found in the leaves, stems, barks, flowers, roots, fruit and seeds of plants. These tannins bind with starch, cellulose and minerals and act as precipitating agents for proteins. Tannins are visible as flaky, powdery, yellowish clumps. They are present in numerous plants all over the world, showing variations in amount and concentration, but lower plants like fungus and algae have minimal tannin concentrations. Tannins are also abundant in tree barks where they serve as an anti-microbial protection and shield for the tree.

Tannins are found in *Pentaclethra macrophylla* where they also show powerful antimicrobial action by disrupting cell membrane and proteins of microbial cells. These tannins have additional astringent and antibacterial properties which may be useful for this plant in wound healing and treating infections (Udo *et al*., 2021).

2.5.4 Saponins

Saponins are naturally occurring phytochemicals that are present in plants .They are known for their distinctive foaming characteristic, their bitter taste and astringency. They are found in both edible and inedible parts of plants such as the leaves, stem and bark. They are also produced by some bacteria and lower marine animals. They are naturally occurring glycosides that composed of carbohydrate part (glycone) and non-carbohydrate part (aglycone).The aglycone moieties are

often called sapogenins .

Saponins are surface active agents with foaming, detergent, emulsifying and wetting properties.

They contain phytoconstituents that are useful in pharmaceutical industries, food production and in cosmetics. They are present in *Pentaclethra macrophylla* where they exhibit antimicrobial, anti-inflammatory, and immune-boosting and Immunomodulation properties. Saponins may also contribute to the plant's anti-fungal activities by disrupting fungal cell membranes (Nwokeke *et al*., 2021).

2.5.5 Terpenoids

Terpenoids which are also known as isoprenoid form a large and diverse category of organic compounds that occur naturally and are derived from five carbon isoprene units. Terpenoids are similar to terpenes in structure but unlike terpenes, terpenoids have other functional groups which mostly include oxygen (Gershenzon and Dudareva, 2023).

Terpenoids studies have shown them to have antibacterial, antitumor, anti-inflammatory, and antioxidant activities (Lange and Turner, 2022). Likewise, these chemicals are used in the production of agrochemicals, perfumes and pharmaceutical products (Chaturvedi *et al*., 2021).

The synthesis pathways of terpenoids, predominately in plants, are of current scientific interest because they could be applied in biotechnology, medicine and pharmaceuticals because of their ability to modify key biological processes, these compounds have also been explored for their medicinal use with emphasis on cancer treatment (Xu *et al*., 2023).

The leaves of *P. macrophylla* contain terpenoids, they have been shown to possess antibacterial,

antioxidant and anti-inflammatory and immunomodulating properties.Terpenoids enhances the plant's defense system against fungal and bacterial pathogens (Akaneme and Ofor, 2023).

2.5.6 Phenolic Compounds

It is well known that phenolic compounds possess potent antioxidant properties which assist in minimizing cellular oxidative damage. In *Pentaclethra macrophylla* these compounds are also believed to work towards antimicrobial activities possibly by means of blocking bacterial enzymes and breaking down cell walls (Okonkwo *et al*., 2022).

The rich phytochemical profile of *Pentaclethra macrophylla*, including alkaloids, flavonoids, tannins, saponins, terpenoids, and phenolic compounds, underpins its diverse biological activities. These compounds collectively contribute to the plant's antimicrobial, antioxidant, and anti-inflammatory properties, making it a valuable resource for traditional and modern medicinal applications.

2.6 Pharmacological Properties of *P. macrophylla* Benth

2.6.1 Anti- ulcer properties

Recent research has found that *P. macrophylla* possess some anti-ulcer properties and this can be attributed to the presence of flavonoids, tannins, alkaloids and saponins present in it. These phytochemicals are believed to contribute to protection of the gastric mucosa.

Studies have shown that the *P. macrophylla* plant has cytoprotective effects as it increases the production or secretion of gastric mucus which serves to protect against irritants thereby

preventing the formation of ulcers (Nwosu *et al*., 2024)

Gastric acids are stomach fluids and digestive fluids that are responsible for aiding food digestion. They are secreted by the gastric glands in the stomach lining and consists primarily of Hydrochloric acid (HCL), Potassium Chloride (KCL) and Sodium Chloride (NaCl).An excess secretion of gastric acid causes damage to the protective lining of the stomach and ultimately ulcer. *P. macrophylla* is said to reduce gastric acid secretion in the stomach thereby reducing the formation of ulcers (Anosike *et al*., 2025)

A study discovered that the methanol extract of *Pentaclethra macrophylla* showed significant anti-ulcer activities in experimental rats by reducing ulcer index score. This is said to be as a result of the flavonoid content of the plant which aids in mucosal defense (Ugochukwu *et al*., 2020)

Another study reported that the ethanol extract of *P. macrophylla* demonstrated anti-ulcerogenic properties in pre-clinical trials, particularly in stress induced ulcer models (Obidike and Salawu, 2018)

These studies lend support to the use of *Pentaclethra macrophylla* in traditional medicine for treating ulcers, and its effects are comparable to standard anti-ulcer drugs.

2.6.2 Anti-inflammatory and analgesic activities

Pentaclethra macrophylla has been explored for its anti-inflammatory and analgesic properties. These properties are attributed to the rich content of bioactive compounds particularly flavonoids and saponins. These compounds modulate inflammatory processes by inhibiting pro-

inflammatory cytokines and mediators such as prostaglandins, nitric oxide (NO) and tumor necrosis factor-alpha (TNF-α) that are relevant in inflammatory responses.

Some studies suggest that the extracts of *P. macrophylla* can stabilize cell membranes, preventing the release of lysosomal enzymes that contribute to tissue inflammation (c *et al*., 2014).

Studies have discovered that the methanol extract of *Pentaclethra macrophylla* reduced carrageenan-induced paw edema in rats. The study linked the anti-inflammatory effect to the inhibition of cyclooxygenase (COX) enzymes, which are keys in prostaglandin synthesis (Eze *et al*., 2021).

2.6.3 Anti-oxidant activity

Oxygen is essential for all living things and it plays a major role in enzymatic processes and oxidation-reduction .Oxygen plays an important role in the metabolism of humans and is responsible for electron transport that results in ATP production. When unpaired single electrons are transferred they lead to the production of free radicals. Free radicals are highly reactive and unstable molecules that are eager to interact chemically with other molecules. Oxygen, nitrogen and sulfur are the elements from which free radicals are formed. Reactive oxygen species ROS which includes superoxide (02), hydroxyl(OH), alkoxyl (RO), peroxyl (ROO) and nitric acid (NO) are examples of oxygen centered free radicals. Living things produce ROS as a byproduct of metabolism and the maintenance of the internal environment of the body depends on the production of these ROS (Huyut *et al*., 2022). When there is an imbalance between ROS and anti-oxidant defenses, oxidative stress occurs.

Anti-oxidant properties of any plant is considered relevant in curbing oxidative stress and so far studies have focused on the stem bark, seed and leaves of *P. macrophylla* and this has led to the discovery of bio active substances such as flavonoids ,phenolics and flavonoids that are useful in neutralizing free radicals and oxidative stress related diseases.

A 2023 study on the methanol extract of the stem bark revealed that it efficiently scavenged DPPH radicals, with inhibition rates reaching 93.93% at a concentration of 250 µg/ml. It also demonstrated nitric oxide scavenging activity (49.47%) and modulated antioxidant enzymes like catalase and superoxide dismutase in vivo, suggesting its role in reducing oxidative stress and improving antioxidant defenses in biological systems (Nnamani *et al*., 2023).

2.6.4 Anti-microbial activities

Research has shown that *Pentaclethra macrophylla* Benth contains bioactive compounds that are responsible for its antimicrobial activity. These compounds include tannins, saponins, alkaloid and flavonoids and they exhibit potential as broad-spectrum antimicrobial agent against gram-positive and gram -negative bacteria and some fungi (Nwokeke *et al*., 2021).

Research has shown the antibacterial effects of *Pentaclethra macrophylla* on different pathogenic bacteria such as *Escherichia coli* (*E. coli*), *Salmonella typhi*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*. A study on the ethanol extract of *P. macrophylla* seeds and results showed a significant inhibition of *Staphylococcus aureus* and *E. coli*, comparable to standard antibiotics (Uhegbu *et al*., 2024).

A study noted the methanol extract of the leaves of *Pentaclethra macrophylla* showed substantial antibacterial activity against multi drug resistant *Pseudomonas* strains, making it a promising

choice for future drug development (Okorie *et al*., 2022).

These studies and many others showcase the anti-microbial properties present in *Pentaclethra macrophylla*.

Another research study carried out investigated the antibacterial effects of methanol extracts from *Pentaclethra macrophylla* seeds. The research revealed considerable activity against *Staphylococcus aureus*, *Escherichia coli*, and *Salmonella typhi*. The results showed that *P. macrophylla* extract was effective and showed similar traits with standard antibiotics (Okonkwo *et al*., 2022)

The plant *P. macrophylla* Benth has been found to contain certain anti-fungal properties. A study on the anti-fungal properties of the plants seed extracts against *Candida albicans* and *Aspergillus* fumigatus showed a dose dependent inhibition of fungal growth with higher concentrations of the plant extract showing greater anti-fungal activity (Udo *et al*., 2021)

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Collection of Plant samples, Identification and Authentication

The plant sample *Pentaclethra macrophylla* bark was collected from capitol behind University of Benin, Benin city. The plant sample was authenticated by Professor H.A Akinnibosun.

3.2 Drying of the Plant

The bark of the plant were cut to smaller pieces and air dried at room temperature for 7 days. After 7 days of air drying, the sample was placed into an oven (model: COV-8320-C) for 72 hours at 45^{o}C. The dried sample was crushed into fine powder using an electronic blender (model: Silver Crest SC-1589). The fine powder was placed in an air tight container for further analysis (Obaro *et al*., 2024).

3.3 Extraction Process Using Cold Maceration Method

The 500 g powdered plant sample was weighed and placed into two (2) glass container each and

2.5 litres of methanol solvent and water was added respectively to each glass container. The respective mixtures were macerated with spatulas, then covered tightly and shaken vigorously and frequently. The container with the sample and methanol was then kept in a dark cupboard for 72 hours while the other was kept for 24 hour. Keeping the mixture in a dark cupboard was done to prevent distortion and damage of the phytochemicals present.

The mixtures were respectively brought out of the dark cupboard, shaked and poured into different cheese clothes over two bowl to filter. The shaft was macerated and squeezed out leaving behind the concentrates. The respective concentrates were poured into separate crucibles and placed in a water bath and concentrated at 45^0C for 24 and 72 hours respectively. The concentrates were completely dried in the oven at 45^0C for another 24 and 72 hours respectively. The dried extracts were stored in air tight containers and placed in the refrigerator (model: TR-131L) for further use.

3.4 Anti-bacterial Assay

Anti-bacterial assay was studied using the methods described by Pâmela *et al*. (2012). pathogenic bacteria cultured for twenty-four hours comprising of gram-negative (*Pseudomonas aeruginosa* and *Escherichia coli*) and gram-positive (*Staphylococcus aureus* and *Bacillus cereus*) bacteria were used for the *in-vitro* antibacterial assay. All micro-organisms were obtained from the laboratory stock of the Department of Pharmaceutical Microbiology, Faculty of Pharmacy, University of Benin.

Antimicrobial agents: pefloxacin 5µg/ml, clotrimazole cream 1mg/ml were used as standard reference drugs.

3.5 Procedure for Antimicrobial Activity Determination

Overnight broth cultures were used to obtain 0.5 standards of bacterium which were used to seed sterile

Mollen nutrient agar medium maintained at 45^0 C. Sabour and dextrose agar medium were similarly seeded with fungi. Seven holes (6 mm) respectively were bored in each of the plates (9 cm diameter) with an aseptic cork borer, when seeded plate had solidified 500, 200, 100, 50, 25, 12.5 and 6.5 mg/ml of the extract was prepared with distilled water by preparing a stock solution and carrying out double-fold dilutions on it. With the aid of a syringe, the wells were filled with 0.25 ml (5 drops) of different dilutions of the extract while the centre wells were filled with 20 ug/ml of standard drug. Diameters of zones of inhibition were determined after incubating plates at 37^o C for 24 hours. This was done for the two (2) different extracts.

3.3 Data Analysis

Results from the studies were taken as the Mean ± SEM. Statistical analysis was arrived at using graph pad prism 8 version software (UK). Comparisons amongst treated and control groups were analysed using one-way ANOVA by, Dunnett's multiple comparisons test. $P < 0.05$ was regarded as indicating significant differences.

CHAPTER FOUR

RESULTS

4.1 Effect of *Pentaclethra macrophylla* bark on Antibacterial assay

4.1.1 Effect of Methanol and Aqueous Extracts of *Pentaclethra macrophylla* bark on *Pseudomonas aeruginosa*

Table 4.1: Effect of Methanol and Aqueous Extracts of *Pentaclethra macrophylla* bark on *Pseudomonas aeruginosa*

Results are presented in mean ± SEM (n=3), * **=** $p \leq 0.05$ ** **=** $p \leq 0.01$; *** **=** $p \leq 0.001$ **compared to minimum inhibitory concentration (62.5 mg/ml)**

Keys:

MEPMB= ***Methanol Extract of Pentaclethra macrophylla*** **Bark**

AEPMB= ***Aqueous Extract of Pentaclethra macrophylla*** **Bark**

4.1.2 Effect of Methanol and Aqueous Extracts of *Pentaclethra macrophylla* bark on *Staphylococcus aureus*

Table 4.2: Effect of Methanol and Aqueous Extracts of *Pentaclethra macrophylla* bark on *Staphylococcus aureus*

Results are presented in mean ± SEM (n=3),*= $p \leq 0.05$ **= $p \leq 0.01$; ***= $p \leq 0.001$ compared to minimum inhibitory concentration (62.5 mg/ml)

Keys:

MEPMB= *Methanol Extract of Pentaclethra macrophylla* Bark

AEPMB= *Aqueous Extract of Pentaclethra macrophylla* Bark

4.1.3 Effect of Methanol and Aqueous Extracts of *Pentaclethra macrophylla* bark on *Bacillus cereus*

Table 4.3: Effect of Methanol and Aqueous Extracts of *Pentaclethra macrophylla* bark on *Bacillus cereus*

Results are presented in mean ± SEM (n=3),*= $p \leq 0.05$ **= $p \leq 0.01$; ***= $p \leq 0.001$ compared to minimum inhibitory concentration (62.5 mg/ml)

4.1.4 Effect of Methanol and Aqueous Extracts of *Pentaclethra macrophylla* bark on *E. coli*

Table 4.3: Effect of Methanol and Aqueous Extracts of *Pentaclethra macrophylla* bark on *E. coli*

Results are presented in mean ± SEM (n=3),*= $p \leq 0.05$ **= $p \leq 0.01$; ***= $p \leq 0.001$ compared to minimum inhibitory concentration (62.5 mg/ml)

CHAPTER FIVE

DISCUSSION

CONCLUSION

This study has provided the basis for the use of *Pentaclethra macrophylla* bark in the treatment of diarrhoeal, dysentery, and wound infection. This study does not only show the scientific basis for some of the therapeutic uses of this plant in traditional medicine, but also confirms the fact that ethno-botanical approach should be considered when investigating antimicrobial properties of plants. There is also need for the incorporation of its methanol and aqueous extracts of *P. macrophylla* bark in the manufacture of new drugs against common infections caused by the organisms whose growth was inhibited. Thorough works still needs to be done to harness the full antibacterial potentials of this plant for an improved health status of man as well as its nutritive potentials. These findings therefore justify its high acceptability in traditional medicine and the

therapeutic uses.

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APPENDIX I

TABLE 1.0 : Methanol extract against *Pseudomonas aeruginosa*

METHANOL (mg/ml)	500	250	125	62.5	31.25
PLATE 1	25mm	14mm	6mm	NA	NA
PLATE 2	24mm	12mm	10mm	5mm	NA
PLATE 3	22mm	16mm	8mm	4mm	NA

TABLE 2.0 : Aqueous extract against *Pseudomonas aeruginosa*

AQUEOUS EXTRACT (mg/ml)	500	250	125	62.5	31.25
PLATE 1	17mm	10mm	8mm	NA	NA
PLATE 2	20mm	15mm	10mm	NA	NA
PLATE 3	18mm	13mm	6mm	NA	NA

TABLE 3.0: Hot water extract against *Pseudomonas aeruginosa*

HOT WATER (mg/ml)	500	250	125	62.5	31.25
PLATE 1	30mm	17mm	12mm	NA	NA
PLATE 2	27mm	20mm	8mm	NA	NA
PLATE 3	26mm	21mm	6mm	NA	NA

TABLE 4.0: Ethanol extract against *Pseudomonas aeruginosa*

ETHANOL (mg/ml)	500	250	125	62.5	31.25
PLATE 1	26mm	19mm	11mm	NA	NA
PLATE 2	31mm	14mm	7mm	NA	NA
PLATE 3	28mm	15mm	7mm	NA	NA

TABLE 5.0: Methanol extract against *Staphylococcus aureus*

METHANOL (mg/ml)	500	250	125	62.5	31.25
PLATE 1	20mm	18mm	6mm	NA	NA
PLATE 2	28mm	16mm	12mm	NA	NA
PLATE 3	26mm	14mm	10mm	NA	NA

TABLE 6.0: Cold water extract against *Staphylococcus aureus*

COLD WATER (mg/ml)	500	250	125	62.5	31.25
PLATE 1	31mm	25mm	12mm	NA	NA
PLATE 2	33mm	28mm	10mm	NA	NA
PLATE 3	31mm	24mm	8mm	NA	NA

TABLE 7.0: Hot water extract against *Staphylococcus aureus*

HOT WATER (mg/ml)	500	250	125	62.5	31.25
PLATE 1	28mm	11mm	NA	NA	NA

PLATE 2	27mm	13mm	NA	NA	NA
PLATE 3	29mm	12mm	NA	NA	NA

TABLE 8.0: Ethanol extract against *Staphylococcus aureus*

ETHANOL (mg/ml)	500	250	125	62.5	31.25
PLATE 1	26mm	19mm	8mm	NA	NA
PLATE 2	27mm	17mm	10mm	NA	NA
PLATE 3	29mm	14mm	7mm	NA	NA

TABLE 9.0: Methanol extract against *Bacillus cereus*

METHANOL (mg/ml)	500	250	125	62.5	31.25
PLATE 1	16mm	15mm	3mm	NA	NA
PLATE 2	22mm	14mm	8mm	NA	NA
PLATE 3	21mm	16mm	5mm	NA	NA

TABLE 10.0: Cold water extract against *Bacillus cereus*

Methanol Extract	500	250	125	62.5	31.25
PLATE 1	16mm	9mm	NA	NA	NA
PLATE 2	21mm	11m	NA	NA	NA
PLATE 3	17mm	7mm	NA	NA	

TABLE 11.0: Hot water extract against *Bacillus cereus*

Aqueous Extract (mg/ml)	500	250	125	62.5	31.25
PLATE 1	29mm	14mm	9mm	NA	NA
PLATE 2	26mm	18mm	7mm	NA	NA
PLATE 3	27mm	12mm	8mm	NA	NA

TABLE 12.0: Ethanol extracts against *Bacillus cereus*

METHANOL (mg/ml)	500	250	125	62.5	31.25
PLATE 1	26mm	12mm	4mm	NA	NA
PLATE 2	28mm	11mm	6mm	NA	NA
PLATE 3	26mm	10mm	4mm	NA	NA

APPENDIX II

MÜLLER-HINTON AGAR

- Beef extract: 2g
- Casein hydrolysate: 17.5g
- Starch: 1.5g
- Agar: 17.0g

Dissolved in 1 liter of distilled water

CIPROFLOXACIN

- Croscarmellose sodium
- Microcrystalline cellulose
- Povidone
- Magnesium stearate