# ANTIMICROBIAL ACTIVITY AND PHYTOCHEMICAL CONSTITUENTS OF *Justicia carnea* (JEHOVAH'S WITNESS PLANT)

**BY**

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**UNIVERSITY OF BENIN,**

**BENIN CITY.**

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**B.Sc (Delsu, Abraka)**

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**DECEMBER, 2023**

## CERTIFICATION

This is to certify that this research was carried out by Omamuyovwi Blessed IGHO-DARO in the Department of Microbiology, Faculty of Life Science, University of Benin, Benin City.

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## CERTIFICATION OF THESIS

We the undersigned attest and declare that the thesis of Omamuyovwi Blessed IGHO-DARO titled; Antimicrobial Activity and Phytochemical Constituents of *Justicia carnea* (Jehovah's Witness Plant) has successfully passed the anti-plagiarism test and does not violate any copyright regulations.

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## DEDICATION

This work is dedicated to God Almighty for His grace, mercy and provision granted unto me to complete this research. May His name be highly exalted.

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## ABSTRACT

*Justicia carnea* (Jacobinia or Jehovah's witness plant in Nigeria) is a medicinal plant used widely in Nigeria and reported to have blood-boosting ability. It is also reported to have diverse antimicrobial functions.

The plant, *Justicia carnea* was subjected to the soxhlet method of extraction. The aqueous and ethanol extracts were screened for antimicrobial effects against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Proteus mirabilis*. Antimicrobial activity was determined using agar well diffusion method. The minimum inhibitory concentration (MIC) and minimum bacteriocidal concentration (MBC) were determined by broth microdilution method. Phytochemical constituents were also evaluated. Toxicity of the extracts was done by evaluating the haematological and histopathological effects on Wistar albino rats. The animals were randomly grouped into seven groups of three rats with each group receiving distilled water (control), MIC of aqueous and ethanol extract, MBC of aqueous and ethanol extract, four times the MIC of aqueous extract and four times MIC of ethanol extract respectively.

The ethanol extract was seen to be the most active against all the species. Zones of inhibition for the aqueous extract ranged from 8.1mm to 21.4mm, while that of the ethanol extract ranged from 10.2mm to 21.8mm. The lowest MIC and MBC were observed against *Proteus* mirabilis. Phytochemicals present were alkaloids, flavonoids, saponins, terpenoids, glycosides, anthraquinones, phenolics, tannins and steroids. There was a slight increase in red blood cells, platelet counts, and packed cell volume of blood, but however not significant. A decrease was observed with the white blood cells. Histopathological examination of the liver and kidney showed an adverse pathological effect. The result of the study suggests that both extracts of *Justicia carnea* have high antimicrobial activity.

# CHAPTER ONE
# INTRODUCTION

## 1.1 BACKGROUND OF STUDY

*Justicia carnea* is a medicinal plant used widely in Nigeria which is reported to have antimicrobial functions including blood-boosting potential.

The traditional form of medicinal practice is an important part of the primary health care delivery system in most developing countries. Plants have been used in traditional medicine and it has been estimated that 70-80% of the world population rely on herbal medicine for their primary health care needs (Adenuga *et al.,* 2020).

The plant *Justicia carnea* also called the Jehovah's witness plant, Brazilian plume flower, Brazilian-plume, flamingo flower or Jacobinia, is a perennial plant growing up to 1.5 to 2m tall and is native to the Atlantic Forest Ecoregions of Eastern Brazil (Natural Resources Conservation Service, 2015). It is a tender ornamental shrub with soft-felt leaves and stunning pale pink, plume-like flowers. It belongs to the *Acanthaceae* family, and is widely distributed in the tropics and subtropics (Correa and Alcantara, 2012). It is also generally rich in vitamins and minerals (Faiza *et al.,* 2013).

*Justicia carnea* is a medicinal plant used widely in Nigeria and reported to have a blood-boosting potential (Onyeabo *et al.,* 2017). Several species of *Justicia* has also been reported to have diverse antimicrobial functions (Igbinaduwa *et al.,* 2020). In various parts of Africa, several species of *Justicia* are used in traditional medicine for treatment of anaemia, inflammation, respiratory and gastrointestinal diseases (Onyeabo *et al.,* 2017). These antimicrobial functions possessed by the plant is associated with its bioactive constituents comprising mainly phenols and flavonoids (Uroko *et al.,* 2017).

Previously, it has been reported that the leaves of *Justicia carnea* contains high quantity of phytochemical constituents such as saponins, alkaloids and terpenoids (Onyeabo *et al.,* 2017). Phytochemical constituents are important compounds found in medicinal plants that are not essential for the normal functioning of the human body, but are active and possess beneficial effects on health or in the elimination of diseases (Boyer and Liu, 2004). These phytochemical constituents are derived from plant extracts and it is reported that 85% of traditional medicine is also derived from plant extracts (WHO, 2002).

In Nigeria, the leaves of *Justicia carnea* are usually prepared with edible vegetables to make food, boiled separately in water or prepared by cooking with other medicinal plants for therapeutic purposes. Despite the various uses of medicinal plants, preliminary toxicity studies remains an essential tool to ensure the safe consumption of plants and prevent unexpected toxicity that could arise from long term exposure.

## 1.2 AIM AND OBJECTIVES

The aim of this study was to evaluate the antimicrobial activities of three selected plants; *Bryophyllum pinnatum, Justicia carnea* and *Phyllanthus nururi* on the organisms; *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* and *Proteus mirabilis,* with a view to conducting further studies on the most active one.

The specific objectives were to:

1. perform a preliminary antimicrobial studies on the three (3) plants.
2. determine the qualitative and quantitative phytochemical constituents of the most active plant; *Justicia carnea.*
3. determine the antimicrobial activity of the aqueous and ethanol extracts of the plant; *Justicia carnea* on *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* and *Proteus mirabilis.*
4. assess the toxicity of *Justicia carnea* by hematological analysis of Wistar albino rats administered with aqueous and ethanol extracts of *Justicia carnea.*
5. study the histopathological effects of *Justicia carnea* on the liver and kidneys of Wistar albino rats, if any.

# CHAPTER TWO

# LITERATURE REVIEW

## 2.1 *Justicia carnea:*

*Justicia* is the largest genus of flowering plants in the family *Acanthaceae*. It has about six hundred(600) recognized species and are found in the pantropical and tropical climate areas, some of which are; *Justicia flava, Justicia gendarussa, Justicia adhatoda, Justicia secunda, Justicia sanguis, Justicia pectoralis, Justicia carnea*, e.t.c. The genus, Justicia is named after the eighteenth century Scottish Botanist, James Justice and it is an ornamental plant widely distributed in various parts of Africa. It is cultivated in home gardens in West and Central Africa especially in Nigeria, Ghana, Guinea, Togo, Benin Republic, Sierra Leone, Cameroon and Congo (Osioma and Hamilton-Amachree, 2017). The *Justicia* plants belong in the tropical to warm temperate regions, including Africa, America and india, and are evergreen perennial plants. They are shrubs or sub-shrubs with strongly-veined leaves and lip-shaped corolla, (Austin, 2004; Dorling, 2008).

The plant, *Justicia carnea* belonging to the family of *Acanthaceae*, is native to the tropics and subtropics (Correa, 2012). As reported by Wasshausen and Wood (2004), its common name in Brazil and South America, includes; Brazilian plume, flamingo flower, Jacobinia, pine-bur begonia, pink Jacobinia, pink tongue's, Kings crown and cardinal's guard. In Ghana, it is known as ‘Ntumunum’, and in Cameroon as, ‘Ewomalajia’. In Nigeria, it is commonly called, 'Hospital too far’ or ‘bloodroot’. It is known by the Igbos, an ethnic group in Nigeria, as ‘ogwu-obara’ meaning blood builder, by the Yorubas as ‘ewe eje’ meaning blood leaf or ‘ewe ajeri’ meaning

Jehovah witness leaf, because it is seen as a substitute for blood boosting for Jehovah's witnesses, and by the Urhobos as 'ebe obara' also meaning blood leaf.

Parker and Pearson, 2012 stated that the plant, *Justicia carnea* is generally considered as an ornamental plant; an upright evergreen shrub with large, dark-green leaves about ten(10) inches long (25cm) and pink –flower plumes of about eight (8) inches long (20cm). It is best grown in moist, humus, well-drained soils and it is also grown in Nigeria around homes.

### 2.1.1 Taxonomy:

The *Justicia carnea* plant is classified according to its taxonomic units into the Kingdom - plantae, subkingdom – viridiplantae, infrakingdom – streptophyta, superdivision – embryophyta, division – tracheophyta, subdivision – spermatophyta, class – magnoliopsida, superorder – asteranae, order – lamiales, family – acanthaceae, genus – justicia, and species – carnea.

Plate 1.1: *Justicia carnea*

**2.2 Medicinal uses of plants:**

Traditional medicine, which is the use of plants or herbal products, is known as the primary health care system mostly preferred in many rural communities, because of its efficacy and easy accessibility. As defined by the World Health Organization (WHO) (2002), it is the total knowledge, skills and practices based on the theories and beliefs of the ways of life of different people, used in the maintenance of health, prevention and treatment of diseases (Adeeyo *et al*., 2018).

Plants, aside being a source of food are also used for medicinal purposes. Plants or herbal products are used generally in traditional medicine for the treatment of diseases, especially infectious diseases (World Health Organization, 2002). Infection is a common source of problem for man, and infectious diseases are the sixth major cause of deaths prematurely in the world, as they bring about threat to human life (Howard and Fletcher, 2012). Though, conventional medicines have effective therapy for the treatment of these infectious disease, they do have the problem of resistance to microorganisms. However, plants and herbal products are used all over the world for the treatment of these infectious diseases, caused by various microorganisms.

The results of Oladele *et al*., (2019), stated that the root and leaf extracts of the plant, *Terminalia glaucescens* was effective against *Salmonella* infections, and also against the organism, *Eschericha coli*. Hassan (2016), also reported that the leaves of *Ficus exasperata* are used for the treatment of infectious diseases and inflammatory conditions, due to its result on the inhibition of the growth of gram-positive organisms. Also some medicinal plants like *Terminalia chebula*, commonly known as black or chebulic myrobalan, native to South Asia; *Syzygiumcumini*, known as Jam; *Euphorbia serpens*, native to South America; and *Artemisia*

*ludoviciana*, when investigated showed great activity on the bacteria, *Vibriocholera* and its infection (Patra and Bag, 2009; Sharma *et al*., 2009; Payne *et al*., 2015; Sanchez *et al*., 2010). Findings from Cichewicz and Thorpe (1996), proved that chile peppers (capsaicin), though used as food items, has an inhibitory activity against bacterial infections. Jones et al., (1997), also reported that chile peppers (capsaicin) showed a bacteriocidal effect on the bacteria, *Helicobacter pylori*. The latex of pawpaw (*Carica papaya*), when investigated by Osato *et al*., (1993), was found to be inhibitory against the organism, Bacillus subtilis, *Enterobacter cloacae, Escherichiacoli, Salmonella typhi, Staphylococcus aureus* and *Proteus vulgaris*. The investigation of the plant, *Vernonia amygdalina* also showed its antimicrobial activity against *Vibrio cholerae* (Barbier *et al*., 2017).

**2.3 Active ingredients in plants:**

Phytochemicals, meaning plant chemicals are biologically active natural chemical properties of plants, such as amino acids, proteins, chlorophyll, sugar, alkaloids, flavonoids, phenol etc. These phytochemicals are active ingredients in plants with therapeutic properties. There are over four thousand (4000) phytochemical constituents that have been documented and classified by their physical, chemical and protective characteristics, of which a hundred and fifty (150) constituents have been studied (Sharma and Kumar, 2013). Majority of these phytochemicals possess beneficial activities like anti-microbial, anti-malarial, anti-cancerous etc., and these properties have helped in the use and exploration of plant resources in recent years (Vivekraj *et al*., 2017). These constituents also provide plants with protection against attack by insects and diseases caused by microorganisms. They include alkaloids, flavonoids, phenols, steroids, glycosides, etc.

### 2.3.1 Modes of action of the active ingredients in plants:

**i. Alkaloids:**

These are a class of naturally occurring basic organic compounds with at least one nitrogen atom. They contain carbon, hydrogen and nitrogen and may also contain oxygen, sulphur, chlorine, phosphorus and bromine. They are also classified into the following group; true alkaloids, which contains nitrogen in the heterocyle and originates from amino acids eg. atropine, nicotine and morphine, these besides having nitrogen in the heterocycle also contains terpene eg. evonine, or peptide fragments eg. ergotamine; protoalkaloids, which contains nitrogen, but not from the heterocyle and originates from amino acids, eg. mescaline, adrenaline, ephedrine; polyamine alkaloid derived from spermidine, spermine, putrescine; peptide and cyclopeptide alkaloids; and pseudoalkaloids, which are alkaloid compounds that do not originate from amino acids eg. steroid like alkaloids, purine-like alkaloid eg. caffeine, theobromine, theacrine and theophylline (Dimitris *et a*l., 1997).

Alkaloids are present in plants; they are found in the roots, seeds, fruits, leaves or bark, and depending on the type of plant, the highest amount can be found in the leaves. Accordimg to the reports of Robbers *et al*., (1996), most alkaloids are used in traditional or modern medicine for drug discovery, and they can also be toxic for example, atropine. They serve as defence chemicals in plants against herbivores, bacteria, fungi and viruses. In fulfilling this function, they react with specific targets in herbivores, predators, microorganisms, thereby inhibiting or disrupting the vital processes of these organisms (Wink and Latz-Bruning, 1995). They also act as protection of plants from attack by predators and also help in the regulation of plant growth (Chik *et al*., 2013). Chik *et al*., (2013) also stated that alkaloids perform various pharmacological

activities such as antimalarial eg. quinine, anticancerous, eg. homoharringtonine, antibacterial eg. chelerythrine, etc.

**ii. Flavonoids:**

These are a group of phytochemicals. They are secondary metabolites that are present in plants, fruits and seeds, and they also provide colour, fragrance and flavour to plants. Flavonoids are also found in beverages and foods such as wine, beer and tea, but fruits, vegetables, flowers and seeds have the highest amount (Rodriguez-Garcia *et al*., 2019). They are classified into flavones, flavanones, flavanols (catechins) and anthocyanins.

They function in the regulation of plant growth, attract pollinating insects and protect plants against biotic stress and abiotic stress (De-Luna *et al*., 2020). They also protect plant from the ultraviolet rays of sunlight (Winkel-Shirley, 2001).

De-Groot (1994), reported that the most described property of flavonoids is its antioxidant activity, that is, scavenging oxygen-derived free radicals, that are produced during the metabolism of oxygen. Due to its antioxidant activity, they are used in food, cosmetic and pharmaceutical industries (Kumar and Pandey, 2013). They also act as being bacteriocidal and bacteriostatic by destroying the cell membrane of bacteria, disrupting the synthesis of nucleic acid and energy metabolism against different microorganisms. The reports of Zhao *et al*., (2018) stated that they also possess anti-cancer, anti-oxidant, anti-inflammatory and anti-microbial properties.

**iii. Saponins:**

The name, Saponins is derived from the Latin word, ‘sapo’ meaning ‘soap’. They have the characteristic nature of producing foams, which is a soapy nature, they possess diverse pharmacological properties and produce inhibitory effects on inflammation. They act as anti-

microbial, anti-inflammatory, insecticidal and pesticidal (Francis *et al*., 2002). Elekofehinti *et al*., (2021), also reported that they also have anti-cancerous activity by inducing cytotoxic effects on cancer cells.

**iv. Terpenoids:**

These are also known as 'isoprenoids', they are organic chemicals occurring naturally. They are the largest class of plant secondary metabolites and they function in plant growth and development, and also defend plant against attack by predators and pathogens, and abiotic stress factors. Thay act as anti-tumour, anti-inflammatory, anti-malarial, anti-bacterial, anti-fungal and anti-viral agents; and also possess hypoglycemic activity (Graziose *et al*., 2010).

**v. Phenols:**

These are the largest group of phytochemicals. They are compounds with one or more aromatic rings (eg. benzene ring) and one or more hydroxyl group. In plants, they offer protection against ultraviolet radiation, pathogens, and also contribute to their colouration. From the reports of Li *et al*., (2014), phenols make up foods such as fruits, vegetables, cereals, olive, legumes, chocolates etc, and beverages such as tea, coffee, etc. The investigations of Tanwar and Ranji (2012), stated that they possess anti-microbial, anti-inflammatory and anti-oxidant activities.

**vi. Steroids:**

These group of secondary metabolites in plants are derived from cholesterol. They possess a broad range of biological activities, and are essential for the growth and development of plant, reproduction and plant responses (Amoo *et al*., 2011).

**vii. Tannins:**

They are called tannic acid. They are found in the roots, barks, leaves and fruits of many plants. They defend plants against herbivores and regulate plant growth. Praveen and Kumud (2012),

reported that these chemical components of plants, tannins, act as anti-nutrients by disrupting the absorption of iron content and other nutrients in plants by the process of reducing the action of digestive enzymes. They also possess anti-microbial and anti-oxidant activities.

**viii. Glycosides:**

These components are natural substances that contain carbohydrate. They protect plants from bacteria and diseases are are involved in the cell wall metabolism of plants. They also possess anti-inflammatory activity. (Mamta *et al*., 2013).

These active ingredients in plants, known as phytochemicals, act through different mechanisms of action, such as damage bacterial membrane, suppress virulence factors, inhibit the activity of enzymes and toxins, inhibit the synthesis of peptidoglycan and bacterial biofilm formation as reported by Rasooli *et al*., (2008).

## 2.4 Phytochemicals in *Justicia carnea*:

The reports of Igbinaduwa *et al*., (2020), stated that the plant, *Justicia carnea* contains phytochemicals such as saponins, glycosides and flavonoids. Ajuru et al., (2021), also reported that *Justicia carnea* contains phytochemicals such as tannins, flavonoids, alkaloids, saponins, glycosides, phenols, terpenoids, steroids etc, and also stated that the presence of these phytochemicals in the plant is an indication that it is a good source of essential nutrients and phytonutrients. Essential nutrients which could be used in diets to supplement the nutritional status of humans and animals, and as phytonutrients which indicates that the plant possesses a strong pharmacological and antimicrobial activity which makes it of good therapeutic use. Phytonutrients have numerous health benefits such as antimicrobial, anti-inflammatory, anti-diabetic and anti-hypertensive properties as reported by Kasote *et al*., (2015).

Most medicinal properties exhibited by plants are as a result of their bioactive constituents of phytochemicals of mainly phenol and flavonoids (Uroko *et al*., 2017). Flavonoids and phenols as powerful water-soluble anti-oxidants, makes the plant, *Justicia carnea* able to prevent oxidative cell damage, reduce the possibility of having conditions caused by excess oxidative stress, and this property is attributed to it's anti-oxidant activity (Okwu, 2004). Other phytochemicals present in the plant, such as alkaloids, terpenoids, saponins, steroids etc, makes the plant effective against microorganisms, due to their antimicrobial and anti-inflammatory activities (Igbinaduwa *et al*., 2020). Uroko *et al*., (2015), also investigated and reported that the presence of saponins in the plant makes it of good medicinal use.

Anarado *et al*., (2021), reported the presence of flavonoids with the highest percentage of 11.83% followed by steroids with 11.67%, saponins with 4.58%, alkaloids with 3.63%, phenolics with 3.50% and tannins with 0.51%. Onyeabo *et al*., (2017), also reported the presence of steroids (4.18%), alkaloids (12.21%), flavonoids (4.90%), terpenoids (15.78%), phenols (5.51%), with saponins (17.02%) as the highest and tannins (0.01%) as the lowest percentage present. The reports of Orjiakor *et al*., (2019), in the "Nutritive properties of aqueous extract *Justiciacarnea* leaves", stated the presence of phytochemicals and their percentages as follows; alkaloids (5.77%), flavonoids (7.06%), glycosides (5.39%), carbohydrate (4.10%), saponins (1.18%), reducing sugars (5.91%), terpenoids (0.30%), with phenols having the highest percentage of 9.19% and tannins having the lowest percentage of 0.70%. Other investigations also stated the presence of alkaloids (3.6%), saponins (4.6%), phenolics (3.5%), steroids (11.7%), with a highest percentage of 11.8% for flavonoids and the lowest percentage of 0.5% for tannins (Udedi *et al*., 2020).

## 2.5 Uses of *Justicia carnea:*

*Justicia carnea* is a medicinal plant that is generally used as a blood tonic for a long time in Nigeria. It has been reported to be rich in both macronutrients such as nitrogen, potassium, calcium, magnesium etc, and trace elements such as chlorine, iron, boron, manganese, zinc, copper etc, of which calcium and iron are in high quantities (Faiza *et al*., 2013).

The plant, *Justicia carnea*, functions in having a blood boosting ability. It boosts or increases the production of blood, by increasing red blood cells, platelet counts and haemoglobin levels in the body, due to the high concentration of iron present in it. Its function of having a blood-boosting ability was also investigated and confirmed by Onyeabo *et al*., (2017). In this study, the extract of the plant was able to reverse the anaemic conditions of the rats.

The plant is also used as a powerful antioxidant due to the presence of various phytochemical components. The leaves of the plant has also been reported to possess anti-diabetic properties in a study that showed that the methanol leaves extract of Justicia carnea exhibited anti-diabetic activities against alloxan-induced diabetes in albino rats (Ukpabi-Ugo *et al*., 2020).

## 2.6 Antimicrobial activity:

The antimicrobial activity of *Justicia* is linked to the presence of various phytochemicals such as flavonoids, terpenoids, saponins etc, as stated by Sonal *et al*., (2011). Correa (2012), reported that several species of *Justicia* are widely used in the treatment of inflammation, respiratory and gastrointestinal diseases, caused by various microorganisms.

Previous studies by Hernando *et al*., (2002), on the invitro bioactivity of the plant, *Justicia secunda* leaves extracts, a species of *Justicia* from the *Acanthaceae* family, demonstrated an effective activity against gram-positive organisms with no activity against gram-negative strains of *Enterobacteriaceae*. Ayodele *et al*., (2020), in their research on the *Justicia secunda* leaves

extract stated that the extract of the plant had activity against the organisms; *Bacillus cereus* and *Listeriamonocytogenes* at 37.5mg/ml, *Staphylococcus aureus* at 150mg/ml and *Pseudomonas aeruginosa* at 18.75mg/ml, while the methanol extract had a minimum inhibitory concentration of 18.75mg/ml on all the organisms, and the ethanol extract had the same minimum inhibitory concentration of 18.75mg/ml for all the organisms except *Pseudomonas aeruginosa* at 75mg/ml.

Another specie of *Justicia, Justicia adhatoda* leaf extract also showed antibacterial activity against *Salmonella typhi, Pseudomonas aeruginosa* and *Bacillus substilis* (Shinwari *et al*., 2009). Agyare *et al*., (2013), investigated the antibacterial activity of *Justicia flava*, another specie on the organisms; *Staphylococcus aureus, Bacillus substilis, Escherichia coli and Pseudomonas aeruginosa*, and reported that the plant extract was active against the organisms at their inhibition zones of 10mm, 10.50mm, 10mm and 10mm respectively.

*Justicia carnea* has been investigated to be used for the treatment of malaria, cancer, inflammation, anaemia, diabetes and typhoid (Komlaga *et al*., 2015; Udedi *et al*., 2020). Anarado *et al*., (2021), reported that the extracts of the plant, *Justicia* carnea showed antibacterial activity against *Staphylococcus aureus, Aspergillus spp* and *Bacillus spp* when investigating the antimalarial activity of the plant, and this antimalarial activity was validated and confirmed. Other investigations of Anarado *et al*., (2021), also showed activity against *Staphylococcus aureus* (21.50mm) and *Escherichia coli* (18mm).

The reports of Ojeaga (2023), stated that the aqueous leaf extract of *Justiciacarnea*, in also determining it's antibacterial activity was effective against the organisms; *Staphylococcus aureus, Klebsiella pneumonia, Salmonella typhi, Escherichia coli* and *Pseudomonas aeruginosa.*

**2.7 Anti-inflammatory activity:**

Inflammation is the mechanism of the body in response to infections by microorganisms, burns, injuries etc, that may endanger human health (Shah *et a*l., 2011).

Jacob *et al*., (2013), stated that, inflammation can be acute, which occurs as a quick response to trauma, or chronic which occurs as response to a long-term condition and can lead to death. The reports of Chen *et al*., (2017), states that clinically, it can be characterised by edema, redness, pain and loss of tissue function. This process of inflammation involves changes in the flow of blood, tissue destruction, increased vascular permeability and local inflammatory mediators such as prostaglandins, leukotrienes, cyclooxygenases etc. (Christiakov *et al*., 2018).

A Specie of *Justicia, Justicia gendarussa* possesses anti-inflammatory activity by inhibiting the release of the hormone, prostaglandin or other inflammatory mediators from the cell membrane by initiating the stability of the membrane (Mamta *et al*., 2013). Other species of *Justicia* such as, *Justicia flava, Justicia adhatoda* also possess anti-inflammatory activity (Correa and Alcantara, 2012).

**2.8 Anti-oxidant activity:**

Oxidation is the combination of a substance (chemical) with oxygen, and it involves the loss of electrons. Anti-oxidants are substances that prevent or act against oxidation.

The plant, *Justicia carnea* has been reported to have an antioxidant ability. The reports of Igwe *et al*., (2022), showed that *Justicia carnea* had a higher nitric oxide scavenging ability when compared with another plant, *Costus afer*, in the invitro antioxidant screening of their ethanol leaves extracts, and the result of this research confirmed that *Justicia carnea* could act as an antioxidant. Udedi *et al*., (2020), also investigated the antioxidant activity of the ethanol extract of *Justiciacarnea* by determining the presence of flavonoids, phenols, ascorbic acid and beta-

carotene, and by using diphenyl-1-picrylhydrazyl (DPPH) reducing antioxidant power as a control. The result of this research showed that the leaf extract contained a high amount of flavonoids, phenols, ascorbic acid and beta-carotene, and also displayed a more powerful antioxidant activity. Other reports also demonstrated its antioxidant activity (Iwetan *et al*., 2022). Other species of *Justicia*, such as *Justicia adhatoda, Justicia gendarussa, Justicia flava* and *Justicia acuminatissima*, also possess antioxidant activity (Correa and Alcantara, 2012).

**2.9 Haematological activity:**

Haematology is the study of blood and its components, such as white blood cells (WBC), red blood cells (RBC), platelets etc.

Following the conventional use of the plant as a blood booster, Onyeabo *et al*., (2017), in the research on the haematological and biochemical studies on *Justicia carnea* leaves extract in phenylhydrazine-induced anaemia in albino rats stated that, the plant was able to reverse the anaemic conditions of the rats by increasing their packed cell volume (PCV) of blood level, red blood cell, haemoglobin and platelet count level. Orjiakor *et al*., (2019), also proved and confirmed the blood-boosting ability of the plant by showing an increase in the blood level of albino rats when the haematological parameters were analysed. The investigations of Igbinaduwa *et al*., (2020), also confirmed the haematological and anti-anaemic properties of the plant.

The reports of Akintimehin *et al*., (2021), stated and further concluded that, due to the plant's abundance in iron content, it is able to increaXse red blood cells.

Many drugs have been formulated from plants, for example, the drug, aspirin developed from the plant, *Filipendula ulmara*, known as the 'queen of the meadow', is an anti-inflammatory drug as reported by Balick (1996). Emetine, also known as isoquinolone alkaloid, is developed from the

plant, *Cephaelis ipecacuanha*, which is an antifungal drug and also used for the treatment of abscesses caused by *Escherichia histolytica* infections. Others are morphine from the plant, opium poppy *(Papaver somniferum)*, etc.

Despite the therapeutic importance of medicinal plants, toxic substances have been shown to be present in large numbers of plants examined (Mouanaga *et al*., 2015). However, contamination of plants should be avoided as there have been reports of such.

Olaniyan *et al*., (2016), reported that contaminatioXn of plants may occur due to the presence of contaminants, such as heavy metals, aflatoxin and pathogenic microorganisms from the soil or in their process of herbal preparations. These contaminated plants being consumed without determining it's efficacy and safety can lead to unexpected toxic effects, which results in the changes in the functions of different organs in the body, as hepatic and renal damage has been linked recently to the use of medicinal plants in the treatment of various diseases (Mapanga and Musabayane, 2010).

# CHAPTER THREE

# MATERIALS AND METHODS

## 3.1 Materials:

### 3.1.1 Collection and identification of plants:

The fresh leaves of the three (3) selected plants; *Bryophyllum pinnatum, Justicia carnea* and *Phyllanthus nururi* were collected from Upper-Agbarho town, Ughelli-North Local Government Area, Delta State, Nigeria. The leaves were identified in the Department of Plant Biology and Biotechnology, University of Benin, Benin City and a voucher number, UBH-J386 was deposited.

### 3.1.2 Collection of test organisms:

The organisms used in this study includes three (3) isolates each of *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* and *ProXteus mirabilis,* isolated fromclinical samples and obtained from the Microbiology LaboraXXX X tory, University of Benin Teaching Hospital, Benin City, Edo state. The isolates were transported to the laboratory in sterile plastic bags and were then tested for viability by resuscitating them in peptone water, subcultured onto a nutrient agar medium and incubated at 37$^{o}$C for 24 hours. Their identities were also confirmed using cultural, morphological and biochemical tests (Cheesebrough, 2000).

## 3.2 Methods:

### 3.2.1 Preparation of plants for extraction:

The leaves were washed with distilled water and air dried for two weeks. They were then grounded (pulverized) into powdered form with a milling machine and weighed.

### 3.2.2 Extraction procedure:

The leaves were extracted using the soxhlet extraction methodXX . The solvents used were aqueous(distilled water) and ethanol. For the aqueous extraction of the powdered form of *Bryophyllum pinnatum* leaves, the solvent (distilled water) was poured into the round bottom flask, which is placed on the heating mantle and the apparatus was fixed. One hundred and fifty (150) grams of the sample was poured into the extraction thimble, which was tied and placed in the soxhlet apparatus, coupled with the jolambo cooling system. The extraction was done for 6 hours until the solvent leaving the extraction thimble into the round bottom flask became clear. It was then filtered using a filter paper to remove residues. The filterate was evaporated to dryness using a water bath and the extract was obtained. The extract was then weighed and placed in a sample container. This process of extraction was repeated for the ethanol solvent extraction of *Bryophyllum pinnatum.*

This soxhlet extraction procedure was also repeated for the leaves of *Justicia carnea,* using one hundred and ten grams (110g), and *Phyllanthus nururi* using one hundred and fifty grams (150g), both of which also used aqueous (distilled water) and ethanol as solvents.

The yields of the aqueous and ethanol extracts of the plants; *Bryophyllum pinnatum, Justicia carnea* and *Phyllanthus nururi* were weighed, placed in sample containers and kept in the refrigerator at 4$^{o}$C for use (Luque de Castro and Garcia-Ayuso, 1998).

### 3.2.3 Preparation of Media:

The media used were prepared according to the manufacturer's instructions. They include Nutrient agar, Nutrient broth and Mueller-Hinton agar, and were sterilized by autoclaving at 121$^{o}$C, 15psi for fifteen (15) mins.

### 3.2.4 Antimicrobial activity:

The agar-well diffusion method was used to determine the antimicrobial activity as described by the Clinical and Laboratory Standard Institute (2012). From the aqueous and ethanol extracts of the plants; *Bryophyllum pinnatum, Justicia carnea* and *Phyllanthus nururi*, concentrations of 25mg/ml, 50mg/ml, 100mg/ml and 200mg/ml were prepared. The test organisms: *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* and *Proteus mirabilis* were standardized and matched to the 0.5 turbidity of McFarland's standard, and were used to seed the Mueller-Hinton agar plates already prepared. In each of these plates, six (6) wells, 6mm and about 2cm apart were made using a sterile cork borer. The wells were filled with a drop, about 0.1ml of the different concentrations of the various plants extracts using a micropipette, with distilled water as negative control and ciprofloxacin as a positive control, and allowed to diffuse at room temperature. The plates were then incubated at 37$^{o}$C for 24 hours. The experiments were carried out in triplicates. The observed zones of inhibition were measured using a transparent meter rule. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) was also determined.

### 3.2.5 Determination of the minimum inhibitory concentration (MIC):

The minimum inhibitory concentration (MIC) of the aqueous and ethanol extracts of *Justicia carnea*, the most active of the three (3) plants in the preliminary test against the organisms, was determined according to the broth microdilution method. 10ml of Nutrient broth were prepared in sterile bijou bottles. From the lowest concentration of the extracts, 25mg/ml, different volumes based on the calculations of the different concentrations of 21, 19, 17, 15, 13, 11, 9, 7, 5, 3, 1, 0.8mg/ml respectively were added to the nutrient broth using a sterile syringe. 0.1ml of the test organisms after standardization and comparing with the 0.5 turbidity of McFarland's standard

were inoculated into the bottles and incubated at 37°C for 24 hours. The bottles were observed for turbidity that is presence of microbial growth. The minimum inhibitory concentration (MIC) was then reported as the lowest concentration that inhibited the growth of organisms, that is showed a clear medium (Andrews, 2001).

**3.2.6 Determination of the minimum bacteriocidal concentration (MBC):**

The minimum bacteriocidal concentration (MBC) of the aqueous and ethanol extracts of *Justicia carnea* was also determined. Nutrient agar plates were prepared and the bijou bottles that showed no turbidity that is showed a clear medium during the minimum inhibitory concentration (MIC) test were subcultured on the agar plates by streaking. The plates were incubated at 37°C for 24 hours and the minimum bacteriocidal concentration (MBC) was recorded as the lowest concentration where no growth was observed, that is, the concentration that killed the organisms (National Committee for Clinical Laboratory Standards, 1999).

**3.2.7 Phytochemical analysis:**

The qualitative and quantitative phytochemical analysis of the aqueous and ethanol extract of *Justicia carnea* for alkaloids, glycosides, tannins, anthraquinones, saponins, phenolics, steroids, terpenoids and flavonoids were carried out.

**3.2.7.1 Qualitative analysis:**

The qualitative screening of the phytochemical components of the plant extracts was carried out using the method modified by Saxena *et al.,* (2013). Essentially, specific weights of the extracts were made up to 10ml in a test tube and different reagents were added as specified. Colour change and the formation of precipitates indicated a positive result, which were compared against standards. The extracts were tested for the presence of alkaloids, glycosides, tannins, anthraquinones, saponins, phenolics, steroids, terpenoids and flavonoids.

**i. Test for saponins:**

One gram (1g) of the plant extracts were introduced into test tubes and 20ml of distilled water was added. The test tubes were shaken thoroughly to dissolve and heated for five (5) minutes. Four milliliter (4ml) of the solutions were measured into another test tubes and two milliliter (2ml) of distilled water was also added with vigorous shaking, after which the tubes were allowed to stand for six (6) minutes. A stable foaming indicated the presence of saponins.

**ii. Test for anthraquinone:**

A gram (1g) of the extracts were shaken vigorously with 10ml of chloroform and filtered. To 4ml of the chloroform filtrates, 2ml of 10% ammonium hydroxide ($NH_4OH$) solution was added, followed by the addition of 3-5% nitric acid. A colour change to orange indicated a positive result.

**iii. Test for steroids:**

One gram (1g) of the extracts were dissolved in 20ml of methanol and briefly heated for about ten (10) minutes in a water bath. They were filtered and the filtrate was evaporated to dryness. A little quantity of the residues obtained from the filtrates was dissolved in 2ml of chloroform. Sulphuric acid was added carefully by the side of the test tubes to form a lower layer of precipitate which indicated a positive result.

**iv. Test for tannins:**

A gram (1g) of the extracts was dissolved in test tubes using the corresponding extraction solvents in sufficient amounts. Then, 1ml of the extracts were added to three (3) drops of lead acetate in another test tubes and the presence of tannin was indicated by the formation of a creamy gelatinous precipitate.

**v. Test for flavonoids:**

To two milliliters (2ml) of the filtrate obtained above, 1ml of sodium hydroxide (NaOH) was added and 1ml of concentrated hydrochloric (HCl) acid was also added. The formation of a cloudy precipitate indicated a positive result.

**vi. Test for phenolics:**

One milliliter (1ml) of the dissolved extracts in their corresponding extraction solvents was added to 1ml of 10% $FeCl_2$ and mixed together. The presence of a blue precipitate indicated a positive result.

**vii. Test for alkaloids:**

Two grams (2g) of the extracts were dissolved in 5ml of 1% sulphuric acid and filtered. The filtrates were tested with alkaloidal reagents; Dragendorff, Wagner, Mayer and Hager. The filtrates were collected in various test tubes. To a tube, a few drops of Wagner's reagent (Potassium-iodine solution) were added, and a reddish-brown precipitate formed indicated a positive result. Generally, the formation of a specific precipitate and colouration upon adding drops of Dragendorff, Wagner, Mayer and Hager's reagents indicates a positive result or the presence of alkaloids.

**viii. Test for glycosides:**

To five milliliter (5ml) of the extracts in tubes treated with glacial acetic acid containing a drop of ferric chloride (0.1%) was added 1ml of concentrated sulphuric acid ($H_2SO_4$). A brownish to brick-red ring or violet colour at the interphase indicated the presence of glycosides.

**ix. Test for terpenoids:**

A quantity (9ml) of ethanol was added to a gram (1g) each of the extracts, and refluxed for a few minutes and filtered. Each of the filtrates was concentrated to 2.5ml in a boiling water bath. Five milliliter (5ml) of distilled water was added to each of the concentrated solutions and each

of the mixtures was allowed to stand for an hour (1 hour) and the waxy matter was filtered off. Each of the filtrate was extracted with 2.5ml of chloroform using a separating funnel. To 0.5ml each of the chloroform extract was evaporated to dryness on a water bath and heated with 3ml of concentrated sulphuric acid for ten (10) minutes on a water bath. A grey colour indicated a positive result.

**3.2.7.2 Quantitative analysis:**

After preliminary analysis to determine the presence of phytochemicals, quantitative analysis to determine the percentage of each of these compounds in the leave extracts of *Justicia carnea* was carried out according to the following procedures:

**i. Determination of total phenolics compounds:**

The total phenol content was determined using a standard calibration curve, as described by Saxena *et al*., (2013). One milliliter (1ml) of the extracts in a test tube, was mixed with methanol (5g/l) and further mixed with ethanol solution of gallic acid (1ml; 0.025-0.400 mg/ml) with 5ml of Folin-Ciocalteau reagent (diluted tenfold) and sodium carbonate (4ml, 0.7M) solution, and finally the volume was made up to 8ml with distilled water, followed by vigorous shaking and was allowed to stand for thirty (30) minutes, after which absorbance values were measured at 765nm using a spectrophotometer and the standard curve was plotted to determine the total phenolic contents. The total phenolics components in the extracts in gallic acid equivalents (GAE) were calculated by the formular;

$$T = \frac{C \times V}{M}$$

Where; T - total phenolic content, milligram per gram of sample extract in GAE

C - the concentration of gallic acid established from the calibration curve (mg/ml)

V - volume of extract (ml)

M - the weight of sample/extract (g)

Or

percentage phenol extracted from the powdered sample, thus;

$$\text{Phenols (\%)} = \frac{100}{W} \times \frac{C}{1000} \times \frac{VF}{VA} \times \frac{D}{1}$$

Where;

W - weight of sample analysed

C - concentration of standard in mg/ml

VF - total filtrate volume

VA - volume of filtrate analysed

D - dilution factor where applicable

**ii. Determination of tannin content:**

The tannin content was determined by Folin-Denis colorimetric method described by Sofowora (1982) and Saxena *et al*., (2013). Two grams (2g) of the samples were dissolved appropriately with 40ml of the extraction solvents in volumetric flasks. The solutions were mixed for thirty (30) minutes at room temperature and filtered to obtain the filtrate. A standard tannic acid solution was prepared, 2ml of the standard solution and equal volume of the solvents were dispersed into separate 40ml volumetric flasks to serve as a standard and reagent blank respectively. Then, 2ml of each of the respective samples were measured into their respective labelled flasks. The content of each flask was then mixed with 35ml distilled water and 1ml of the Folin reagent. This was then followed by the addition of 2.5ml of saturated sodium

trioxocarbonate (iv) solution ($Na_2CO_3$) and incubated for ninety (90) minutes at room temperature. After which, their absorbance values were measured at 760nm in a spectrophotometer with the reagent blank at zero (0). The tannin content was calculated as shown below;

$$\text{Tannin (\%)} = \frac{100}{W} \times \frac{au}{as} \times C \times \frac{Vt}{Va}$$

Where; W - weight of sample

au - absorbance of test sample

as - absorbance of standard tannin solution

C - concentration of standard tannin solution

Vt - total volume of extract

Va - volume of extract analysed

**iii. Determination of total flavonoids:**

The method is based on the formation of the flavonoids-aluminium complex which has an absorptivity maximum at 415nm. One hundred microliter (100ul) of the extracts in methanol (10mg/ml) was mixed with 100ul of 20% aluminium trichloride in methanol and a drop of acetic acid, and then diluted with methanol to 5ml. The absorbance value was read at 415nm after forty (40) minutes. Blank samples were then prepared from 100ml of the plant extracts and a drop of acetic acid was added, and then diluted to 5ml with methanol. The absorbance of the standard rutin solution (0.5mg/ml) in methanol was measured under the same condition.

**iv. Total terpenoids:**

The samples were appropriately dissolved in 40ml of the extraction solvents, filtered and to 2ml of the filtrates and 0.2ml of linalool solution, 1.5ml of chloroform was added. The mixture was then serially diluted using methanol followed by the addition of 0.1ml concentrated sulphuric acid ($H_2SO_4$) and incubated in a water bath at 25$^oC$ for ninety (90) minutes. After incubation, the reddish-brown precipitate was dissolved, and 2ml was transferred into cuvettes for spectrophotometric readings which were plotted in a standard curve from where the quantity of terpenoids were estimated.

**v. Determination of total alkaloids:**

Five grams (5g) of the extracts were weighed into a 250ml beaker and 200ml of 10% acetic acid in ethanol was added, covered and allowed to stand for four (4) hours. They were filtered and the extracts were concentrated on a water bath to one-quarter of their original volumes. Concentrated ammonium hydroxide ($NH_4OH$) was added in drops to the extracts until their precipitations were complete. The solutions were allowed to settle, and the precipitates were collected, washed with dilute ammonium hydroxide ($NH_4OH$) and filtered. The residues, alkaloid was then dried and weighed.

Percentage alkaloids were calculated as follows;

$$\text{Alkaloids (\%)} = \frac{W2 - W1}{\text{weight of sample}} \times 100$$

Where; (W2 - W1) - weight of residue

**vi. Determination of total saponins:**

The total saponin content was measured by the double solvent extraction gravimetric method, as described by Saxena *et al*., (2013). Five grams (5g) of the extracts were mixed with 50ml of 20% aqueous-ethanol solution and incubated for twelve (12) hours at a temperature of 55$^{o}$C with constant agitation. Then, the mixtures were filtered using a filter paper. The residues were then re-extracted with 50ml of ethanol solution for thirty (30) minutes and the extracts were weighed together. The combined extract was reduced to about 40ml by evaporation and then transferred to a separating funnel and equal volume (40ml) of diethyl ether was added. After mixing, there was a partition and the other layer was discarded, while the aqueous layer was reserved. The aqueous layer was re-extracted with the ether, after which its hydrogen-ion concentration (pH) was adjusted with the addition of drops of dilute sodium hydroxide (NaOH) solution. Saponin, in the extract was taken up in successive extraction with 60ml and 30ml portion of normal butanol. The combined extract was washed with 5% sodium chloride (NaCl) solution and evaporated to dryness in a previously weighed evaporating dish. The saponin was then dried in the oven at 60$^{o}$C, to remove any residual solvent, cooled in a dessicator and re-weighed. The saponin was determined and calculated as a percentage of the original samples.

$$\text{Saponin (\%)} = \frac{W2 - W1}{W} \times 100$$

Where; W - weight of sample used

W1 - weight of empty evaporating dish

W2 - weight of dish + saponin extract

**vii. Determination of total glycosides:**

The digested glycoside content of the sample (extract) was determined using the method described by Giuliani *et al*., (2007) and Saxena *et al*., (2013). Five grams (5g) of the samples (extracts) were dissolved in 250ml of distilled water and treated with glacial acetic acid containing a drop of ferric chloride (0.1%), and introduced into a beaker containing 1ml of concentrated sulphuric acid ($H_2SO_4$), with continuous agitation for three (3) hours, followed by filtration. Then, 10ml of freshly prepared 0.10% Anthrone reagent was added and mixed thoroughly by gently shaking. The experiment was repeated to obtain a blank using distilled water in place of the extract. Thereafter, the samples obtained were transferred to a spectrophotometer and the absorbance value was read at 630nm against the blank. The total available glycosides were then calculated as follows;

$$\text{Glycoside (\%)} = \frac{25A_1}{W} \times \frac{100}{A_2}$$

Where; W - weight of sample

25 - constant

$A_1$ - absorbance of diluted sample

$A_2$ - absorbance of diluted standard

**3.2.8 Experimental animals:**

A total of twenty-one (21) healthy male Wistar albino rats, weighing 116g and above, obtained from the animal house of the Department of Anatomy, University of Benin, Benin city were used in this study. They were kept in plastic cages with wire screen tops at room temperature and were acclimatized for a week. They had free access to their feeds and water *ad libidum*; and fresh wood shavings used as bedding materials in the cages were replaced each day.

### 3.2.9 Treatment of animals:

The animals were placed into seven (7) groups of three (3) rats each. Group one (1), the control received distilled water, Group two (2) received aqueous minimum inhibitory concentration (MIC) of *Justicia carnea* extracts (15mg/kg), Group three (3) received ethanol minimum inhibitory concentration (MIC) of *Justicia carnea* extracts (13 mg/kg), Group four (4) received the aqueous minimum bacteriocidal concentration (MBC) of the extract (17mg/kg), Group five (5) received ethanol minimum bacteriocidal concentration (MBC) of the extract (15mg/kg), Group six (6) received four times the aqueous minimum inhibitory concentration (MIC) of the extract (60mg/kg) and Group seven (7) received four times the ethanol minimum inhibitory concentration (MIC) of the extract (52mg/kg). Both extracts of *Justicia carnea* (aqueous and ethanol) and distilled water (control) were orally administered to the rats daily for fourteen (14) days using an oral gavage. The rats were observed daily for signs of morbidity and mortality and were weighed on the first and last days of administration.

### 3.2.10 Haematological analysis:

At the conclusion of the administration of the extracts, on the fifteenth (15th) day, the blood samples of the animals were collected for haematological analysis. They were first anaesthetized in a closed chamber containing chloroform to render them unconscious for dissection. The blood samples were collected from the heart (cardiac puncture) using sterile needles and syringes and placed in anticoagulant bottles containing ethylene diamine tetraacetic acid (EDTA). The bottles containing the blood samples were transported to the Department of Haematology, University of Benin Teaching Hospital, Benin city for analysis. The parameters analyzed were white blood cells (WBC), lymphocytes (LYM), granulocytes (GRAN), red blood cells (RBC), haemoglobin

(HGB), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), platelets (PLT) and packed cell volume (PCV). This haematological analysis was carried out using an automated haematology analyzer (AHA) machine in which the blood samples were agitated to uniformly distribute the cells, then blood smears were made and placed in the machine which counted the number of blood cells called complete blood count (CBC) or full blood count (FBC). This analysis is also carried out manually in which blood smears are made on glass slides and placed under the microscope using a hemocytometer to view and count the number of cells such as white blood cells (WBC), red blood cells (RBC), platelets, etc., found in the blood (Tefferi *et al.,* 2005; D'Souza *et al.,* 2015).

**3.2.11 Histopathological analysis:**

After administering the extracts and distilled water for fourteen (14) days, the animals were sacrificed in order to harvest their selected organs; liver and kidney in their anaesthetized state for histopathological analysis. The organs were weighed and placed in different universal containers containing formalin solution to preserve them, and were transported to the Department of Histopathology, University of Benin Teaching Hospital, Benin city for analysis.

For this histopathological analysis, the organs (tissues) were dehydrated and embedded in melted paraffin wax and mounted on a microtome to cut into thin slices. These slices were fixed on microscopic glass slides at which point the wax was removed with a solvent and then rehydrated to be ready for staining. The haematoxylin and eosin (H&E) staining method was used which involved the application of the haematoxylin dye followed by rinsing in a weak acid (HCL) solution to remove excess staining and bluing in alkaline water (eg. Scott's tap water). Thereafter, the counterstaining with eosin dye was done. Then, the slides after drying were observed under the microscope (Kiernan, 2018).

### 3.2.12 Data Analysis:

The data obtained from this research were analyzed using statistical package for social scientist (version 21), and Microsoft excel (version 2019). Values were expressed as mean ± standard deviation (Ogbeibu, 2015).

# CHAPTER FOUR

# RESULTS

## 4.1 Yield of plant extracts:

The results obtained from the yield of extraction of the three (3) plants; *Bryophyllum pinnatum, Justicia carnea and Phyllanthus niruri*, are presented in Table 4.1.

The solvent, aqueous(distilled water) of the three (3) plants yielded more than the ethanol extracts; with aqueous *Bryophyllum pinnatum* yielding 20.7% which is more than the ethanol extract yielding 12.4%. Aqueous *Justicia carnea* yielding 6.3% which is more than the ethanol extract yielding 6.2%; while aqueous *Phyllanthus niruri* yielding 20.3% more than the ethanol extract which yielded 18.7%.

$$\text{Percentage yield (\%)} = \frac{\text{Weight of extract (g)}}{\text{Weight of plants (g)}} \times \frac{100}{1}$$

## 4.2 Antimicrobial activity:

The antimicrobial activity of the aqueous and ethanol extracts of the three (3) plants; *Bryophyllum pinnatum, Justicia carnea and Phyllanthus niruri*, at different concentrations with their measured zones of inhibition are presented in the figures 4.1, 4.2 and 4.3 respectively.

These preliminary studies show that the aqueous and ethanol extracts of *Justicia carnea* were most active against the organisms at all concentrations with *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* and *Proteus mirabilis* having zones of inhibition of various diameters. The *Justicia carnea* aqueous and ethanol extracts being most active was followed by *Phyllanthus niruri*, while *Bryophyllum pinnatum* was least active against the organisms at all

concentrations with *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* and *Proteus mirabilis* having few or no zones of inhibition for both the aqueous and ethanol extracts.

**Table 4.1: Percentage yield of plant extracts**

| Plants | Extraction solvents | Weight of plants (g) | Weight of extracts (g) | Percentage of yield (%) |
|---|---|---|---|---|
| *Bryophyllum pinnatum* | Aqueous | 150 | 31.1 | 20.7 |
| | Ethanol | 150 | 18.6 | 12.4 |
| *Justicia carnea* | Aqueous | 110 | 6.9 | 6.3 |
| | Ethanol | 110 | 6.8 | 6.2 |
| *Phyllantus niruri* | Aqueous | 150 | 30.5 | 20.3 |
| | Ethanol | 150 | 28.2 | 18.7 |

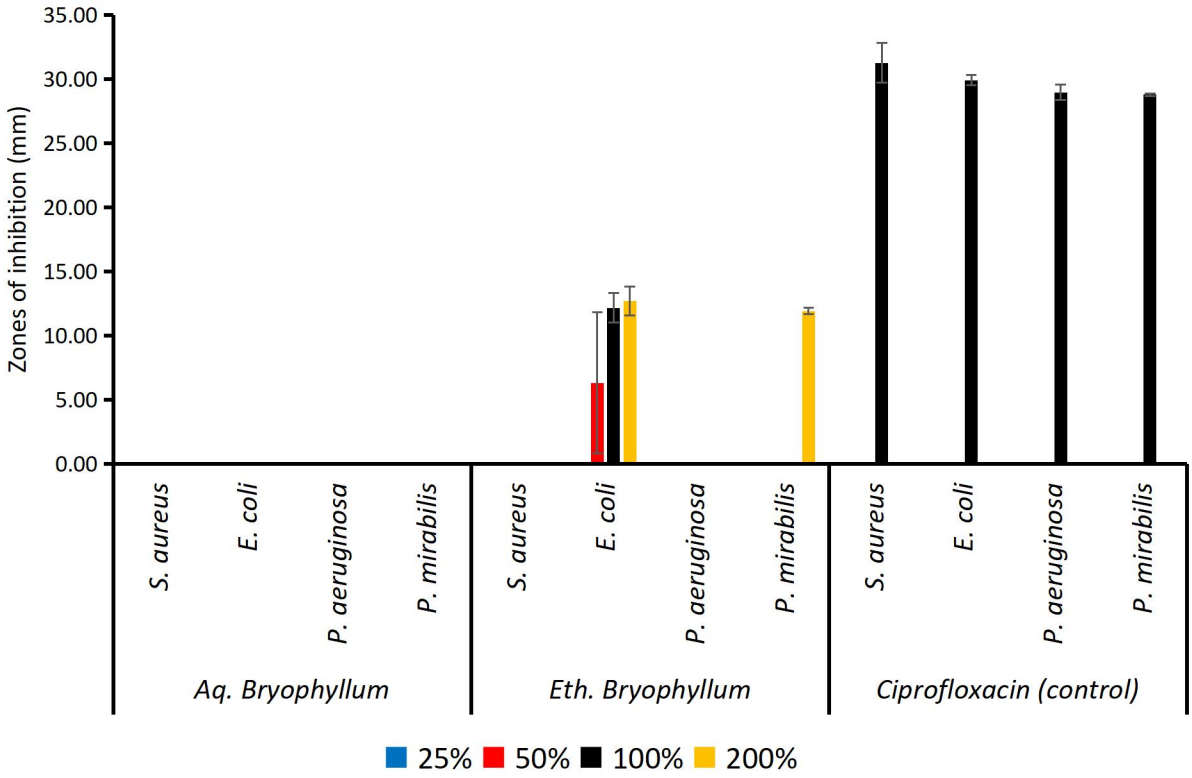



Figure 4.1: Antibacterial effect of the aqueous and ethanol extract of *Bryophyllum pinnatum* at different concentration levels of 25mg/ml, 50mg/ml, 100mg/ml and 200mg/ml.

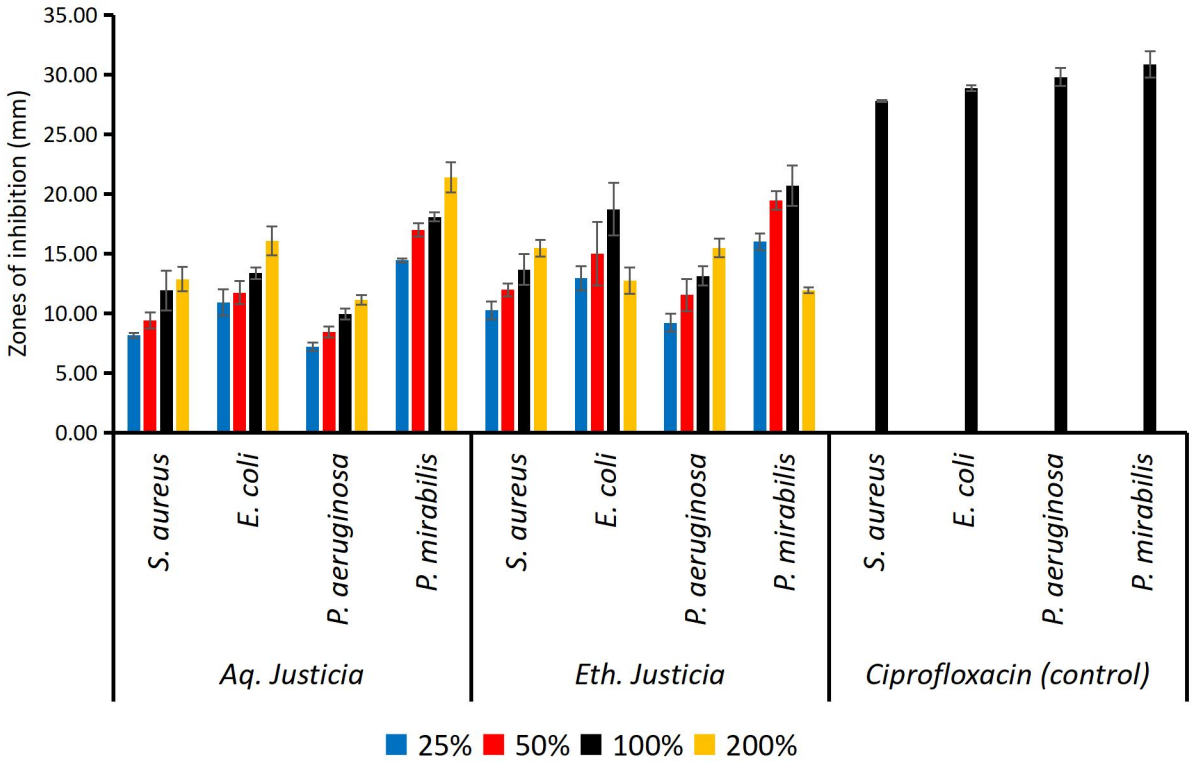



Figure 4.2: Antibacterial effect of the aqueous and ethanol extract of *Justicia carnea* at different concentration levels of 25mg/ml, 50mg/ml, 100mg/ml and 200mg/ml.

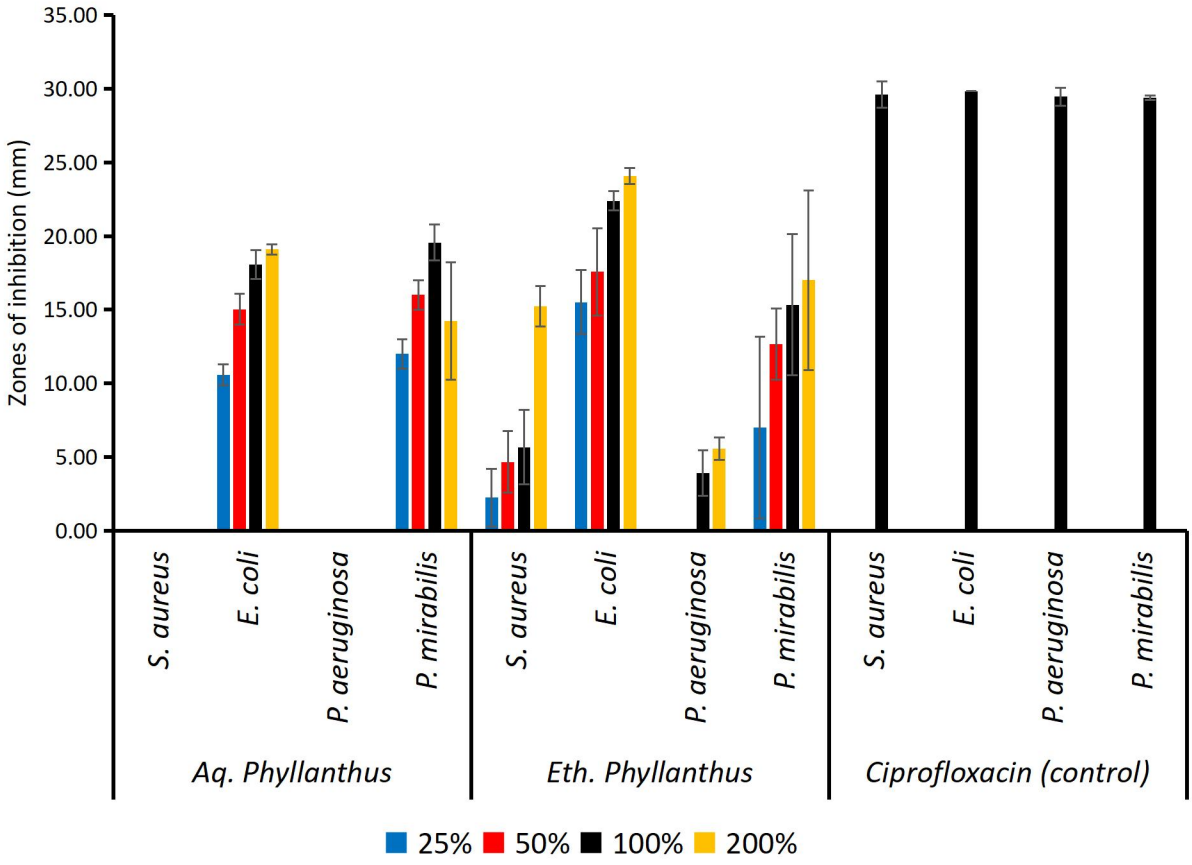



Figure 4.3: Antibacterial effect of the aqueous and ethanol extract of *Phyllanthus niruri* at different concentration levels of 25mg/ml, 50mg/ml, 100mg/ml and 200mg/ml.

### 4.3 Minimum inhibitory concentration (MIC) and Minimum bacteriocidal concentration (MBC):

The minimum inhibitory concentration and minimum bacteriocidal concentration of the aqueous and ethanol extracts of *Justicia carnea* are presented in Table 4.2.

From the aqueous extract, the least minimum inhibitory concentration was 7mg/ml against *Proteus mirabilis*, while the highest was 15mg/ml against *Pseudomonas aeruginosa*. From the ethanol extract, the least minimum inhibitory concentration was 5mg/ml also against *Proteus mirabilis*, while the highest was 13mg/ml also against *Pseudomonas aeruginosa*.

For the minimum bacteriocidal concentration, the least concentration (11mg/ml) and highest concentrations (17mg/ml) were against *Proteus mirabilis* and *Pseudomonas aeruginosa* for the aqueous extract, while for the ethanol extract, the least concentration (7mg/ml) and highest concentrations (15mg/ml) were also against *Proteus mirabilis* and *Pseudomonas aeruginosa* respectively.

**Table 4.2: Minimum inhibitory concentration (MIC) and minimum bacteriocidal concentration (MBC) of aqueous and ethanol extracts of *Justicia carnea* against bacteria spp from clinical samples.**

| Organisms | Aqueous extract | | Ethanol extract | |
|---|---|---|---|---|
| | MIC (mg/ml) | MBC (mg/ml) | MIC (mg/ml) | MBC (mg/ml) |
| *Staphylococcus aureus* | 13 | 15 | 11 | 11 |
| *Escherichia coli* | 13 | 15 | 9 | 11 |
| *Pseudomonas aeruginosa* | 15 | 17 | 13 | 15 |
| *Proteus mirabilis* | 7 | 11 | 5 | 7 |

**4.4 Phytochemical constituents of aqueous and ethanol extracts of *Justicia carnea*:**

The qualitative and quantitative phytochemical constituents of the aqueous and ethanol extracts of *Justicia carnea* are presented in Table 4.3.

The plant constituents; tannins and steroids were absent in the aqueous extract of *Justicia carnea*, while they were present in the ethanol extract in addition to all other constituents, such as alkaloids, glycosides, anthraquinones, saponins, phenolics, terpenoids and flavonoids.

The results also show the quantity of the phytochemical constituents present in the aqueous and ethanol extracts of *Justicia carnea*. The values ranged from 23.03% for glycosides to 0.00% for steroids and tannins in the aqueous extract and 18.83% for phenolics to 0.06% for steroids in the ethanol extract.

**Table 4.3: Qualitative and Quantitative phytochemical constituents of *Justicia carnea***

| Plant constituents | Aqueous extract (%) | Ethanol extract (%) |
|---|---|---|
| Alkaloids | 16.27 | 17.16 |
| Glycosides | 23.03 | 12.33 |
| Anthraquinones | 1.80 | 15.60 |
| Tannins | 0.00 | 1.13 |
| Saponins | 8.23 | 8.47 |
| ssPhenolics | 6.57 | 18.83 |
| Steroids | 0.00 | 0.06 |
| Terpenoids | 7.70 | 11.63 |
| Flavonoids | 5.90 | 16.43 |

Key: % - quantity present or absent

**4.5 Haematological parameters for the aqueous and ethanol extracts of *Justicia carnea*:**

The haematological parameters for the aqueous and ethanol extracts of *Justicia carnea* are presented in Table 4.4.

There was an increase in the number of packed cell volume (PCV) of blood from 0.14% to a range of 0.17% to 0.34% at all concentrations of the extracts administered; platelet count also increased from 195.67x10^3/µl to a range of 228.00×10^3 to 435.50x10^3/µl. There was also an increase in mean corpuscular volume (MCV) of blo0od and a marginal increase in red blood cells (RBC) and haemoglobin (HGB). The mean corpuscular haemoglobin (MCH) also increased but there was a decrease in the number of white blood cells (WBC) from 6.40x10^3/µl to a range of 2.77x10^3/µl to 6.33x10^3/µl, and granulocytes (GRAN) from 9.50% to a range of 4.10% to 9.10%, as compared to the control.

**Table 4.4: Haematological parameters of aqueous and ethanol extracts of *Justicia carnea***

| | | | Aqueous extract | | | Ethanol extract | | |
|---|---|---|---|---|---|---|---|---|
| **Parameters** | **GROUP 1 (CONTROL)** | **GROUP 2** | **GROUP 4** | **GROUP 6** | **GROUP 3** | **GROUP 5** | **GROUP 7** | ***P - value*** |
| **WBC** | 6.40±2.14 | 6.10±2.17 | 2.77±1.37 | 4.15±0.35 | 6.33±2.71 | 5.47±2.65 | 5.43±1.64 | *P<0.05* |
| **LYM** | 80.33±9.62 | 85.63±7.26 | 80.07±5.17 | 73.95±16.19 | 86.40±5.88 | 88.17±3.47 | 86.73±1.54 | *P>0.05* |
| **GRAN** | 9.50±6.17 | 4.83±2.54 | 9.10±4.99 | 9.80±6.36 | 4.50±2.01 | 4.17±1.26 | 4.10±0.79 | *P<0.05* |
| **RBC** | 5.49±1.29 | 6.08±0.52 | 4.38±3.39 | 4.72±1.36 | 6.25±0.47 | 6.34±0.26 | 4.61±3.06 | *P>0.05* |
| **HGB** | 11.23±2.97 | 12.93±1.51 | 9.37±7.35 | 10.05±2.33 | 13.03±0.95 | 13.17±0.4 | 9.57±6.64 | *P>0.05* |
| **MCV** | 64.60±3.14 | 67.47±0.75 | 67.87±0.81 | 67.45±8.7 | 66.47±4.2 | 67.00±1.54 | 65.27±2.51 | *P>0.05* |
| **MCH** | 20.33±0.7 | 21.20±0.7 | 20.63±1.39 | 21.40±1.27 | 20.83±0.4 | 20.73±0.29 | 19.80±2.14 | *P>0.05* |
| **MCHC** | 31.60±2.44 | 31.50±0.85 | 30.63±1.46 | 32.00±2.26 | 31.47±1.4 | 31.00±0.3 | 30.53±3.93 | *P>0.05* |
| **PLT** | 195.67±58.5 | 269.67±12.5 | 228.00±195.73 | 435.50±282.14 | 315.67±137.02 | 241.67±116.71 | 334.33±30.14 | *P>0.05* |
| **PCV** | 0.14±0.05 | 0.21±0.01 | 0.17±0.15 | 0.34±0.19 | 0.26±0.13 | 0.19±0.1 | 0.25±0.03 | *P>0.05* |

Values are mean ± standard deviation

**Key:** WBC-white blood cells, LYM-lymphocytes, GRAN-granulocytes, RBC-red blood cells, HGB-hemoglobin, MCV-mean corpuscular volume, MCH-mean corpuscular hemoglobin, MCHC-mean corpuscular hemoglobin concentration, PLT-platelets, PCV-packed cell volume

Group 1- control (distilled water), Group 2-aqueous MIC, Group 3-ethanol MIC, Group 4-aqueous MBC, Group 5-ethanol MBC, Group 6-aqueous 4 × MIC, Group 7-ethanol 4 × MIC

$P>0.05$-not statistically significant, $P<0.05$-statistically significant.

**4.6 Percentage weight gain of animals (rats) and weight of organs:**

The results obtained from the body weight gain of animals (rats) in their percentages and the weights of the organs, liver and kidney are presented in Table 4.5.

The result shows, an increase in the percentage body weight of animals (rats) across all concentrations, as compared to the control. There was also an increase in the weight of the organs, liver and kidney, as compared to the control.

**Table 4.5: Effect of extract on the weight of animals (rats) and weight of organs (P>0.05)**

| GROUPS | PERCENTAGE WEIGHT GAIN (%) | WEIGHT OF LIVER (g) | WEIGHT OF KIDNEY (g) |
|---|---|---|---|
| GROUP1(CONTROL) | 20.38 | 5.6 | 0.7 |
| GROUP 2 | 21.68 | 8.3 | 0.8 |
| GROUP 3 | 23.92 | 6.7 | 0.9 |
| GROUP 4 | 29.69 | 9.0 | 1.2 |
| GROUP 5 | 29.68 | 8.2 | 0.9 |
| GROUP 6 | 29.03 | 7.2 | 0.8 |
| GROUP 7 | 22.80 | 8.6 | 1.4 |

Key: Group 1 (Control) – Distilled water

Group 2 – 15mg/kg of aqueous extract of *Justicia carnea*

Group 3 – 13mg/kg of ethanol extract of *Justicia carnea*

Group 4 – 17mg/kg of aqueous extract of *Justicia carnea*

Group 5 – 15mg/kg of ethanol extract of *Justicia carnea*

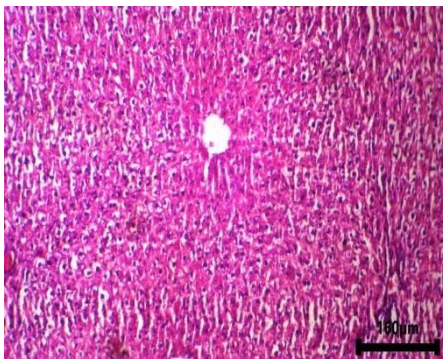
Group 6 – 60mg/kg of aqueous extract of *Justicia carnea*

Group 7 – 52mg/kg of ethanol extract of *Justicia carnea*

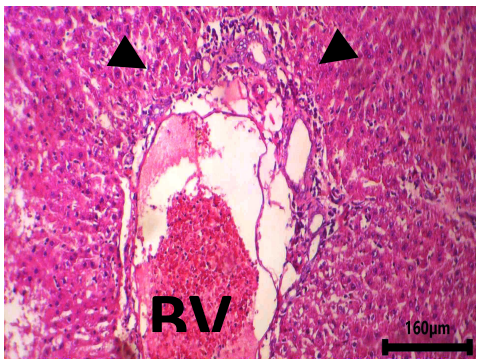
P>0.05 – Not statistically significant

### 4.7 Histopathological analysis:

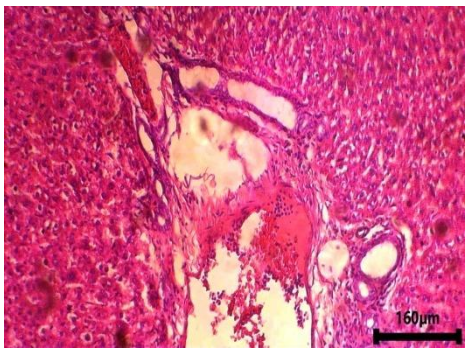
The results obtained from the histopathological analysis of the organs, liver and kidney are presented;



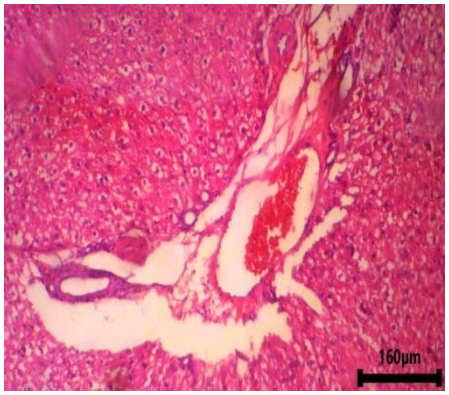

**Group1 (control)**



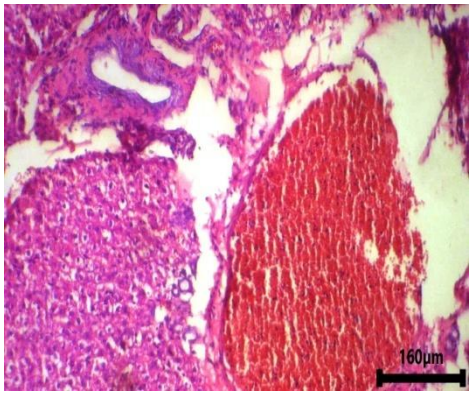

**Group 2**



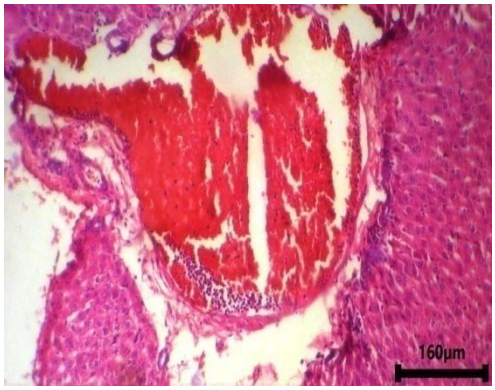

**Group 3**



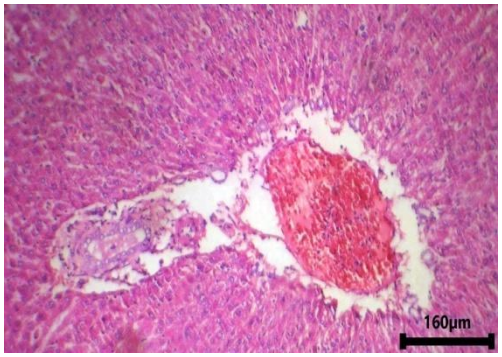

**Group 4**




**Group 5**

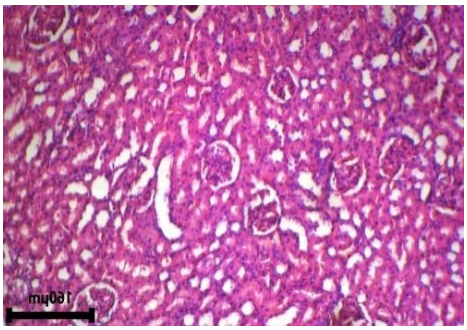



**Group 6**

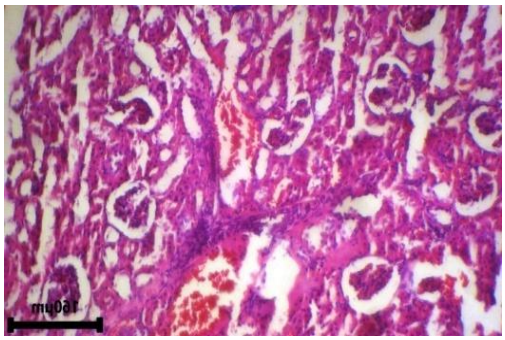



**Group 7**

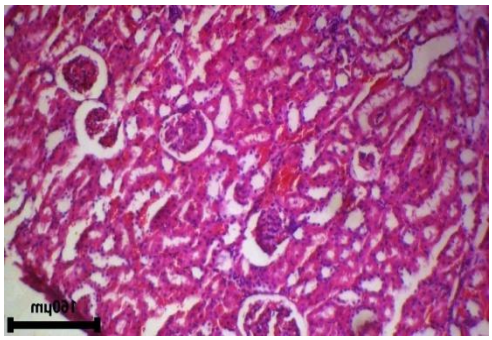
Plate 4.1: Microscopic view of the liver of wistar albino rats (160µm) administered with the aqueous and ethanol extracts of *Justicia carnea*



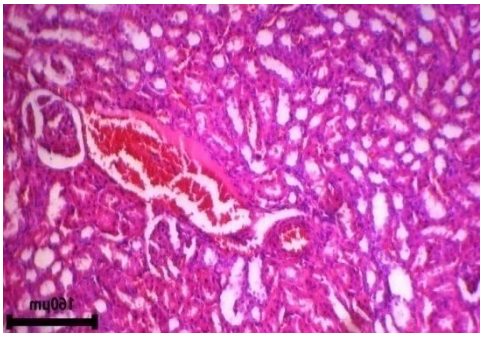
**Group 1(control)**



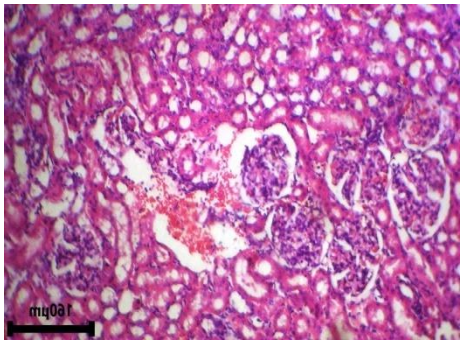
**Group 2**



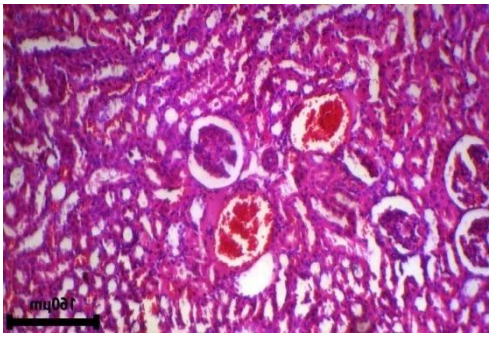
**Group 3**



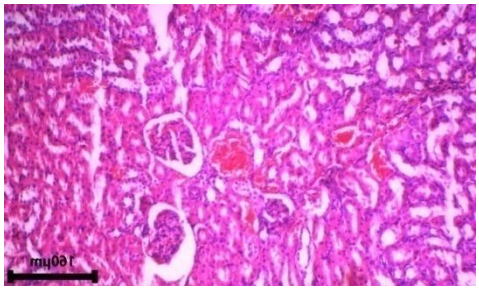
**Group 4**



**Group 5**



**Group 6**



**Group 7**

Plate 4.2: Microscopic view of the kidney of wistar albino rats (160µm) administered with the aqueous and ethanol extracts of *Justicia carnea*

Key: Group 1 (Control) – Distilled water
Group 2 – 15mg/kg of aqueous extract of *Justicia carnea*

Group 3 – 13mg/kg of ethanol extract of *Justicia carnea*

Group 4 – 17mg/kg of aqueous extract of *Justicia carnea*

Group 5 – 15mg/kg of ethanol extract of *Justicia carnea*

Group 6 – 60mg/kg of aqueous extract of *Justicia carnea*

Group 7 – 52mg/kg of ethanol extract of *Justicia carnea*

The photomicrograph of the liver sections stained with haematoxylin and eosin dye, showed the control group (Group 1) appearing normal with the central vein without congestion, interstitial spaces and hepatocytes(cells of the liver) radiating. Groups two (2) and three (3) showed the dilation and congestion of blood vessels with blood and infiltrates of inflammatory cells; Group 4 showing the dilation of the portal triad, which consists of the central vein, bile duct and artwith the congestion of blood; and Groups 5, 6 and 7, also showing the central vein being congested with blood, and the presence of inflammatory cells.

The photomicrograph of the kidney section stained with haematoxylin and eosin dye, showed the control group (Group 1) appearing normal with glomerular cells and interstitial spaces without congestion; and Groups two (2) to seven (7) showing the interstitial spaces and glomerular cells being congested with blood (Haemorrhage).

# CHAPTER FIVE

# DISCUSSION

In this study, the selected plants; *Bryophyllum pinnatum, Justicia carnea* and *Phyllanthus niruri* gave various percentage yields of their aqueous and ethanol extracts, with the aqueous extract having a higher percentage yield than the ethanol extracts. The solvents, water and ethanol were used for extraction because they have different polarities and are also acceptable for human consumption (Waszkowiak and Gliszczynska-Swiglo, 2016). It has also been previously reported by Caleja *et al*., (2016), that the aqueous extracts of plants usually yields higher compared to the ethanol extract due to the higher polarity of water.

These percentage yields are comparable with the reports of Casmir *et al*., (2017), which stated a percentage yield of 4% for *Bryophyllum pinnatum* using only the ethanol solvent by the manual method of extraction; Iwetan *et al*., (2022), on the plant *Justicia carnea* gave a percentage yield of 15% using only the aqueous solvent (water) by the manual method of maceration extraction; Arnold and Nigam (2021), stated a percentage yield of 5.8% for the plant *Phyllanthus niruri* using the aqueous solvent; and Orjiakor *et al*., (2019) who also stated a percentage yield of 18.43% using only distilled water (aqueous) as solvent, also by the manual method of maceration extraction.

From the preliminary study of antimicrobial activity of the selected plants, *Justicia carnea* being the most active of the plants has shown that some plants exerts more antimicrobial activity than others. Reports states that some plants extracts are more active than others against microorganisms because they possess the ability to be used for therapeutic measures due to the amount of active substances, that is phytochemicals, present in them (Ishaku *et al.,* 2017).

Cowan (1999), reported that this could also be due to the differences in their chemical compositions as well as in the mode of action of their biologically active components. Chen *et al*., (2005), also pointed out their richness in phytochemicals, like tannins, anthraquinones, flavonoids, glycosides etc, than in other plants.

The plant, *Justicia carnea* was therefore used for further studies because of its high antimicrobial activity. This result is comparable to the investigations of Anarado *et al*., (2021), who also reported that the extracts of *Justicia carnea* showed an antibacterial activity against the organisms; *Staphylococcus aureus, Escherichia coli, Aspergillus spp* and *Bacillus spp*, when also investigating the antimalarial activity of the plant.

It was observed that the ethanol extract had more antimicrobial activity and inhibitory effect than the aqueous extract. Several authors have reported that compounds with antimicrobial activities such as alkaloids, phenols, flavonoids etc, have a high rate of solubility in ethanol than water, that is, they have the ability to dissolve completely in ethanol and dissolve poorly in water( Onivogui *et al*., 2016; Al Farraj *et al*., 2020). The reports from the investigations of other authors, also pointed out that the ethanol solvent, extracts more antimicrobial compounds from plant materials as opposed to water (Arsene *et al*., 2021; Owczarek *et al*., 2021). This high activity of the ethanol extract of *Justicia carnea* than the aqueous extract can be compared to the results of Al Farraj *et al*., (2020) who also reported a high activity of the ethanol extract on the antimicrobial activities of the extracts obtained from the plant, *Dicapdi viride*.

The antimicrobial activity of *Justicia carnea* may be due to the different components identified in the plant, such as the phytochemical constituents; flavonoids, alkaloids, saponins, etc. as reported by Sonal *et al*., (2011).

Flavonoids, a group of phytochemicals provide flavour and colour to fruits and vegetables (Amadi *et al*., 2006). This can be responsible for the flavour and deep-red colour of the leaves of *Justicia carnea*, when boiled for consumption despite the greenish nature of the plant. They also possess antimicrobial activities through various mechanisms. They act as bacteriocidal and bacteriostatic by inhibiting the synthesis of nucleic acid, energy metabolism and damage the cytoplasm of the bacteria due to cell lysis (Donadio *et al*., 2021; Gorniak *et al*., 2019). They also inhibit the attachment of bacteria to surfaces and biofilm formation, alters the bacteria membrane permeability and it's ability to cause disease, which are of importance for the growth of bacteria (Xie *et al*., 2014). Okwu (2004), resported that flavonoids also possess antioxidant activity and the consumption of foods rich in flavonoids, like the plant, *Justicia carnea*, in the right quantity reduces the possibility of having diseases and conditions caused by oxidative stress. This antioxidant property has also been suspected to contribute to its antibacterial activity as reported by Cushnie and Lamb, (2011).

Phenols, also a group of phytochemicals, possess antimicrobial properties by displaying activity against a wide range of organisms such as bacteria (both gram-positive and gram-negative), thereby inhibiting their growth (Kyaw *et al*., 2012).

Alkaloids are said to be potentially active against bacteria, including the methicillin-resistant *Staphylococcus aureus* (MRSA) which is a common cause of infection (Chen *et al*., 2012). They function in inhibiting the cell wall synthesis, nucleic acid and protein synthesis of bacteria, bacteria metabolism and damage bacterial cell membrane (Kelley *et al*., 2013). They also possess pharmacological activity and are used as antibacterial, antifungal, anti-hypertensive, antimalarial activities, etc. (Uroko *et al*., 2015; Sangodare *et al*., 2015).

Saponins, also possess antibacterial activity by destroying the membrane and cell wall of bacteria and inhibiting their attachment to surface as reported by Montdargent and Letourneur, (2000). Mamta *et al*., (2013) also reported that they reduce serum cholesterol levels in animals when the leaves of plant are consumed.

Tannins, another group of phytochemicals, function in antimicrobial activity by inhibiting the metabolism of bacteria. They show a broad spectrum of activity, especially against *Staphylococcus aureus* and *Enterococcus faecalis* and also against *Streptococcus pyogenes* and *Escherichia coli* (Belhaoues *et al*., 2020; Dabbaghi *et al*., 2009). They inhibit the attachment of bacteria to surfaces and the uptake of sugar and amino acid by bacteria, thereby limiting their growth ( Hull-Vance *et a*l., 2011). Tannis, also act as anti-nutrients and disrupts the assimilation of iron by combining with iron in the gastrointestinal tract to reduce the biological availability of iron. High levels of tannins also decreases the activity of digestive enzymes, such as alpha (α) amylase, trypsin, chymotrypsin and lipase, and may also damage the mucosa of the digestive tract and reduce the absorption of nutrients such as Cyanocobalamin (Vitamin B12) (Praveen and Kumud, 2012).

Therefore, the absence or low level of tannins found in this plant indicates that the absorption or assimilation of its iron content cannot be disrupted, as the iron content and other nutrients in the plant are available for uptake and utilization.

The presence of the various phytochemical constituents in the plant is an indication that it could promote quality human health. These phytochemicals present in *Justicia carnea* agrees with Anarado *et al*., (2021); Onyeabo *et al*., (2017) and Orijakor *et al*., (2019), who also investigated and reported their presence.

From earlier reports of the plant, *Justicia carnea* being a blood booster, the haematological parameters of the experimental animals were observed to be due to the intake of the leave extracts of the plant, as compared with the control. The decrease in the level of white blood cell (WBC) counts and granulocytes was statistically significant ,while the slight increase in the level of lymphocytes was of no significant difference.

White blood cells play a major role in immunity by providing the body with the ability to protect or defend itself against infection and diseases. White blood cell (WBC) counts usually increases in the presence of foreign agents (pathogens)which results in the normal body response of initiating the body's defence system (Eyong *et al*., 2004; Stover and Caudill, 2008). A decrease in the level of white blood cells occurs when the body is not able to produce enough white blood cells thereby leading to an increase in the risk of getting infections. This significant decrease in the level of white blood cells and granulocytes of the experimental animals as compared with the control, could indicate that the intake of the leave extracts of *Justicia carnea* produced a condition that disrupted the production of white blood cells by the body and showed a form of harm or threat to the defence mechanism of the animals. This result is similar with the reports of Kemp and Franco (2002), who also reported a decrease in the white blood cell count when investigating the activity of a plant which was known to be a treatment for upper respiratory tract infections and further concluded that this decrease could be due to the prolonged usage of the plant for treatment. This result also agrees with Akintimehin *et al*., (2021), who reported a decrease in the level of white blood cells (WBC) and granulocytes, when also investigating this plant, *Justicia carnea.*

The increase in platelet count shows that the extract can function in preventing the loss of blood through blood clotting. Platelets also play a role in immunity by capturing and engulfing microorganisms and by the formation of clot, they prevent the spread of bacteria, which is a protective mechanism (Morrell *et al*., 2014).

Although statistically insignificant, the increase in the packed cell volume (PCV) of blood and the marginal increase in red blood cells (RBC) and haemoglobin (HGB) agrees to the plant having a blood boosting potential. The production of blood occurs in the bone marrow and red blood cells, a component of the blood, functions in carrying oxygen (by haemoglobin, the oxygen-carrying pigment in the blood) and nutrients to all parts of the body. According to Orjiakor *et al*., (2019), the blood stimulating effect of this plant could be due to the presence of biologically active constituents in it, facilitating the action of haematopoeitic cells and stabilizing the circulation of blood. These increase in red blood cells (RBC), packed cell volume (PCV) of blood and platelet count (PLT) agrees with the previous work of Onyeabo et al., (2017) on the reversed effect of *Justicia carnea* leaf extract on anaemia-induced experimental rats and Orjiakor *et al*., (2019) on its effect on the haematological indices of anaemia-induced rats.

The increase in the mean corpuscular volume (MCV) and mean corpuscular haemoglobin (MCH) of blood, as compared with the control, were of no significant differences, and therefore are statistically insignificant.

It is therefore observed that the plant, *Justicia carnea*, gave a decrease in the level of white blood cells.

The increase in the body weight (P>0.05) of the animals in respect to their percentage weight gain was of no significant difference, and it is an indication that the plant was not able to disrupt their feeding ability and thus were still able to maintain their adaptive and positive response to the environment. The organ weights of the liver and kidney of the experimental animals showed an increase (P>0.05), which was also of no significant difference, in all concentrations of the extract administered, as compared with the control.

According to Stahl (2013), the relationship between body weight and organ weight in animals is in a linear form, as an increase in the body weight of animals also gives an increase in the weight of organs. Russell and Cullen (2013), stated that a decrease in the weight of the organs, liver and kidney, are not common and they reflect a loss of function associated with atrophy or cellular injury which is more often observed in acute injury, while an increase is more commonly observed in relation with adaptive changes such as hypertrophy or hyperplasia and may reflect generalized accumulations of fat, glycogen and water. These increase agrees with Onyeabo *et al*., (2017), who also reported an increase of no significant difference in the weights of liver and kidney.

From the histopathological result of the organs of the experimental animals, as compared with the control, the dilation and congestion of blood vessels with blood, the dilation of the portal triad which consists of the central vein, bile duct and artery, and the presence of infiltrates of inflammatory cells, as observed in the liver are abnormalities that can lead to a failure in the function of the liver and death, if left untreated (Hellsten *et al*., 2012). The dilation of the blood vessels, a condition called Vasodilation, is a process whereby the blood vessels widens or expands, as a result of the relaxation of the muscular wall of the vessels to allow for the

increased flow of blood, thereby lowering blood pressure, while the dilation of the portal triad (central vein, bile duct and artery) is a form of expansion that can cause the narrowing of other vessels in the liver thereby restricting the supply of blood to them, as reported by Hellsten *et al*., (2012).

However, congestion of blood is the excess accumulation of blood in vessels and it could be due to an increased flow of blood (Elmore, 2006). Elmore (2006), further stated that if abnormalities, such as dilation or congestion of vessels with blood occurs in organs due to the intake of a treatment, then the treatment should be diagnosed and graded.

The congestion of blood and haemorrhage observed in the glomerular cells and interstitial spaces of the kidney across the groups administered as compared with the control, can lead to a failure in the function of the kidney and possibly death if not treated (Russell and Cullen, 2013). As compared with the control, the intake of the extracts by the experimental animals led to a condition of haemorrhage in the kidney, which is a process of active bleeding, by which blood escapes from the blood vessels, either into the organs or tissues in the body (internal bleeding), or outside the body (external bleeding) as a result of injury. Roth (2011), stated that this escape of blood into the organs of the body, can be due to the pressure of blood flowing through the blood vessels leading to a tear or a burst of the vessels causing haemorrhage.

From these histopathological results, the administration of the *Justicia carnea* plant extracts without any form of morbidity or mortality of the experimental animals but with slight abnormalities on their organs (liver and kidney) could be due to the daily dosage of the plant for a period of two (2) weeks. This result is comparable with Akintimehin *et al*., (2021), who

reported a liver and kidney structure with slight abnormalities of mild fat congestion and haemorrhage.

## 5.1 SUMMARY

The aqueous and ethanol extracts of *Justicia carnea* had a higher antimicrobial activity, with zones of inhibition from 8.1mm to 21.4mm and 10.2mm to 21.8mm respectively relative to the aqueous and ethanol extracts of *Bryophyllum pinnatum* (0mm to 13mm and 0mm to 12.7mm respectively), and *Phyllanthus niruri* (10.6mm to 14.2mm and 7mm to 13.7mm respectively), and was therefore used for the completion of this study. The qualitative and quantitative phytochemical constituents present in the aqueous extract were alkaloids, glycosides, anthraquinones, saponins, phenolics, terpenoids, flavonoids, with the absence of tannins and steroids, while those presents in the ethanol extracts were alkaloids, glycosides, anthraquinones, saponins, phenolics, terpenoids, flavonoids, tannins and steroids. There was a mean increase in the packed cell volume (PCV) and platelet count, a marginal increase in the red blood cells (RBCs) and haemoglobin which were of no significant differences ($P>0.05$) and a mean decrease in the level of the white blood cells (WBCs) and granulocytes which were of statistical significance(significant difference) ($P<0.05$). The aqueous and ethanol extract of *Justicia carnea* leaves showed abnormalities in the liver and kidney of the wistar albino rats.

## 5.2 CONTRIBUTION TO KNOWLEDGE

The following are the contributions to knowledge:

1. Due to the strong antibacterial activity of the leave extracts of the plants, *Justicia carnea* on the test organisms, it could be used as a source of preventive and curative measures to infectious diseases.
2. The ethanol extract of the plant *Justicia carnea*, exerts more antimicrobial activity compared to the aqueous extract.
3. The ethanol extract of the plant also contained more phytochemicals as compared to the aqueous extract.
4. The haematological and histopathological study of the plant has shown various abnormalities and dosage and duration should be taken into consideration.

## 5.3 CONCLUSION/RECOMMENDATION

The presence of phytochemical constituents in the plant suggests that the leaves of this plant can make important contributions and increment to nutrition and quality of human health. The results of this study indicate that the plant has high antimicrobial activity and no morbidity or mortality was observed in the experimental animals. The haematological analysis of the plant led to a decrease in the level of white blood cells while the histology of the organs showed pathological conditions in the organs investigated. This study has therefore stated that the plant, *Justicia carnea* possess a high antimicrobial activity. However, more research into the adequate dosage and duration of treatment is required.

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## APPENDIX I

Table 4.2a: Antimicrobial activity of aqueous and ethanol extracts of *Bryophyllum pinnatum* at different concentrations of 25mg/ml, 50mg/ml, 100mg/ml and 200mg/ml respectively

| Organisms | Aqueous extract | | | | Ethanol extract | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Zones of Inhibition (mm) | | | | | | | | | |
| | Concentrations (mg/ml) | | | | | | | | Cip. (µg/ml) | Dis. |
| | 25 | 50 | 100 | 200 | 25 | 50 | 100 | 200 | | |
| ***S. aureus*** | - | - | - | - | - | - | - | - | 31.3±1.0 | - |
| ***E.coli*** | - | - | - | - | - | 6.3±3.2 | 12.2±0.7 | 12.7±0.6 | 28.8±0.3 | - |
| ***P. aeruginosa*** | - | - | - | - | - | - | - | - | 32.3±0.8 | - |
| ***P. mirabilis*** | - | - | 9.3±1.3 | 13.0±0.7 | - | - | - | 11.9±0.1 | 32.8±1.0 | - |

**Key:** Cip- Ciprofloxacin (positive control)

Dis- Distilled water (negative control)

\- - No activity

Zones of inhibition = mean ± standard error

Table 4.2b: Antimicrobial activity of aqueous and ethanol extracts of *Justicia carnea* at different concentrations of 25mg/ml, 50mg/ml, 100mg/ml and 200mg/ml respectively.

| **Organisms** | **Aqueous extract** | | | | **Ethanol extract** | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **Zones of Inhibition (mm)** | | | | | | | | | |
| | **Concentrations (mg/ml)** | | | | | | | | **Cip.** | **Dis.** |
| | **25** | **50** | **100** | **200** | **25** | **50** | **100** | **200** | **(µg/ml)** | |
| ***S. aureus*** | 8.1±0.1 | 9.4±0.4 | 11.9±0.9 | 12.9±0.6 | 10.2±0.4 | 11.9±0.3 | 13.6±0.7 | 15.5±0.4 | 27.8±0.2 | - |
| ***E. coli*** | 10.9±0.6 | 11.7±0.6 | 13.4±0.3 | 16.1±0.7 | 12.9±0.6 | 15.0±1.5 | 18.7±1.3 | 21.7±0.3 | 30.9±0.5 | - |
| ***P. aeruginosa*** | 7.2±0.2 | 8.4±0.3 | 9.9±0.3 | 11.1±0.2 | 9.2±0.4 | 11.5±0.8 | 13.1±0.5 | 15.5±0.6 | 27.4±0.4 | - |
| ***P. mirabilis*** | 14.4±0.1 | 17.0±0.3 | 18.1±0.2 | 21.4±0.7 | 16.0±0.4 | 19.5±0.4 | 20.7±0.9 | 21.8±1.1 | 33.2±0.8 | - |

**Key:** Cip- Ciprofloxacin (positive control)

Dis- Distilled water (negative control)

- - No activity

Zones of inhibition = mean ± standard error

Table 4.2c: Antimicrobial activity of aqueous and ethanol extracts of *Phyllanthus niruri* at different concentrations of 25mg/ml, 50mg/ml, 100mg/ml and 200mg/ml respectively.

| **Organisms** | **Aqueous extract** | | | | **Ethanol extract** | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **Zones of Inhibition (mm)** | | | | | | | | | |
| | **Concentrations (mg/ml)** | | | | | | | | **Cip.** | **Dis.** |
| | **25** | **50** | **100** | **200** | **25** | **50** | **100** | **200** | **(µg/ml)** | |
| ***S. aureus*** | - | - | - | - | 2.2±2.2 | 3.8±3.8 | 4.8±4.8 | 15.2±0.8 | 29.6±1.1 | - |
| ***E. coli*** | 10.6±0.4 | 15.0±0.6 | 18.1±0.6 | 19.1±0.2 | 15.5±1.3 | 17.6±1.7 | 22.4±0.4 | 24.1±0. 3 | 29.4±0.6 | - |
| ***P. aeruginosa*** | - | - | - | - | - | - | 3.9±3.9 | 5.6±5.6 | 29.7±0.2 | - |
| ***P. mirabilis*** | 12.0±0.6 | 16.0±0.6 | 19.4±0.6 | 14.2±2.3 | 7.0±3.6 | 9.3±4.7 | 12.0±6.0 | 13.7±6.8 | 31.2±1.2 | - |

**Key:** Cip- Ciprofloxacin (positive control)

Dis- Distilled water (negative control)

- - No activity

Zones of inhibition = mean ± standard error