COMPARATIVE QUANTITATIVE AND QUALITATIVE PHYTOCHEMICAL STUDIES ON THE AQUEOUS ROOT EXTRACT OF *JUSTICIA CARNEA*

BY

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AN UNDERGRADUATE PROJECT SUBMITTED TO THE DEPARTMENT OF ENVIRONMENTAL MANAGEMENT AND TOXICOLOGY, FACULTY OF LIFE SCIENCES, UNIVERSITY OF BENIN, BENIN CITY, EDO STATE, NIGERIA; IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR AWARD OF BACHELOR OF SCIENCE (B.Sc) DEGREE IN ENVIRONMENTAL MANAGEMENT AND TOXICOLOGY.

NOVEMBER, 2025

CERTIFICATION

This is to certify that this research titled **"COMPARATIVE QUANTITATIVE AND QUALITATIVE PHYTOCHEMICAL STUDIES ON THE AQUEOUS ROOT EXTRACT OF *JUSTICIA CARNEA*"** was carried out by ANDREW IYERE and presented to the Department of Environmental Management and Toxicology, Faculty of Life Sciences, University of Benin, Benin City; in partial fulfillment of the requirements for the award of Bachelor of Science (B.Sc) in Environmental Management and Toxicology. It was conducted under suitable conditions, was carefully supervised and subsequently approved as having met the requirements for the award of Bachelor of Science degree in Environmental Management and Toxicology.

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OSAKPAMWAN UZAMA-AVENBUAN **Date**

Project Supervisor

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Prof. (Mrs.) E. T. Aisien **Date**

Head of Department

DECLARATION

I **"ANDREW IYERE"** declare that "**COMPARATIVE QUANTITATIVE AND QUALITATIVE PHYTOCHEMICAL STUDIES ON THE AQUEOUS ROOT EXTRACT OF *JUSTICIA CARNEA*"** is my own work and that all sources that I have used or quoted have been acknowledged by means of complete references and that this work has not been submitted before for any other degree at any other University.

ANDREW IYERE

_____

Date

DEDICATION

This Topic titled **"COMPARATIVE QUANTITATIVE AND QUALITATIVE PHYTOCHEMICAL STUDIES ON THE AQUEOUS ROOT EXTRACT OF *JUSTICIA CARNEA***" is dedicated to God Almighty, and to my supervisor **OSAKPAMWAN UZAMA-AVENBUAN**, my parents, siblings and all those who provided unwavering love, support and contributed to the success of this work.

ACKNOWLEDGEMENT

All glory, honour and praise belong to God Almighty, the source of my strength, wisdom and peace. Without His grace, none of this would have been possible. Through every challenge, delay and moment of uncertainty, God's faithfulness remained constant, and for that, I am eternally grateful.

To my amazing parents, Mr. and Mrs. IYERE, words will never be enough to thank you. Your love, prayers, sacrifices and constant encouragement have shaped me into who I am today. You have both shown me what hard work, humility and faith truly look like, and I will always carry those values with me wherever I go.

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To my Kezmond, I thank you for being a constant light and calm through this journey. Your kindness, patience and support have meant more than words could ever describe. You've been there in ways that truly mattered, and I'll always be grateful for that.

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To my course advisor, Dr A. F. Eghomwanre, thank you for your steady support, mentorship and words of wisdom that helped shape not just my academic journey but my personal growth as well.

Lastly, this one's for me, for showing up every single time, for holding on when it got tough, and for turning every doubt into drive. I've learned to grow through the pressure and to trust my journey even when the path wasn't clear..

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ABSTRACT

This study investigates the phytochemical compositiopn and antimicrobial potential of *Justicia carnea* root extracts. The research aims to evaluate the presence of key bioactive compounds and assess their efficacy against selected pathogenic microorganisms. Standard laboratory techniques were employed for phytochemical screening and antimicrobial testing using various solvent extractsq. Results revealed the presence of alkaloids, tannins, saponins, flavonoids, and phenolic compounds, indicating the plant's rich medicinal value. The extracts exhibited notable inhibitory effects against both Gram-positive and Gram-negative bacteria, suggesting broad-spectrum antimicrobial activity. These findings support the traditional use of *Justicia carnea* in herbal medicine and highlight its potential as a source of natural therapeutic agents.

CHAPTER ONE

INTRODUCTION

For centuries, plants and herbs have been central to human health care. Historical accounts show that in the nineteenth century, more than 80% of medicines were obtained from herbal sources, highlighting their vital role in drug discovery (Arsad *et. al.*., 2013). Herbal plants serve either as crude preparations used in traditional medicine or as sources of purified active compounds developed into modern pharmaceuticals. Their growing relevance is linked to the limitations in access to conventional medicine, which has encouraged renewed scientific interest (Cordell, 1993).

It has been estimated that over 2,500 flowering plants contribute medicinally useful substances, and roughly one-quarter of drugs in clinical use today are of plant origin (Dutta, 1999; Taylor *et. al.*., 2001). According to Saad and Said (2011), at least 25% of orthodox medicines contain one or more herbal-derived ingredients. Several studies further affirm that these natural compounds have provided the foundation for modern pharmacotherapy (Edeoga *et. al.*., 2005; Akinmoladun *et. al.*., 2007; Rout *et. al.*., 2009).

The continued global use of herbal remedies demonstrates their importance in health systems. In many developing countries, indigenous medicine remains the main form of therapy because it is affordable, accessible, and culturally accepted. The World Health Organization (2003) acknowledges their economic value and social acceptance, particularly in the management of chronic diseases such as diabetes, obesity, cardiovascular disorders, arthritis, and liver or kidney complications (Kunle *et. al.*., 2012). Akharaiyi and Boboye (2010) further note that these remedies are favored because they are readily available, low in toxicity, and generally well

tolerated. Their therapeutic properties are attributed to biologically active metabolites that exert diverse pharmacological effects (Firenzuoli and Gori, 2007; Doughari *et. al.*., 2009).

The popularity of herbal and alternative medicine has risen in recent decades due to factors such as cost-effectiveness, safety perception, and easy accessibility. Ethnopharmacological investigations, supported by experimental validation, have provided reliable evidence of their medicinal benefits. Studies continue to demonstrate their potential in disease prevention and treatment across different populations. In Nigeria, for example, over 325 species from 95 families are documented as being commonly used for treating ailments (Kunle *et. al.*., 2012). Herbal medicines can take various forms, ranging from crude plant materials to standardized extracts and finished products.

The health benefits of medicinal plants are largely due to their phytochemicals. Compounds such as alkaloids, tannins, flavonoids, terpenoids, steroids, saponins, and phenolics have been identified as active agents with antioxidant, anti-inflammatory, and detoxification-supporting properties (Fasuyi, 2006; Kumar *et. al.*., 2009; Fahey and Talalay, 1995). Although synthetic drugs are available for most conditions, their high costs and adverse effects have made natural alternatives increasingly attractive (Chattopadhyay and Bandyopadhyay, 2005; Oze *et. al.*., 2008).

Nevertheless, despite their wide application, many herbal remedies lack rigorous clinical evaluation. The World Health Organization (2008) has stressed the need for standardization and safety testing of medicinal plants. Obidike and Salawu (2013) argue that safety concerns remain, even as demand continues to grow. While herbal medicines are often perceived as harmless because of their natural origin (Latha *et. al.*., 2010), toxicological assessments are necessary to establish their safety profiles. Without such evaluations, the potential risks associated with

bioactive compounds remain uncertain. Toxicology therefore plays a key role in determining the safe clinical application of herbal-based drugs and should accompany pharmacological studies before widespread therapeutic use.

1.1 AIM

This study is aimed at the extraction and qualitative and quantitative determination of the phytochemical in water extracts of *Justicia carnea roots.*

1.2 OBJECTIVES

To achieve the above aim, the following objectives were set to:

i) Collect, dry, and pulverize the roots of *Justicia carnea.*

ii) Extract the pulverized root of *Justicia carnea* using water as solvent by maceration process.

iii) To determine the quantitative and qualitative phytochemical consistent of the aqueous root extract of *Justicia carnea*

CHAPTER TWO

LITERATURE REVIEW

2.1.1 *JUSTICIA CARNEA*

Justicia carnea is a medicinal plant used widely in Nigeria reported to have diverse functions including blood-boosting potential. *Justicia carnea* has been used in traditional medicine in Nigeria in the treatment and management of various diseases including: inflammation, gastrointestinal disorders, anemia, respiratory tract infection, cancer, malaria, sickle cell disease, diabetes, diarrhea, typhoid, hepatitis, liver diseases, etc. (Badami, *et. al.*., 2003 and Corrêa, *et. al.*., 2012).

Plate 1: Justicia carnea leaf (Photo credit)

Plate 2: *Justicia carnea* root (Photo credit)

2.1.2 Taxonomy/Classification of *Justicia carnea*

Kingdom: Plantae

Clade: Tracheophytes (vascular plants)

Clade: Angiosperms (flowering plants)

Clade: Asterids

Clade: Eudicots

Order: Lamiales

Family: Acanthaceae

Genus: *Justicia* L

Species: *Justicia carnea* Lindl.

(Source: Mabberley, 2017; The plant List,2023)

The leaves of the plant *Justicia carnea* when soaked in boiled water in a closed container for about 15minutes, when boiled, the green leaves release a purplish-red juice. The plant J. secunda

belongs to the Acanthaceae family. It is locally called ‘Asindiri’or ‘Ohowaazara’ (meaning medicine that gives blood) by the Ogbia people of Otuoke Otuaba community in Ogbia Local Government Area of Bayelsa State, and other communities in the Niger Delta region of Nigeria (Osioma *et. al.*., 2017; Perveen and Qaiser, 2010). It is used for the treatment of anaemia. Other common names are ‘Hospital too far’, ‘Blood leaf’ or Blood tonic. Its aqueous extract is normally served as a tea drink to the anaemic patients in these communities. Justicia secunda (Acanthaceae) is a plant used in traditional medicine in Cameroon in the treatment of anemia (Fongod *et. al.*., 2013). Some studies have also shown that the plant possesses antianemic and antimicrobial properties (Chen *et. al.*., 2012; N'Guessan *et. al.*., 2010). Compared to many plants whose iron contents are known, Justicia secunda appears to be a very important source of iron. These high iron contents (240 mg/g 19) justify its use as anti-anemic agent in the Congolese popular medicine (Achi *et. al.*., 2017; Moswa *et. al.*., 2008). Decoctions of the leaves of J. secunda are used traditionally for the treatment of anemia in humans (Fongod *et. al.*., 2013). Usually these decoctions are prepared only when need arises and in quantities that can be consumed before deterioration. It is worth noting that empirical observations in the field have shown that it is difficult to store this beverage beyond 3 days. Since the leaves are equally very perishable, the availability of the leaves and/or its decoctions out of season is very limited. To prolong the shelf-life of this drink, there is need to pasteurze and to stabilize it by the addition of an anti-bacteria agent which can delay the development of microorganisms during storage. *Justicia carnea* is a flowering plant that belongs to Acanthaceae family, widely distributed in various parts of Africa. In Nigeria, the shrubs of *J. carnea* commonly called ‘Hospital Too Far or Blood of Jesus’ and are grown around homesteads and act as fences (Sharma, 2004), which are easy to grow and propagate from stem cuttings by pushing the stems 1 to 2 inches into the soil.

Onyeabo *et. al.*. 2017 reported a survey among the Igbo local populace in Nigeria revealed that the plant under study is locally called “ogwu obara” meaning blood tonic. The deep purple coloured juice from the leaves of this plant is extracted either by soaking or boiling in water, which can be drunk as tea (Khan *et. al.*., 2017; Onyeabo *et. al.*., 2017). In other localities in Nigeria, the raw leaves are chewed and used together with “nchu anwu” as culinary vegetables to garnish yam porridge. It is generally considered as an ornamental plant. This pecies of Justicia, like others, are widely used in folk medicine for the treatment of inflammation, respiratory, and gastrointestinal disorder (Parker and Pearson, 2012). Most of the medicinal properties exhibited by plant extracts such as antimicrobial, antioxidant, hypocholesterolemic and anti-cancerous are associated with their bioactive constituents mainly phenols and flavonoids (Chandon *et. al.*., 2011; Janifer *et. al.*., 2010; Uroko *et. al.*., 2017). It has also been reported to be rich in both macronutrients and trace elements of which calcium and iron are in high quantity (Faiza *et. al.*., 2013). *Justicia carnea* is used as blood tonic locally in many parts of Nigeria and there is no sufficient scientific evidence to support its use as blood tonic by traditional consumers. This research work is therefore focused on the determination of proximate and phytochemical contents of *Justicia carnea* and J. secunda in order to evaluate its pharmacological and nutritional values. Ethnomedicinal uses of different parts *Justicia carnea Justicia carnea* is from the family of Acanthaceae Justicia and it is the largest genus of Acanthaceae having about 700 species. It is cultivated in West and Central Africa especially in countries like Nigeria, Ghana, Guinea and Togo (Anorado, *et. al.*., 2021). *Justicia carnea* is commonly called “Hospital is too far” or “blood of Jesus”. It is nown as “Ogwu obara” (blood tonic) by the Igbo speaking tribe of Nigeria while the Yoruba people of Nigeria call it “ewe eje” (blood leaf) as a reminder of the fact that the leaf is a substitute for blood transfusion. In folkloric medicine the decoction from the

leaves of J.carnea is employed in the treatment of muscle spasms and anemia (Olufunke, 2021). The decoction from boiled root of *J. carnea* is used in the treatment of menstrual pain (Oladele and Elem, 2018). *Justicia carnea* plant is also generally considered as an ornamental plant (rjiakor, *et. al.*., 2019). In some parts of Nigeria, the leaves of *J. carnea* are used as vegetable in the preparation of edible soup (Olufunke, 2021). *Justicia carnea* Lindl. (Lamiales: Acanthaceae) commonly referred to as the Brazilian plume flower, Brazilian-plume, flamingo flower or jacobinia is a flowering perennial plant native to the Atlantic forest ecoregions of eastern Brazil. The genus Justicia was named after a Scottish Gardner, James Justice, in the 18th century (Onyeabo *et. al.*., 2017). In Nigeria, the shrubs of *J. carnea* are grown around homesteads and act as fences, which are easy to grow and propagate from stem cuttings by pushing the cut stems 1 to 2 inches into the soil (Mabberley, 1997). It has been reported that *J. carnea* is rich in macronutrients and trace elements such as iron and calcium (Rasheed *et. al.*., 2013). Presently, the interest in studying and understanding the constituents of plants with healing properties have increased. It is widely distributed in Nigeria and used in homes as a decorative plant. It is well used in eastern Nigeria as blood tonic made by decoction (Onyeabo *et. al.*., 2017). Traditionally, several species of Justicia are used in the management of several ailments such as: inflammation, gastrointestinal disorders, diarrhoea, liver diseases, rheumatism and arthritis (Corrêa and Alcântara, 2012; Onyeabo *et. al.*., 2017). They also possess anti-inflammatory, anti-allergy, anti-tumor, anti-viral and analgesic activities (Radhika *et. al.*., 2013) including antioxidant activity (Medapa *et. al.*., 2011) and hepatoprotective activity (Ukpabi-Ugo *et. al.*., 2019). The phytochemical analysis showed that phenols, tannins, alkaloids, anthraquinone, saponins, flavonoids and reducing sugars were present in the leaves of *Justicia carnea* (Makunga *et. al.*., 2008; Onyeabo *et. al.*., 2017). It has been widely use as antimicrobial, antioxidant,

hypocholesterolemic and anti-cancerous and this may associated with the bioactive constituents like phenols and flavonoids present in it (Oloruntola *et. al.*., 2022). The reason for increasing interest in herbal medicines is the belief that because these medicines are natural and have been traditionally used, they are safe and harmless (Arsad *et. al.*., 2013). Nevertheless, their natural origin is not a guarantee of safety, as many studies concerning the risks associated with the use of herbal products and its dosage have been reported (Vaes and Chyka, 2000; Whiting *et. al.*., 2002). Hence, scientific information regarding the safety of this plant for use as an alternative medicine is very important before it is further developed into a new medicinal herbal therapy. Therefore, the objective of the present study was to determine the subacute toxicity of methanol extract of *J. carnea* leaves in Wistar albino rats. The genus Justicia, named after the 18th-century Scottish botanist James Justice, belongs to the large family of Acanthaceae consisting of about 600 species of herbs and shrubs native to the tropics and subtropics (Corrêa and Alcântara, 2012; Durkee, 1986). *J. carnea* (*Justicia carnea*) is a flowering plant, widely distributed in various parts of Africa. In Nigeria, the shrubs of *J. carnea* are grown around homesteads and act as fences, which are easy to grow and propagate from stem cuttings by pushing the stems 1 to 2 inches into the soil (Mabberley, 1997). A survey among the Igbo local populace in Nigeria revealed that the plant under study is locally called “ogwu obara” meaning blood tonic. The deep purple colored juice from the leaves of this plant is extracted either by soaking or boiling in water, which can be drunk as tea. In other localities in Nigeria, the raw leaves are chewed and used together with “nchuanwu” as culinary vegetables to garnish yam porridge. Traditionally, several species of Justicia are used in the management of inflammation, gastrointestinal disorders, respiratory tract infection, fever, pain, diabetes, diarrhea, liver diseases, rheumatism and arthritis (Badami *et. al.*., 2003; Corrêa and Alcântara, 2012). They also possess anti-inflammatory,

anti-allergic, anti-tumoral, anti-viral and analgesic activities (Radhika *et. al.*., 2013). Species of Justicia found in India, such as Justicia traquebareinsis and Justicia wynaadensis, have been reported to possess cardioprotective properties (Radhika *et. al.*., 2013) and antioxidant activity, respectively (Medapa *et. al.*., 2011) Onyeabo *et. al.*., (2017) reported that *J. carnea* leaf extracts possess anti-anemic potential, lending credence to the use of these plant extracts in folk medicine for the management of hemolytic anemia. The observations from the study revealed that leaves of *J. carnea* not only possess anti-anemic properties as reportedly used by traditional healers, but have hypolipidaemic potential, which could be beneficial to individuals predisposed to cardiovascular diseases. Further studies are warranted to determine the bioactive component present in *J. carnea* leaves that could be responsible for both anti-anemic and hypolipidaemic effects. *Justicia carnea* (Jacobinia or Jehovah's Witness plant in Nigeria) is a medicinal plant used widely in Nigeria and reported to have a blood-boosting potential (Onyeabo *et. al.*., 2017). Several species of Justicia have 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 the treatment of anaemia, inflammation, and respiratory and gastrointestinal diseases (Onyeabo *et. al.*., 2017). These functions possessed by the plant are associated with its bioactive constituents comprising mainly phenols and flavonoids (Uroko *et. al.*., 2017). It has been reported that some species of Justicia possess 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). Inflammation is the response of the body to infections by microorganisms, burns, and injuries that may endanger human health (Shingala *et. al.*., 2021). The reports of Cheng *et. al.*., (2017), state that clinically, it can be characterized 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, and cyclooxygenases (Christiakov *et. al.*., 2018). Other species of Justicia such as, J. flava, and J. adhatoda also possess anti-inflammatory activity (Correa and Alcantara, 2012). 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 *J. carnea* leaf extract in phenylhydrazine-induced anaemia in albino rats reported 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 analyzed. 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 can increase red blood cells. Despite the therapeutic importance of medicinal plants, toxic substances are present in large numbers of plants examined. Olaniyan *et. al.*. (2016) reported that contamination 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 their efficacy and safety can lead to unexpected toxic effects, which results in 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). Herbal plant usage has been on the increase due to its medicinal potential, hence the scientific investigation on the safety of *J. carnea* for use as an alternative medicine. This study aimed to evaluate the toxicity of

J. carnea in Wistar albino rats with a focus on hematological analysis and histopathological effects of *J. carnea* on the liver and kidneys of Wistar rats. *Justicia carnea* is an unconventional medicine, traditionally used to manage several diseases (Onyeabo *et. al.*., 2017; Akintimehin *et. al.*., 2021). The assumption that herbal preparations/remedies are safe and effective has influenced the indiscriminate use of such remedies, most especially among rural communities, where these remedies can be administered for a long time without considering the dose or concentration that will bring about toxic side effects (Ben-Arye *et. al.*., 2016). The genus Justicia belongs to Acanthaceae family and was named after the 18th century Scottish horticulturist James Justice (Correa and Alcantara, 2012). This perennial shrub has a characteristic pink to purple or orange flower colour which blooms all through the summer. It grows 3-4 feet wide and height of 1.8m (Wasshausen and Wood, 2004). The flowering plant is a native of South Africa, precisely Brazil and widely distributed in Africa, even Nigeria. It is propagated by pushing 1-2 inches of the stem into the soil and kept until new leaves appear (Mabberley, 1997). *Justicia carnea* function mostly as ornamental plant (Parker and Pearson, 2012).Medicinal properties of several species of Justicia include; respiratory, gastrointestinal disorder, inflammation including application in rheumatism and arthritis, headache and fever which may be associated with sedative and analgesic properties, cancer, diabetes and HIV (Correa and Alcantara, 2012). Justicia neesii has been reported to exhibit antitumoral activity (Rajasekhar and Subbaraju, 2010).). Characterization based on GC-MS analysis of ethanol extract of *Justicia carnea* suggested the presence of isonicotonic acid N-oxide, phosphinodithioic acid, palmitic aci, 7H-purine, 7-benzyl-2,6-dichloro,9,12,15-octadecatrien-1-ol and2,2,3,3,4,4,5,5,5-Nonafluoro-pentanoic acid methyl ester (Otuokere *et. al.*., 2016). The haematological and lipid modulatory effect of aqueous and ethanol extract of *Justicia carnea*

yielded a positive effect as described by Orjiakor, (2014); Onyeabo *et. al.*., (2017). According to Alozie *et. al.*., (2018), aqueous extract of *Justicia carnea* demonstrated considerable antiplasmodial activity. Leaves of *Justicia carnea* contains in abundant different phytochemicals with different therapeutic potentials which could serve as precursors in the synthesis of orthodox drugs as well as herbal agents in treatment of diseases..(Peters et.al., 2022). *Justicia carnea* contains lignans which one the major components of the active extracts of the species of Justicia, exhibiting important pharmacological properties (Ubaoji *et. al.*. 2020). Study carried out by Ubaoji *et. al.* (2020) that *Justicia carnea*leaf have compounds with thepotential to scavenge free radicals by donating electrons that stabilizes free radicals. These free radicals cause oxidative stress. Therefore, *Justicia carnea* leaves are rich sources of antioxidants. The genus Justicia named after Scottish Gardner James Justice in the 18th century, established by Linnaeus in 1753 (Otuokere *et. al.*. 2016; Udedi *et. al.*. 2020; Ukpabi-Ugo et. al, 2019; House, 2015) are scandent or erect annual or perennial subshrubs or herbs. The leaves are petiolate with an entire leaf margin. They are known by their spicate inflorescences which could be compound or simple, two stamens with asymethrical anthers each, 3 lobed anterior lip, 2 lobed posterior lip, bilabial corolla, four seeded capsule and a basalsterile portion (Kitadi *et. al.*. 2019; Corrêa and Alcântara, 2012; House, 2015). Justicia, a tribe of Ruellieae and subtribe of Justiciinae is the largest genus of Acanthaceae family, having about 600 species (Kitadi *et. al.*. 2019; Corrêa and Alcântara, 2012; House, 2015), contrarily to the seven hundred species reported by House (2015) and Danie (2020). Justicia possesses 695 species with scientific names, out of which 376 are accepted species names and 32 scientific names of intraspecific. Justicia species are reported to occur in tropical to warm temperate regions of the Americas, India, Indonesia, Southeast Asia, Malaysia, Pakistan and Africa (Manokar, 2019). Justicia comprises of 700 species and are predominantly

pantropical, while many still occur in subtropical and temperate region (Danie, 2020). *Justicia carnea* is an evergreen erect perennial shrub or herb that grows up to 4 feet, it has petiolate leaves with an entire leaf margin. It is known for its bilabial corolla, 2-lobed posterior lip, 3-lobed anterior lip, two stamens, four seeded capsule and pinkish flowers. The plant grows well in loamy soil with dappled sunlight (Nduche and Offor, 2019). Ethnomedicinal Uses *Justicia carnea* commonly known in Nigeria as: ‘’blood root’’ (Oladele and Elem, 2018), “hospital too far” (Udedi *et. al.*. 2020), “ogwu obara” in Igbo (Onyeabo *et. al.*., 2017; Anigboro *et. al.*. 2019), ‘Oso-afia in Ogba/Egbema/Ndoni (Oladele and Elem, 2018). In Cameroon as: “Ewolamajia” in Bakweri (Nji et. al, 2020), in Ghana as: “Ntumunum” in Bosomtwe and Sekyere East. Also known in Brazil and South America as: “Jacobinia”, “Pink jacobinia” Cardinal’s guard”, “Brazilian plume”, “Pine-bur begonia” and flamingo flower” (Orjiakor et. al, 2019). In Nigeria, the root is used in treating menstrual pain (Oladele and Elem, 2018), the decoction of the leaf as blood supplement and management of anemia (Ani *et. al.*. 2020; Onyeabo *et. al.*., 2017; Udedi *et. al.*. 2020; Ukpabi-Ugo et. al, 2019; Orjiakor et. al, 2019; Igbinaduwa *et. al.*. 2019; Akintimehi et, al., 2021). The plant has also been used as hallucinogens, sedative (Nduche and Offor, 2019), ornamental (Orjiakor et. al, 2019) and in treating HIV, cancer, diabetes, whooping cough, epilepsy, bronchitis cold (Nduche and Offor, 2019), respiratory tract diseases, gastro intestinal infections, inflammation (Ukpabi-Ugo *et. al.*. 2019; Nduche and Offor, 2019; Orjiakor et. al, 2019), sickle cell disease, hepatitis, typhoid, (Udedi *et. al.*. 2020), malaria (Udedi *et. al.*. 2020; Ukpabi-Ugo *et. al.*. 2019; Komlaga, 2015) rheumatism, liver disease, arthritis and diarrhea (Ukpabi-Ugo et. al, 2019;). The plant has also exhibited hypocholesterolemic, anti microbial, anti cancer, (Orjiakor et. al, 2019), antioxidant (Ukpabi-Ugo et. al, 2019; Orjiakor et. al, 2019), anti tumor, anti allergy, anti inflammatory and analgesic properties (Ukpabi-Ugo et. al, 2019).

Chemical Constituents The preliminary phytochemical analysis of aqueous leaf extract of *J. carnea* revealed that terpenoids, tannins, alkaloids, carbohydrates, flavonoids, saponins, phenols, reducing sugar and glycosides. Phenols and flavonoids were found in high concentration which could be responsible for the anticancer and antioxidant activities exhibited by the plant (Orjiakor et. al, 2019). The presence of terpenoids and carbohydrates in the aqueous leaf extract of the plant was against the findings of Anigboro *et. al.*., (2019), who reported their absence in aqueous leaf extract of the same plant. Also present in the aqueous leaf extract are vitamin A, B1, B12, B6, B9, B2, C and E containing highest concentration of vitamin C, and high concentration of iron and calcium while Magnesium, zinc and copper were present in low concentration (Orjiakor et. al, 2019). Similarly, Onyeabo *et. al.*. (2017) in their work had already reported that phenols, alkaloids, tannins, flavonoids, terpenoids, saponins and steroids were present in the ethanol leaf extract of *J. carnea*, with high percentage of terpenoids, alkaloids and saponins. Also present in the extracts were vitamins C, E and A. Proximate analysis of the extract revealed the presence of high percentage of carbohydrate and protein, also present were moisture, crude fibre, ash and low percentage of fat (Igbinaduwa *et. al.* (2019) in their work also reported presence of alkaloids, flavonoids, tannins, phenols and saponins in the methanol leaf extract of *J. carnea*. Result of proximate analysis revealed carbohydrates (30%), protein (24.31%), crude fibre (18.71%), Fe, Zn, K, Ca and Pb were also found in the extract. In another research, flavonoids, alkaloids, phenols, tannins, carbohydrates, glycosides, gum, protein, fixed oils and fat were reported to be present in methanol leaf extract of *J. carnea*. While alkaloids, fixed oils were absent in the root extract of *J. carnea*, sterols, gum were reported in both stem and root extracts [30]. The absence of saponins in the methanol leaf extract of *J. carnea* was against the reported of Igbinaduwa *et. al.*. (2019) who in their work reported the presence of saponins. The presence of vitamin B12,

vitamin C, Ca, Zinc, tannins and iron found in various extracts of *J. carnea* lends credence to the ethnomedicinal use of the plant in treating anaemia (N'guessan, 2010). The presence vitamins A, C and E which are antioxidants in the extract was attributed to reduction in the oxidative stress caused by the phenylhydrazine, and thereby reversing the anemic condition in the rats. Saponins and alkaloids have been reported to have anti anemic potentials. Phenolic were found in all the extracts of *J. carnea*, with largest quantity found in leaf, the phenolics have attributed to antioxidant property of leaf of the plant. The use of *J. carnea* in the treatment of cancer, anaemia and diabetes could be attributed to the presence of alkaloids, saponins, flavonoids and phenols. In another work, the ethanol leaf extract of *J. carnea* was said to have contained six compounds- 2,2,3,3,4,4,5,5,5- Nonafluoropentanoic acid methyl ester, palmitic acid, Phosphorodithioic acid, diphenyl, Isonicotinic acid N-oxide, 7H-Purine, 7-benzyl-2,6-dichloro and 9,12,15-Octadecatrien-1-ol using GC-MS (Otuokere, 2016). While Anigboro *et. al.*. (2019) in their work identified 2-Cyclopenten-1-one, 3-ethyl-2-hydroxy-, 2-Methoxy-4-vinylphenol, Formic acid, 2,6-dimethoxyphenyl ester, 9-Undecen-2-one, 6,10-dimethyl-, oleic acid, 5-Cyclohexadecen-1-one, 2-Heptadecenal, 7,11-Hexadecadienal in aqueous leaf extract using also GC-MS. These compounds were similar to compounds in the NIST library software. Pharmacological Uses Anti diabetic/ Hypoglycemic Activity The fasting blood sugar, the liver and kidney function tests as well as the lipid profile of ethanol leaf extract of *J. carnea* on alloxan-induced albino rats were analysed, the results revealed that the extract caused an in vivo reduction of blood glucose level at both 100mg/kg and 200mg/kg. It was also reported that that there was significant reduction ($p<0.05$) of Serum levels of ALT, creatinine and AST, while there was significant increase in serum levels of total protein, urea and ALP, and non-significant decrease in serum levels of chloride, sodium, bicarbonate and potassium. At, 200mg/kg, serum

levels of TC, TG, LDL and VLDL were also significantly reduced (Ani *et. al.*. 2020). Similarly, the methanol leaf extract of *J. carnea* also showed significant in vivo reduction in blood glucose levels in alloxan-induced rats treated with 200, 500 and 1000mg/kg body weight of *J. carnea* (Ukpabi-Ugo *et. al.*. 2020). Also antidiabetic potential of aqueous leaf extract of *Justicia carnea* was investigated using α-amylase inhibition model from the linear plot of extract concentration (µg/mL) against percentage α-amylase inhibition, the extract significantly ($p<0.05$) decreased α-amylase activity with increasing concentrations of the extract (Anigboro *et. al.*. 2019). The results confirmed the folkloric use of *J. carnea* in treating diabetes (Nduche and Offor, 2019). Antioxidant Meanwhile, the antioxidant activity of methanol leaf extract of *J. carnea* on CCl4-induced oxidative stress in female albino rats showed that the extract at 200, 500 and 100mg/kg bw significantly increased the concentrations of CAT, GSH and SOD, but also decreased the serum MDA at the same concentrations (Ukpabi-Ugo et. al, 2019). Since reactive oxygen could be produced by CCl4, which could also lead to lipid peroxidation, concentration of MDA which was a secondary product of such peroxidation was increased by CCl4 which was due to increased lipid peroxidation. Aqueous leaf extract of the plant also showed exhibited high scavenging activities against DPPH and nitric oxide radicals, with significantly increased ($p<0.05$) ferric reducing antioxidant power (FRAP) and total anti-oxidant capacity (TAOC) values in a dose-dependent manner (Anigboro *et. al.*. 2019). Phenols and flavonoids present in the leaf of *J. carnea* were attributed to the antioxidant property of the extract (Ukpabi-Ugo et. al, 2019). Similarly, the in vitro antioxidant activity of ethanol leaf extract of *J. carnea* using DPPH scavenging assay, total phenols, total ascorbic acid, total β-carotene, total lycopene, total flavonoids assays, showed that the extract showed both scavenging and reducing power to both DPPH and Ferric ion with EC50 of 200µg/ml and 40µg/ml respectively. The extract was also

contained high concentration of content of, total phenols, total ascorbic acid, total β-carotene, total lycopene, total flavonoids (Udedi *et. al.*. 2020), which justified its antioxidant property. Flavonoids are potent antioxidants, and act as free radical scavengers so can exhibit antioxidant activity (Velayutham *et. al.*. 2009). Phenolic compounds have been shown to exhibit antioxidant properties(Araújo *et. al.*. 2015; García-Alonso *et. al.*. 2015; Erge and Karadeniz, 2011) polyphenols are antioxidants which induce cytoprotective proteins which act in a variety of antioxidant actions from reduction of oxidants to the production of endogenous direct antioxidants(. (Christensen and Christensen, 2014), flavonoids prevent diseases related to oxidative stress in living systems (Čvorović, 2018), terpenoids exhibit antioxidant activity in their interactions with free radicals (Ayodele *et. al.*. 2020), flavonoids are strong polyphenolic antioxidants which reduce oxidative stress by acting as free radical scavengers, they inhibit the aggregation of plasma platelet which participate in pathogenesis of cardiovascular disease (Orjiakor et. al, 2019; Babu and Liu, 2009). So the use of the plant in the treatment of high blood pressure is attributed to the presence of flavonoids. Similarly, terpenoids such as lycopenes and β- carotenes containing conjugated double bonds have been reported to have both singlet oxygen and free radical quenching abilities, protect cellular components from oxidative damage, and inhibit lipid peroxidation by scavenging peroxyl radicals produced by the peroxidation reaction, and therefore are strong antioxidants (Hussein *et. al.*., 2016; Novelina and Adrian, 2016; Nwaichi, 2015; Sidhu *et. al.*. 2017). Hepatoprotective Activity Anti hepatotoxicity assay of methanol leaf extract of *J. carnea* was conducted on CCl4-induced albino rats. The result indicated that at 200, 500 and 100mg/kg, the leaf extract showed significant decrease ($p<0.05$) in bilirubin, ALP, AST and ALT content. It was reported that there was better liver framework and in better necrosis of the tested liver from histopathological survey by the extract, when compared

with the liver from CCl4- induced rat only, showing that the extract exhibited hepatoprotective activity (Ukpabi-Ugo et. al, 2019). The result lends credence to the use of the plant in treating hepatitis (Udedi *et. al.*. 2020). Anti hyperlipidaemic Activity The ethanol leaf extract of *J. carnea* was reported to have shown anti hyperlipidaemic activity through significantly reducing serum cholesterol, LDL, VLDL, triacylglycerol concentrations and increasing the HDL concentration in phenylhydrazine –induced anemic rats with respect to anemic and non anemic rats. The decrease in the serum cholesterol concentration was attributed to the presence of steroids- which was said to contain phytosterols that could reduce plasma cholesterol concentration and saponins that has been said to be anti hyperlipidemic by reducing the uptake of cholesterol in the gut in the extract (Onyeabo *et. al.*., 2017). Methanol leaf extract of *J. carnea* also showed anti hyperlipidemic by significantly reducing VLDL, LDL, serum cholesterol and triacylgycerol and increased the HDL ((Ukpabi-Ugo et. al, 2020), which is in line with the report of Onyeabo *et. al.*. (2017) on ethanol leaf extract. The reduction of triacylglycerol was also attributed to the presence of some metabolites such as saponins in the extract. Increase in HDL and decrease in the LDL by extract would in general reduce cholesterol and reduce the risk of heart attack, cardiovascular diseases, atherosclerosis and stroke (Onyeabo *et. al.*., 2017). Anti Anaemic and Antisickling Activity In a work, the haematological assay of ethanol leaf extract of *J. carnea* on phenylhydrazineinduced anemic rats showed that the extract at 500mg/kg and 1000mg/kg showed significant increase in Packed Cell Volume, RBC, Hb and WBC counts. The increase in RBC was attributed to the presence of some metabolites such alkaloids, saponins and flavonoids. The increase in the heamatological parameters reversed the anemic condition of phenylhydrazine-induced anemic rats from 14 day treatment (Onyeabo *et. al.*., 2017). Confirming the report of Onyeabo *et. al.*. (2017) and Igbinaduwa et. al, (2019) revealed that

methanol leaf extract of *J. carnea* also reversed the phenylhydrazine- induced anaemia by increasing the reduced RBC, WBC, Hb, PCV, platelet, monocytes, lymphocytes and Neutrophil levels 100-1000mg/kg body weight of extract. The level of monocytes decreased on administration of higher concentrations of the extract and was attributed to absence of anaemia at those concentrations. The increase in the RBC and Hb was understandably due to high concentration of iron present in the extract. In line with their findings, Nji *et. al.*. (2020) in their work confirmed the heamatinic activity of *J. carnea*, when they reported that the leaf powder of the plant also significantly increased the same parameters including platelet counts. Saponins and alkaloids found in the extract have been reported to also have anti anemic activity which could have caused the increment in the haematological parameters levels. These works validated the use of *J. carnea* as blood tonic and in treating anaemia (Ani *et. al.*. 2020; 12,Udedi *et. al.*. 2020; Orjiakor *et. al.*. 2019). Toxicity Studies Generally, the leaf extract of *J. carnea* was reported to be non toxic and could be used in traditional medicine (Onyeabo *et. al.*., 2017), which is in line with the findings of Akintimehin *et. al.*. (2021) that the LD50 of ethanol extract of *J. carnea* was greater than 5000 mg/kg body weight. Higher doses (> 500 mg/kg) of extract significantly ($p < 0.05$) increased RBC, hemoglobin and platelet compared to the control. Liver superoxide dismutase (SOD) activity was significantly ($p < 0.05$) increased at 1200 mg/kg while other tested doses caused no detrimental effect on glutathione, catalase, SOD and malondialdehyde level in liver and kidney. Histopathological examination of liver and kidney showed mild to severe pathological lesion in a dose dependent manner. They concluded that the extract of J. is relatively safe, could be beneficial in alleviating hematology related abnormalities without causing adverse effects on endogenous antioxidant system. Contrarily to the report of Onyeabo *et. al.*. (2017) and Akintimehin *et. al.*. (2021), the photomicrographs of processed kidney liver

tissues showed that kidney tissue from animals treated with 100 and 200mg/kg of methanol leaf extract of *J. carnea* showed lobulation of glomerular tuft and collapsed tubules, and there was infiltration of interstitial tissue with inflammatory cells which indicated distorted kidney, while in the liver, the portal vein were congested with blood cells, micro vesicular steartosis and cytoplasm were replaced with fatty cells, and the nuclei were centrally placed, which indicated that there was a distortion in the liver. The toxicity of the extract could be attributed to the presence of Pb, which had been shown to cause histological changes in the kidney and hydropic degeneration in the liver of dog, and Diminution and even depletion of total proteins in the kidney and liver of Oreochromis mossambicus, caused dramatic changes in the subcellular distribution and expression of rat kidney glutathione S-transferase, caused vacuolation, fatty degeneration, congestion within central veins, hemorrhage and infiltration of inflammatory cells in the liver of rats (Daggett *et. al.*. 1998; Dehkord et. al, 2015; Meshram *et. al.*. 2019; White, 1997). *Justicia carnea* functions mostly as ornamental plant (Parker *et. al.*. 2011). It grows 3-4feet wide and a height of 1.8m (Wasshausen and Wood, 2004). It is propagated by pushing 1-2 inches of the stem into the soil and kept until new leaves appear (Mabberly, 1997). The ethnopharmacological properties of several species of Justicia include respiratory, gastrointestinal disorder, inflammation including application in rheumatism and arthritis, headache and fever which maybe is associated with sedative and analgesic properties, cancer, diabetes and HIV (Corrêa and Alcântara, 2012; Badami et, al., 2003). According to Alozie *et. al.*. (2018), aqueous extract of *Justicia carnea* demonstrated considerable antiplasmodial activity. Presently, the study determined the oral median lethal dose of ethanol leaf extract pf *Justicia carnea* as well as its adverse effect on liver histopathology, haematological and biochemical parameters.A great diversity of chemical classes is found in the Justicia family of plant, mainly

alkaloids, lignans, flavonoids, and terpenoids (Corrêa and Alcântara, 2012; Trease and Evans, 2009). Several species of the Justicia family are used traditionally in the management of various ailments such as inflammation, gastrointestinal disorders, respiratory tract infection, fever, pain, diabetes, diarrhea, liver diseases, rheumatism and arthritis (Corrêa and Alcântara, 2012; Badami et, al., 2003). The ethno pharmacological potentials of J. species lay on the bioactive ompounds that exhibit physiological action on the body. Peter *et. al.* (2022) reported that oral administration of both aqueous and ethanol extracts of *Justicia carnea* is safe at the doses of 500 and 1000mg/kgb.wt. The extracts stimulate hemopoiesis and thrombopoieis hence could be used in management of hemophilia. *Justicia carnea* is a member species of the genus Justicia in the family Acanthaceae. Herbaceous and perennial in nature and commonly called Jacobinia or Flamingo plant or Hospital too far. Morphologically, *J. carnea* grows up to 3-6feet tall with evergreen leaves and highly branched stem. The leaves are simple and opposite, elliptic, acute with entire margins, deciduous and pinnate venation. The inflorescence is spike with clustered flowers. The fragrant flower colors are white, pink, apricot, yellow and lavender [7,9]. The difference in flower colour explains that this could be variety forms of *J. carnea*. The fruits are brown, pod-like in structure with dry or hard surface. The medicinal properties of *J. carnea* are enormous and are paramount in southern Nigeria. Recent research has shown that it possesses anti-inflammatory, antimicrobial, hepatoprotective effects, anti-cancerous properties (Uroko *et. al.*. 2017; Orjiakor *et. al.*. 2019; Ukpabi-Ugo *et. al.*. 2019). In trado-medicine, decoction of *J. carnea* is used to boost blood level, aid digestion, dysentery, boost the immune system, influenza, diarrhea etc. (Otuokere, 2016; Joshi and Josh, 2000i). Although it is used to boost blood level in southern Nigeria, this application has not been certified and without clinical tests (Orjiakor *et. al.*. 2019). *Justicia carnea* (belonging to Acanthaceae family) is a flowering plant consisting ~ 600

species that are widely distributed in the tropics and subtropics (Corrêa and Alcântara, 20`2). In various parts of Africa, several species of Justicia are used in traditional medicine for the treatment of anemia, inflammation, fever, diarrhea, liver diseases and arthritis respiratory and gastrointestinal disorder (Badam *et. al.*. 2003; Onyeabo *et. al.*. 201`7). Recently, it has also been reported that the species possess cardioprotective properties, antioxidant and are generally rich in vitamins and minerals (Faiza *et. al.*. 2013; Radhika *et. al.*. 2013; Medapa *et. al.*. 2011). In Nigeria, the leaf of *Justicia carnea* is usually prepared with edible vegetables to make soup, boiled separately in water to make tea or prepared by cooking with other medicinal plants for therapeutic purposes. Despite the avalanche use of medicinal plant, preliminary toxicity studies remain essential tools to ensure safe consumption and prevent unexpected toxicity that could arise from long term exposure (Akintimehin et. al, 2021). Commonly referred to as pink jacobinia, famingo fower, or pine-bur begonia in English, and known as "Ewe eje" in Yoruba, *Justicia carnea* (Acanthaceae family) is a delicate perennial shrub that is extensively grown in tropical and subtropical regions. According to reports, Justicia species can help with a variety of health issues, including cancer, diabetes, gastrointestinal issues, and arthritis (Onyeabo *et. al.*., 2017). Additionally, it has positive efects on hematological parameters (Asakizi *et. al.*., 2020; Peters *et. al.*., 2022). Active phytochemicals, minerals, and essential micronutrients like iron, copper, zinc, folic acid, and selenium have all been connected to the health advantages of *J. carnea* leaves (Asogwa *et. al.*., 2020). Although *J. carnea* has been used for a variety of medical purposes in folklore, there is no scientifc or experimental proof that it can reduce oxidative stress in the brain or afect the expression of cellular adhesion molecule-1. Based on these fndings, the current study was created to assess how *Justicia carnea* leaf extract afected the expression of

cellular adhesion molecule-1 and brain oxidative stress in albino mice that had been experimentally exposed to trinitrobenzene sulfonic acid (TNBS).

2.2 BIOACTIVE CHEMICAL CONSTITUENTS IN MEDICINAL PLANTS

These plants contain secondary metabolites (alkaloids, flavonoids, saponins, steroids, tannin and phenolic compounds), vitamins and minerals, which are bioactive, compounds and have been known to have anti-microbial properties (Chung *et. al.*., 1998). Also, these compounds are found in varying concentrations in human and animal diets.

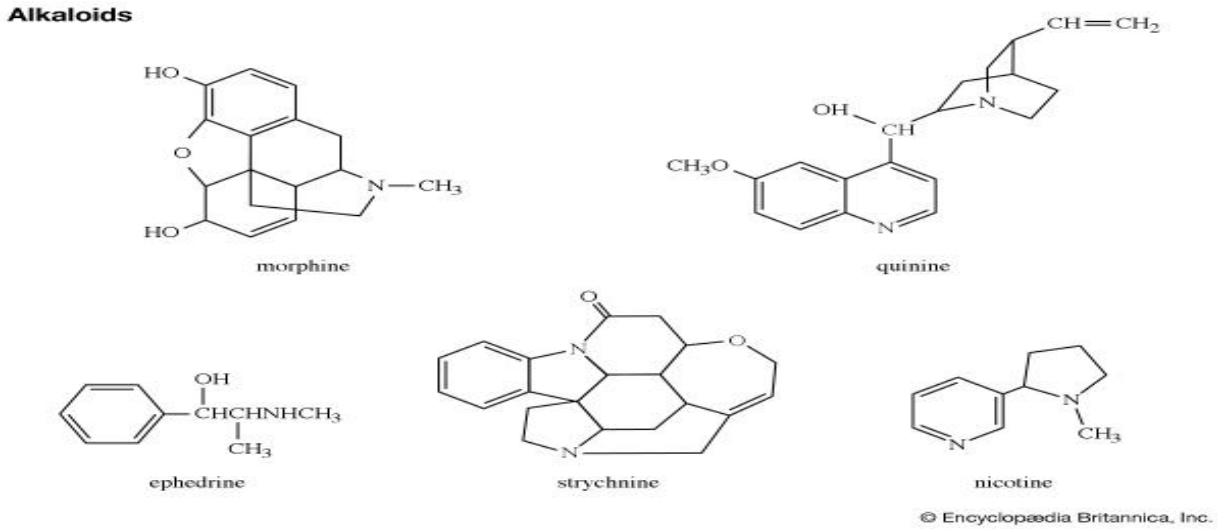
2.2.1 ALKALOIDS

Alkaloid is a class of naturally occurring organic nitrogen-containing base, they have diverse and important physiological effects in humans and other animals. Well-known alkaloid includes, morphine, strychnine quinine, ephedrine and nicotine. Alkaloids are found primarily in plants and are especially common in certain families of flowering plants. More than 3,000 different types of alkaloids have been identified in a total of more than 4,000 plant species. In general, a given species contains only a few kinds of alkaloids, though both the Opium poppy. And the ergot fungus (Claviceps) each contains about 30 different types. Certain plant families are particularly rich in alkaloids; all plants of the family (Papaveraceae) are thought to contain them, for example, the Ranunculaceae (buttercup), Solanaceae.

The chemical structures of alkaloids are extremely variable. Generally, an alkaloid contains at least a nitrogen atom in an amine-type structure i.e., one derived from ammonia by replacing the hydrogen atoms with hydrogen-carbon groups called hydrocarbons. This or another nitrogen atom can be active as a base in acid-base reactions. The name alkaloid (“alkali-like”) was originally applied to the substances because, like the inorganic alkalis, they react with acids to form salts.

Most alkaloids have one or more of their nitrogen atoms as part of a ring of atoms, frequently called a cyclic system. Alkaloid names generally end in the suffix -ine, a reference to their chemical classification as amines. In their pure form most alkaloids are colourless, nonvolatile, crystalline solids. They also tend to have a bitter taste.

Structures of alkaloids



2.1.3 GLYCOSIDES

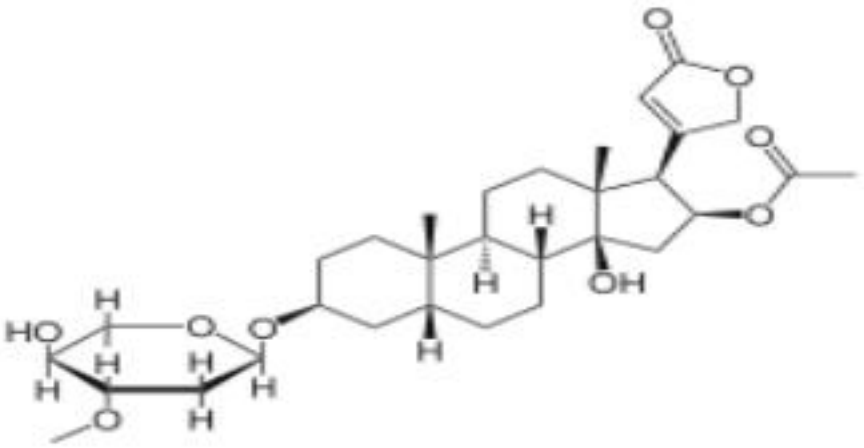
Glycosides are a class of organic compounds that increase the output force of the heart and increase its rate of contractions by acting on the cellular sodium potassium ATPase pump. Their beneficial medical uses are as treatment for congestive heart failure and cardiac arrhythmia; however their relative toxicity prevents them from being widely used. They are most commonly found as secondary metabolites in several plants such as foxglove plants these compounds nevertheless have a diverse range of biochemical effects regarding cardiac cell function and have also been suggested for use in cancer treatment. The general structure of a glycoside consists of a steroid molecule attached to a sugar (glycoside) and a R group The steroid nucleus consists of

four fused ring to which other functional groups such as methyl hydroxyl and aldehyde groups can be attached to influence the overall molecule's biological activity.

Structure of Glycoside

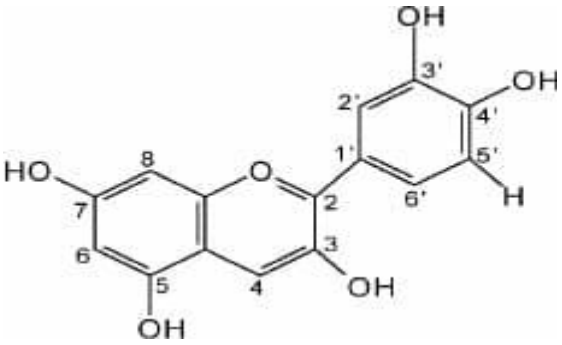
2.3 REDUCING SUGAR

A reducing sugar is any sugar that is capable of acting as a reducing agent because it has



a free aldehyde group or a free ketene group. All monosaccharides are reducing sugars, along with some disaccharides, oligosaccharides, and polysaccharides. The monosaccharides can be divided into two groups: the aldoses, which have an aldehyde group, and the ketoses, which have a ketone group. Ketoses must first tautornerize to aldoses before they can act as reducing sugars. The common dietary moceharidesgalactose, glucose and fructose are all reducing sugars.

2.3.1 FLAVONOIDS



Structures of flavonoids

Flavonoids or bioflavonoids are a term that originated from the latin word flavus (Meaning yellow in their colour in nature) are a class of plant and fungus secondary metabolites. Chemically, flavonids hare the general structure of a 15-carbon skeleton, which consists of two phenyl rings (A and B) and a heterocyclic ring (G) this carbon structure can be abbreviated C_6-C_3-C_6 according to the IUPAC nomenclature, they can be classified into;

- Flavonoids or bioflavonoids
- Isoflavonoids, derived from 3-phenylchromen-4-one (3phenyl 1,2 benzopyrone) structure.
- Neoflavonoids, derived from 4-phenylcoumarine (4-pheny1-1,2-benzopyrone) structure

The three-flavonoid classes above are all ketone-containing compounds and as such are anthoxanthins (flavones and flavonols).

Flavonoids (specifically flavanoids such as the catechins) are the most common group of polyphenolic compound in the human diet and are found ubiquitously in plants. Flavonols the original bioflavonoid such as quercetin, are also foundubiquitously but in lesser quintiles food with a high flavonoid content these include parsley, onions,blueberries berries, black tea, green tea and bananas, all citrus fruits ginkgo biloba, red wine, sea buckthoms, buck wheat, and dark chocolate (with a cocoa content of 70%or greater).

2.3.2 TERPENOIDS

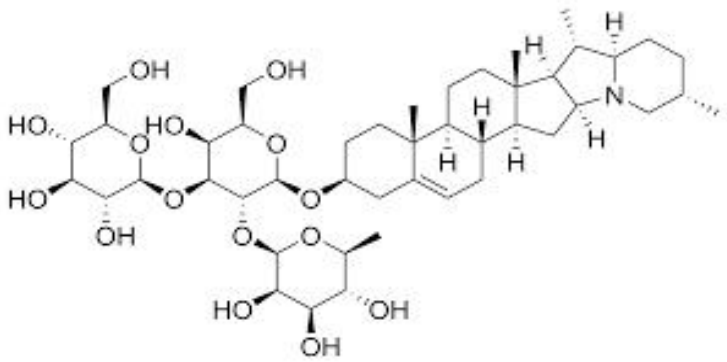
The terpenoids are a class of natural products, which have been derived from five-carbon isoprene units. Most of the terpenoids have multi- cyclic structures that differ from one another by their functional groups and basic carbon skeletons. These types of natural lipids can be found in every class of living things, and therefore considered as the largest group of natural products (Elbien, Molyneux, 1999). Many of the terpenoids are commercially interesting because of their use as flavors and fragrances in foods and cosmetics example; menthol and sclareol or because

they are important for the quality of agricultural products, such as the flavour of fruits and the fragrance of flowers like linalool (Harbome, Tomas Barberan, 1991).

Terpenes are widespread in nature, mainly in plant as constituents of essential oils. Their building block is the hydrocarbon isoprene, $CH_2=C(CH_3)-CH=CH_2$. Terpene hydrocarbons therefore has molecular formular $(C_5H_8)_n$ and are classified according to the number of isoprene units (Langenheim, 1994).

2.4 SAPONINS

Saponins are glucosides with foaming characteristics. Saponins consist of a polycylic aglycones attached to one more sugar chains. The aglycone part, which is also called sapogenin, is either steroid (C_{27}) or a triterpese (C_{30}) the foaming ability of saponins is caused by the combination of a hydrophobic (fat-soluble) sapogenin and a hydrophilie (water-soluble) sugar part Saponins have bitter taste. Some saponins are toxic and are known as sapotoxin (Lazze 2003)



Structure of saponin

Image source: Wikipedia

Saponins are phytochemicals that can be found in most vegetables, beans and herbs. The best known sources of saponins are peas, soybeans, and some herbs with names indicating

foaming properties such as soapwort, soapbark and soapberry Commercial saponins are extracted mainly from Yucca schidigera. (Ronald and Ronaid 1997)

Saponins have many health benefits. Studies have illustrated the beneficial effects on blood cholesterol levels, cancer, bone health and stimulation of the immune system. (Harbone1998)

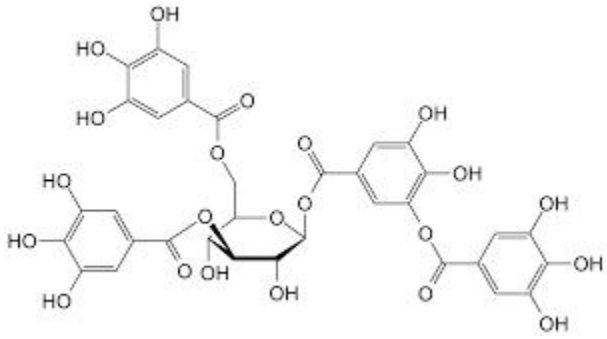
2.4.1 TANNINS

Tannins are complex chemical substances derived from phenolic acids sometimes called tannic acid) they are classified as phenolic compounds which are found in many species of plant, from all climates and all parts of the globe, they are large molecule that bind readily with protein cellulose, starches, and mineral. These resulting substances are insoluble and resistant to decomposition. Tannins occur in many species of coniferous trees as well as a number of flowering plant families. (United State Department of Agriculture USD).

Tannins are found commonly in the bark of trees, wood, leaves, buds, stems, fruits, seeds, root, and plant gall. In all of these plant structures, tannins help to protect the individual plant species. Tannins that become stored in the bark of tree protect the tree from being infected by bacterial or fungi. In these cases, the tannins precipitate out the enzyme and other protein exudants from bacteria and fungi thus not allowing these organisms to infect the tree. Many bud scales on woody plants contain tannins to protect the inner leaf tissues from being consumed and in many seed plants that initial sets of leaves from a germinating seed are also high in tannins (USDA 2000).

Unripe fruits are high in tannin content. The high tannins content discourages fruit eating animals from consuming the fruit until the seeds are mature and ready for disposal. As the fruit ripens the tannin content lessens

Besides fruits tannin are also contained in coffee, tea, red wine and beer. The initial astringent taste when you sip a red wine will actually come from tannins in the wood of the oak barrels in which the wine was aged.



Structure of tannin

Image source: Wikipedia

Since the tannin are well known for their astringent properties they had been used as a base for several herbal treatment. They may be mostly used in the form of tea, certain health expert recommend the use of some tannin-containing herbs to keep away certain ailments. Examples are bayberry, blackberry, raspberry, and sage.

2.4.2 STERIODS

Plants steroids (or steroid glycosides) also referred to as cardiac glycosides are one of the most naturally occurring plant phyto-constituents that have found therapeutic applications as arrow poison or cardiac drugs (Firn, 2010). The cardiac glycosides are basically steroids with an inherent ability to afford a very specific and powerful action mainly on the cardiac muscle when administered through injection into man or animal. Steroids (anabolic steroids) have been observed to promote nitrogen retention in osteoporosis and in animals with wasting illness (Maurya *et. al.*., 2008; Madziga *et. al.*., 2010). Caution should be taken when using glycosides as small amounts would exhibit the much needed stimulation on a diseased heart, whereas

excessive dose may cause even death. Diosgenin and ceradine (from veratrum veride) are examples of plant steroids.

2.5 EUGENOLS

Eugenol is a naturally occurring phenolic molecules from the class of phenylpropanoids found in several plants and the main component of clove It consists of 45 – 90% of its essential oil. Among the plants that contain eugenol, soyabeans, cloves, beans and cinnamon also present the anti-oxidant activity, possibly performed by this compound and other constituents. In addition, clove is also known by anti-inflammatory activity, which may be related to anti-inflammatory action of eugenol. Several pharmacological activities have been reported to eugenol; anti-inflammatory, antitumor, antibacterial, antifungal, antipyretic, anaesthetic and analgesic activities (Jadhave *et. al..,* 2004).

2.5.1 PHENOLICS

Phenolic phytochemicals are the largest category of phytochemicals and the widely distributed in the plant kingdom. The three most important groups of dietary phenolics are flavanoids, phenolic acids and polyphenols. Phenolic are hydroxyl group (-OH) containing class of chemical compounds where the (-OH) bonded directly to an aromatic hydrocarbon group. Phenol (C_2H_5OH) is considered the simplest class of this group of natural compounds. Phenolic compounds are a large and complex group of chemical constituents found in plants (Walton *et. al..*, 2003). They are plant secondary metabolites and they have an important role as defence compounds.

The term ‘Phenolic acids’, in general, designates phenols that possess one carboxylic acid functional group. Naturally occurring phenolic acids contain two distinctive carbon frameworks: the hydroxycinnamic and hydroxybenozoic structures. These compounds have been studied

mainly for the properties against oxidative damages leading to various degenerative diseases, such as cardiovascular diseases.

CHAPTER THREE

METHODOLOGY

3.1 Materials and Equipment:

Materials: Fresh *Justicia carnea* plants, Distilled water, Cotton wool, Whatman No. 1 filter paper, Labels, Ethanol (70%), Amber screw-capped bottles.

Equipment: Analytical balance, Drying rack, Grinder, 2 L conical flask, Magnetic stirrer, centrifuge, Water bath (40°C), Refrigerator (4°C), Glassware, PPE.

3.2 Extraction Procedure(Maceration using water)

The aqueous extraction of *Justicia carnea* was carried out using the maceration technique.

A known weight of 50g of the powdered sample was measured and transferred into a 1000 mL conical flask. One liter (100 mL) of distilled water was added to the flask, maintaining a solvent to sample ratio of 10:1 (v/w). The mixture was stirred thoroughly with a glass rod to ensure even mixing and proper wetting of the plant powder.

The flask was covered lightly with cotton wool to prevent contamination while allowing air exchange. The mixture was left to stand for 72 hours (3 days) at room temperature, during which it was stirred occasionally with a magnetic stirrer to facilitate the dissolution and diffusion of water-soluble bioactive compounds from the plant material into the solvent.

After 72 hours, the mixture was filtered first through cotton wool to remove large particles, and then through Whatman's No. 1 filter paper placed in a funnel to obtain a clear filtrate. The

filtrate was collected into a beaker and then transferred into a clean conical flask using a pipette and measuring cylinder for accuracy.

3.3 PREPARATION OF SAMPLE

1.0g of the sample was weighed and dissolved in 50ml of cool methanol in a 100ml beaker. This was transferred to a 100ml standard flask. The beaker was rinsed into the standard flask three times with about 10ml of the boiled out distilled water. It was then made up to mark with the same distilled. The flask was corked and inverted four times for proper mixing and then set aside for analysis.

3.4 PHYTOCHEMICAL SCREENING

The Phytochemical examinations of the plant extract were carried out using standard methods as employed by Tiwari *et. al.*., 2001, with little modification.

Detection of Alkaloids: This was done by first evaporating 2.0ml of the plant extract to dryness. Then the resultant residues were dissolved in 5ml of HCl (2mol/ dm3) and filtered. The filtrate was divided into two test tubes. To the first test tube, a few drops of Mayer's reagent were added, and the formation of a yellow-coloured precipitate indicates the presence of alkaloids.

The second test tube was treated with a few drops of Wagner's reagent, and the brownish-red precipitate formation indicates alkaloids.

Detection of Glycoside: This was done by dissolving 0.5 mg of the extract in about 1 ml of water and then aqueous NaOH solution was added. The formation of a yellow color indicates the presence of glycosides.

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Detection of Tannins: To 1.0ml of the extract, 1.0ml of 1% gelatin solution containing sodium chloride was added. The formation of a white precipitate indicates the presence of tannins.

Detection of Phenols: This was done by treating 1.0ml of the plant extract with 4 drops of ferric chloride solution. The formation of a bluish-black colour indicates the presence of phenols.

Detection of Saponins: The foam test method and froth test methods were used in the detection of saponins. In the foam test method, 0.5g of the plant extract was shaken with 2.0 ml of distilled water. The formation of foam which persists for 10 minutes indicates the presence of saponins.

In the froth test method, 5.0ml of the extract was diluted with distilled water to 20.0ml and this was shaken in a 50ml graduated cylinder for 15 minutes. The formation of a 1cm layer of foam indicates the presence of saponins.

Detection of Flavonoids: This was done using the alkaline reagent test and the lead acetate test. In the alkaline reagent test, the extract was treated with a few drops of 2mol/dm3 solution of sodium hydroxide. The formation of an intense yellow colour which becomes Colourless with the addition of dilute hydrochloric acid (2mol/dm3), indicates the presence of flavonoids.

In the lead test, the plant part extract was treated with a few drops of lead acetate solution. The formation of a yellow colour precipitate indicates the presence of flavonoids.

Detection of Eugenols: About 2ml of the extract was mixed with 5ml of 5% KOH solution. The aqueous layer was separated and filtered. A few drops of HCl were added to the filtrate. A pale-yellow precipitate was indicative of a positive test.

Detection of Steroids: 2 ml of acetic anhydride was added to 0.5 g of the extract of each with 2 ml of H2SO4. The colour changed from violet to blue or green in some samples indicating the presence of steroids.

Detection of Terpenoid: 0.2 g of the extract of the plant sample was mixed with 2 ml of chloroform (CHCl3) and concentrated H2SO4 (3ml) was carefully added to form a layer. A reddish-brown colouration in the interface indicates positive results for the presence of terpenoids.

Detection of Reducing Sugar: This was done by dissolving 2ml of the plant extract in 2ml of water. The resultant solution was divided into two test tubes. The first test tube was treated with Benedict's reagent and then heated gently. Orange red precipitate indicates the presence of reducing sugars.

The second test tube was treated with 20 drops of boiling Fehling's solution (A and B).

The formation of a brick – red precipitate in the bottom of the tube indicates the presence of reducing sugars.

3.5 DETERMINATION OF TOTAL PHENOLIC CONTENTS

The amount of total phenolics in the extract was determined with Folin–Ciocalteu reagent according to the method of Singleton and Rossi(1965) with slight modification using tannic acid as a standard.

Briefly, 1.0ml of extract solution (250 Ug/ml) was added in a test tube. Then, 1.0 mL of Folin–Ciocalteu reagent was added, and the contents of the flask were mixed thoroughly. After 5 min, 15.0 ml Na2CO3 (20 %) was added and allowed to stand for 2 hours. The absorbance was

measured at 760 nm using a UV-Vis spectrophotometer (Jenway 6100, Dunmow, Essex, U.K). The total phenolic content was determined as Ug of tannic acid equivalent(TAE) using an equation obtained from the standard tannic acid calibration graph.

3.6 DETERMINATION OF ALKALOIDS CONTENT

The total alkaloid content was measured using the method described by Harborne (1973). 5g of the extract was weighed into a 250 mL beaker and 100 mL of 20% acetic acid in ethanol was added and covered to stand for 2 hours. This was filtered and the extract was concentrated using a water bath to one quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate was collected by filtration, washed with 1% ammonia solution, dried and weighed. All samples were analyzed in triplicates.

$$\text{Alkaloid (\%)} = \frac{\text{Weight of residu}}{\text{Weight of sample}} \times 100$$

3.7 FLAVONOID CONTENT DETERMINATION

The flavonoid content was determined on triplicate aliquots of the homogenous cabbage extract (1.5 g) (Ilahy *et. al.*., 2011). Thirty-microliter aliquots of the methanolic extract were used for flavonoid determination. Samples were diluted with 90 µl methanol, 6 µl of 10% Aluminum chloride (AlCl3), 6µl of 1mol/l Sodium acetate (CH3CO2Na) were added and finally 170 µL of methanol was added. The absorbance was read at 415 nm after 30 min. Quercetin was used as a standard for calculating the flavonoid content (mg Qe/kg).

3.8 ESTIMATION OF TOTAL SAPONINS CONTENT

Estimation of total saponins content was determined by the method described by Makkar *et. al.*. based on vanillin-sulphuric acid colorimetric reaction with some modifications. About 50 µL of plant extract was added with 250 µL of distilled water. To this, about 250 µL of vanillin reagent (800mg of vanillin in 10Ml of 99.5% ethanol) was added. Then 2.5mLof 72%sulphuric acid was added and it was mixed well. This solution was kept in a water bath at 60°Cfor 10min. After 10min, it was cooled in ice cold water

and the absorbance was read at 570nm. 0- 25 ppm standard saponin solutions were prepared from saponin stock solution. The standard solutions were treated similarly as test samples. The values were expressed as mg/kg.

3.9 ESTIMATION OF TANNINS CONTENT

Exactly 0.20 mL of sample was added to 20 mL of 50% methanol and placed in a water bath at 77˚C - 80˚C for

1 hr and shaken. The extract was quantitatively filtered using a double layered Whatman No.1 filter paper and

20 mL of distilled water, 2.5 mL Folin-Denis reagent and 10 mL 17% Na_2CO_3 were added and mixed. The mixture was allowed to stand for 20 min. A series of standard tannic acids solutions were prepared in methanol and their absorbance as well as samples was read after colour development on a UV/ Visible spectrophotometer at a wavelength of 760 nm. Total tannin content was calculated from calibration curve.

CHAPTER FOUR

4.0 RESULT

This chapter is divided into two sections, viz, ***Result***, which deals with the presentation of results obtained from the study, and Discussion, which attempts to interpret the results obtained from this study.

4.1.0: RESULT

The results obtained from this study are presented in the tables below:

Table 4.2: The Result of the Phytochemical contents of the seed extract.

PARAMETERS	**A**	**B**	**C**
Total phenolic content m(g TAE/kg)	490.353	492.141	487.432
Flavonoid content (mg QE/kg)	53.363	50.314	56.401
Total Tannins content (mg TEA/kg)	56.228	45.660	50.959
Saponin (mg/kg)	12.315	18.273	15.644
Alkaloid (%)	8.614	6.634	7.338

Table 4.3: THE RESULT OF THE PHYTOCHMICAL SCREENING OF THE EXTRACT

S/N	PARAMETERS	TEST METHODS	OBSERVATION
1	GLYCOSIDES	General Test	+
2	SAPONINS	Frothing	+
3	ALKALOIDS	Picric Acid/Wagner	+
4	PHENOLICS	Ethanol/FeCl3	+
5	EUGENOIDS	KOH/HCL	+
6	STEROIDS	Acetic Anhydride/H2SO4	-
7	TERPENOIDS	Salkowski Test	+
8	FLAVONOIDS	Lead Acetate	+
9	TANNINS	FeCL3	-
10	REDUCING SUGAR	Fehling Solution A&B	+

KEY

+……..PRESENT

-……ABSENT

CHAPTER FIVE

DISCUSSION AND CONCLUSION

5.1 DISCUSSION

The phytochemical screening of the aqueous root extract of *Justicia carnea* revealed the presence of several important secondary metabolites, including glycosides, saponins, alkaloids, phenolics, eugenols, terpenoids, flavonoids, tannins, and reducing sugars, while steroids were absent. These compounds are known to contribute to the pharmacological potential of medicinal plants (Harborne, 1998; Tiwari *et. al.*., 2001).

Quantitative analysis showed that the total phenolic content was the most abundant, ranging between 487.432 and 492.141 mg TAE/kg, followed by flavonoids (50.314–56.401 mg QE/kg) and tannins (45.660–56.228 mg TEA/kg). The saponin content varied between 12.315 and 18.273 mg/kg, while alkaloid concentration ranged from 6.634% to 8.614%. The high phenolic and flavonoid concentrations indicate a strong antioxidant potential of the extract, as phenolic compounds are effective hydrogen donors capable of neutralizing free radicals (Singleton & Rossi, 1965; Falode *et. al.*., 2022).

The moderate presence of tannins supports the potential astringent, antimicrobial, and anti-inflammatory properties of *J. carnea* roots. Tannins are known to form complexes with proteins, thereby contributing to wound healing and anti-diarrheal effects (Trease & Evans, 2002). The detection of saponins suggests that the root extract may exhibit membrane-permeabilizing, expectorant, and cholesterol-binding activities, consistent with findings reported by Ajuru (2021) and Allied Academies (2018), who documented similar saponin levels in *J. carnea* leaves and roots.

The relatively high alkaloid content (up to 8.614%) further supports the therapeutic relevance of the extract. Alkaloids are well documented for their analgesic, antimicrobial, and antimalarial properties (Harborne, 1998). The coexistence of alkaloids and phenolics in the extract may explain the traditional use of *J. carnea* for managing anemia and infections (Falode *et. al.*., 2022; Oboma *et. al.*., 2024).

The absence of steroids in the extract contrasts with some reports from leaf and stem analyses (Peters, 2022), suggesting that steroidal compounds may be less concentrated or absent in the root tissues. However, the positive detection of terpenoids and eugenols implies the presence of volatile constituents that could enhance anti-inflammatory and antimicrobial activities, as terpenoids and phenolic ethers are potent bioactive agents (Oboma *et. al.*., 2024).

Overall, the phytochemical profile obtained in this study aligns with existing reports that *Justicia carnea* is rich in phenolic compounds, flavonoids, alkaloids, and saponins (Ajuru, 2021; Allied Academies, 2018). The presence of these compounds supports its traditional application in the management of blood related disorders, oxidative stress, and microbial infections. The results, therefore, highlight the potential of *J. carnea* root extract as a source of natural antioxidants and therapeutic agents.

5.2 CONCLUSION

The qualitative and quantitative phytochemical analyses of the aqueous root extract of *Justicia carnea* confirmed the presence of several bioactive compounds such as phenolics, flavonoids, tannins, saponins, alkaloids, terpenoids, and glycosides. The quantitative results revealed that phenolic compounds were the most abundant, followed by flavonoids and tannins, indicating that the root extract possesses strong antioxidant potential.

The study findings support previous reports that *J. carnea* contains diverse phytochemicals responsible for its medicinal properties, including anti-inflammatory, antimicrobial, and haematinic activities. The absence of steroids in this study may reflect organ-specific differences in phytochemical distribution.

In conclusion, the aqueous root extract of *Justicia carnea* can be considered a promising natural source of bioactive compounds. Future research should include chromatographic identification (HPLC/LC-MS) of individual phytochemicals, antioxidant and antimicrobial bioassays, and toxicity evaluation to establish its pharmacological efficacy and safety profile

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