CHARACTERIZATION OF ISOLATED OIL FROM JUSTICIA CARNEA

BY

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PSC1908736

SUBMITTED TO THE DEPARTMENT OF CHEMISTRY (INDUSTRIAL)

FACULTY OF PHYSICAL SCIENCE UNIVERSITY OF BENIN

BENIN CITY

APRIL, 2026

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A PROJECT REPORT SUBMITTED TO THE DEPARTMENT OF CHEMISTRY, FACULTY OF PHYSICAL SCIENCE IN PARTIAL FUFILMENT OF THE REQUIREMENT OF A BACHELOR'S DEGREE IN CHEMISTRY (INDUSTRIAL) IN THE UNIVERSITY OF BENIN, BENIN CITY

APRIL, 2026

CERTIFICATION

This is to certify that **MARVELLOUS OMAMUROMU EYUBE**, with the matriculation number **PSC1908736**, a final year student pursuing a Bachelor's Degree in Chemistry (Industrial Chemistry) at the University of Benin (UNIBEN), has successfully completed and presented their final year project titled "**Characterization of Isolated Oil from *Justicia carnea*.**"

_____ _____

Marvellous O. Eyube Date

(Project Student)

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Prof. Osaro Iyekowa Date

(Project Supervisor)

_____ _____

Prof. E. E. Irabor Date

(Head of Department)

DEDICATION

I dedicate this project to the countless individuals whose support, guidance, and inspiration have supported, inspired and have been the driving force behind its completion.

To my beloved family, whose unwavering love and encouragement have been my guiding light and my greatest source of strength and motivation through every twist and turn of this journey. Your sacrifices and belief in me have fueled my determination to excel and to reach this milestone.

To my esteemed professors, doctors and mentors, whose wisdom, invaluable guidance, expertise and encouragement have shaped my academic growth and instilled in me the courage to pursue excellence in this academic journey and beyond.

To my cherished friends whose unwavering support, camaraderie, and laughter have brightened the darkest days and made this journey unforgettable.

To all the participants and contributors of this research whose invaluable insights, cooperation, and collaboration as well as their willingness to share their knowledge and expertise has enriched this project and contributed to its success.

And finally, to myself, for the resilience, perseverance, dedication, and hard work that have propelled me forward, overcoming obstacles and reaching new heights up to this moment of accomplishment.

May this project serve as a testament to the collective effort, passion, and commitment of all who have played part in its realization.

Marvellous O. Eyube

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I extend my heartfelt appreciation to the participants and contributors of this study, without whom this research would not have been made possible. Your cooperation, insights, and willingness to share your expertise have greatly enriched the quality and depth of this project.

I am indebted to my family for their unwavering love, encouragement, and understanding throughout my academic journey. Their support has been a pillar of strength, motivating me to overcome challenges and strive for excellence.

Lastly, I am grateful to my friends, Courage Enuesueke, Orion Arose, Clara Okpala, Michael Adewale, John Ikpeba, Priscilla Maika, Clinton Ogbeide for their encouragement, motivation, and unwavering belief in my abilities. Your friendship and support have made this journey memorable and fulfilling.

To all those mentioned above and to everyone else who has contributed in any way, I offer my sincere gratitude. Your support and encouragement have been invaluable, and I am truly thankful for the opportunity to undertake this project.

ABSTRACT

The research project investigates the phytochemical profile of the oil fraction derived from *Justicia carnea* leaves, utilizing Gas Chromatography-Mass Spectrometry (GC-MS). Methanolic extraction through maceration, followed by rotary evaporation for concentration, was employed to isolate the phytoconstituents of the plant material. A portion of the concentrated crude extract was subjected to a detailed phytochemical screening, which identified bioactive classes such as flavonoids, alkaloids, and saponins, indicating the plant's rich chemical composition. For further analysis, another fraction of the crude extract underwent vacuum liquid chromatography (VLC) using a gradient of solvent systems. The hexane:ethyl acetate fraction was selected for GC-MS analysis, where phytol, a diterpenoid alcohol, was identified as the major compound with a retention time of 23.74 minutes and an abundance of 67.286%. Phytol, a well-documented diterpene, plays a significant physiological role and is associated with multiple biological activities, including anti- inflammatory, antioxidant, and antimicrobial properties. The convergence of phytochemical screening and GC-MS results underscores the chemical complexity and pharmacological relevance of *Justicia carnea's* oil fraction. These findings support further bioactivity-guided studies of this botanical source, which may yield novel bioactive molecules with therapeutic potential for addressing human health concerns.

Keywords: Justicia carnea, phytochemical composition, Gas Chromatography-Mass Spectrometry (GC-MS), Methanol extraction

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LIST OF ABBREVIATIONS

UV - Ultraviolet

IR - Infrared

NMR - Nuclear Magnetic Resonance

MS - Mass Spectrometry

TLC - Thin-Layer Chromatography

GC - Gas Chromatography

HPLC - High-Performance Liquid Chromatography

IC - Ion Chromatography

SEC - Size-Exclusion Chromatography

SPE - Solid-Phase Extraction

DPPH - 2,2-Diphenyl-1-picrylhydrazyl

ABTS - 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)

SFE - Supercritical Fluid Extraction

J. carnea – Justicia carnea

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction

Medicinal plants have been pivotal in human healthcare for centuries, serving as the foundation for traditional remedies and modern drug discovery. They are rich repositories of bioactive compounds, offering structurally diverse molecules that have inspired the development of numerous therapeutic agents (Marrelli, 2021; Salmerón-Manzano, Garrido-Cardenas, & Manzano-Agugliaro, 2020). The ethnopharmacological knowledge associated with these plants provides critical insights into their therapeutic potential and guides the rational exploration of bioactive metabolites (Süntar, 2020).

In recent years, medicinal chemistry has increasingly relied on natural products from plants to identify novel lead compounds with pharmacological relevance. Phytochemical screening and chemical characterization of plant extracts enable researchers to elucidate the molecular structures responsible for biological activities, offering opportunities for the development of nutraceuticals, functional foods, and drug candidates (Fitzgerald, Heinrich, & Booker, 2020; Ijoma, Ajiwe, & Odinma, 2023). Advanced analytical techniques, such as gas chromatography–mass spectrometry (GC-MS), provide a robust platform for profiling complex mixtures of phytochemicals, allowing the identification and quantification of bioactive compounds that underpin medicinal properties.

Among the plants with promising therapeutic potential, *Justicia carnea*, commonly known as the flamingo plant, has attracted attention for its diverse pharmacological activities, including antioxidant, anti-inflammatory, and anti-sickling properties (Osamwonyi *et al.*, 2024; Iyekowa, 2024). Despite its recognized ethnomedicinal use, there remains a need for detailed chemical profiling of its bioactive fractions, particularly to support drug discovery and natural product research in medicinal chemistry. In lieu of this limitation, this study is aimed at determining the phytochemical

constituents and characterization of the hexane:ethyl acetate oil fraction of *Justicia carnea* by GC-MS analysis.

1.1.1. Background of the Study

Plants have historically been a rich source of bioactive molecules that continue to inspire modern pharmacological research. Their chemical complexity and structural diversity make them valuable starting points for discovering novel therapeutics, especially in the context of medicinal chemistry and natural product research (Marrelli, 2021; Salmerón-Manzano, Garrido-Cardenas, & Manzano-Agugliaro, 2020). Traditional knowledge often guides the identification of plants with therapeutic relevance, highlighting species that warrant further chemical investigation (Süntar, 2020; E Anarado *et al.*, 2021).

Justicia carnea Lindley, widely known as the flamingo plant, has been recognized for its ethnomedicinal uses in managing conditions such as oxidative stress, inflammation, diabetes, and obesity (Oloruntola *et al.*, 2022; Anigboro *et al.*, 2021; Ani *et al.*, 2020). Scientific studies have begun to validate these traditional applications, reporting activities that include antioxidant, anti-inflammatory, antidiabetic, anti-obesity, and anti-sickling effects (Osamwonyi *et al.*, 2024; Iyekowa, 2024). Toxicological assessments further indicate that oral administration of its ethanol leaf extracts is generally safe, with no significant adverse effects on hematological, biochemical, or histological parameters in animal models (Akintimehin *et al.*, 2021).

Phytochemical investigations of *J. carnea* have revealed a rich array of secondary metabolites, including flavonoids, terpenoids, phenolics, saponins, and tannins, which are believed to contribute to its bioactivities (Okocha *et al.*, 2023; Ijoma, Ajiwe, & Odinma, 2023). Despite these insights, the chemical profiles of specific solvent fractions, such as the hexane:ethyl acetate fraction, remain underexplored. These fractions are likely to contain lipophilic to moderately polar compounds that

may have distinct pharmacological potentials, making their characterization critical for natural product research and the development of functional molecules in medicinal chemistry.

In light of these considerations, analyzing the hexane:ethyl acetate oil fraction of *Justicia carnea* can provide a more detailed understanding of its chemical constituents, bridging the gap between traditional knowledge and scientific validation. Such characterization not only enhances the chemical understanding of the plant but also informs potential applications in drug discovery and functional food development.

1.1.2. Statement of the Study

Although *Justicia carnea* has been recognized for its medicinal properties, detailed chemical characterization of its hexane:ethyl acetate fraction remains scarce. Without such information, the specific bioactive compounds responsible for its pharmacological effects are not fully understood, limiting its potential application in natural product-based drug development.

This study addresses this gap by investigating the phytochemical composition and characterizing the hexane:ethyl acetate oil fraction of *Justicia carnea* using GC-MS, providing essential data for future medicinal chemistry research and potential therapeutic applications.

1.1.3. Significance of the Study

The chemical characterization of *Justicia carnea*, particularly its hexane:ethyl acetate oil fraction, holds significant relevance for natural product research and medicinal chemistry. Although previous studies have highlighted the plant's antioxidant, anti-inflammatory, antidiabetic, anti-obesity, and anti-sickling activities (Oloruntola *et al.*, 2022; Anigboro *et al.*, 2021; Osamwonyi *et al.*, 2024; Iyekowa, 2024), detailed profiling of specific solvent fractions remains limited. By identifying the phytochemical constituents using GC-MS, this study will enhance understanding of the bioactive molecules responsible for these pharmacological effects, providing a scientific basis for their potential

therapeutic applications (Okocha *et al.*, 2023; Ijoma, Ajiwe, & Odinma, 2023). Furthermore, the research will validate and bridge traditional uses of *J. carnea* with experimental evidence, supporting its integration into drug discovery, nutraceutical development, and functional food research (E Anarado *et al.*, 2021; Süntar, 2020). The findings will also serve as a reference for future studies exploring structure-activity relationships, in silico modeling, and formulation development, while equipping undergraduate researchers with practical experience in phytochemical analysis and advanced analytical techniques (Akintimehin *et al.*, 2021; Fitzgerald, Heinrich, & Booker, 2020). Overall, this study contributes to both scientific knowledge and practical applications, positioning *J. carnea* as a promising source of natural compounds for medicinal chemistry research.

In addition to its scientific value, the study has broader implications for healthcare innovation and local resource utilization. By elucidating the bioactive profile of *J. carnea*, the research could inform the development of standardized plant-based formulations, contributing to safer and more effective natural therapies. This is particularly relevant in regions where access to conventional drugs is limited and traditional medicine remains a primary healthcare resource (Marrelli, 2021; Salmerón-Manzano, Garrido-Cardenas, & Manzano-Agugliaro, 2020). Moreover, the study encourages the sustainable exploration of local medicinal plants, fostering economic opportunities for communities engaged in cultivation, processing, and commercialization of herbal products. Ultimately, the work reinforces the role of medicinal plants in modern pharmacology while promoting the responsible integration of ethnomedicinal knowledge into evidence-based practice (Süntar, 2020; Ijoma, Ajiwe, & Odinma, 2023).

1.1.4. Scope of the Study

This study focuses on the phytochemical investigation and chemical characterization of *Justicia carnea*, with particular emphasis on its hexane:ethyl acetate oil fraction. The research encompasses the collection, drying, and pulverization of the plant leaves, followed by extraction of the powdered

material using methanol through the maceration method. The crude methanol extract is then subjected to preliminary phytochemical screening to identify major bioactive compounds using standard analytical methods. Subsequently, the oil fraction is isolated from the hexane:ethyl acetate fraction via vacuum liquid chromatography, and its chemical constituents are characterized through gas chromatography–mass spectrometry (GC-MS) analysis.

In addition to the experimental procedures, the study includes a comprehensive review of existing literature on the phytochemical composition and reported pharmacological activities of *J. carnea*. By combining experimental characterization with literature insights, the research aims to expand understanding of the plant's bioactive components and highlight their potential pharmacological applications. The scope of the study is therefore limited to the chemical profiling of the hexane:ethyl acetate oil fraction of *J. carnea* leaves, providing foundational data that may inform future investigations, natural product research, and medicinal chemistry-based drug development.

1.1.5. Aim and Objectives of the Study

This study is aimed at determining the phytochemical constituents and characterization of the hexane:ethyl acetate oil fraction of *Justicia carnea* by GC-MS analysis. To achieve the aim above, the specific objectives required are therefore elucidated:

1. To collect, dry and pulverize the leaves of *J. carnea.*
2. To extract the powdered sample of *J. carnea* with methanol by maceration method.
3. To screen the crude methanol extract for phytochemical constituents using standard methods.
4. To isolate the oil fraction by hexane: ethyl acetate fraction using vacuum liquid chromatography.
5. To characterize the oil fraction obtained from the hexane: ethyl acetate fraction by GC-MS analysis.

6. To conduct a comprehensive review of the literature on the phytochemical composition of *Justicia carnea* and its potential pharmacological activities.
7. To contribute to the understanding of the phytochemical composition and pharmacological potential of *Justicia carnea* oil for future research and development

1. 

Figure 1. *J. carnea* leaves and flower

Medicinal plants continue to serve as a rich source of bioactive compounds with therapeutic potential, forming the basis for much of modern pharmacology and drug discovery (Marrelli, 2021; Salmerón-Manzano, Garrido-Cardenas, & Manzano-Agugliaro, 2020). *Justicia carnea*, commonly known as the flamingo plant, has attracted significant attention due to its diverse ethnomedicinal applications, including management of anemia, oxidative stress, inflammation, diabetes, and infections (E Anarado *et al.*, 2021; Oloruntola *et al.*, 2022; Akintimehin *et al.*, 2021).

Several studies have investigated the hematological and biochemical effects of *J. carnea* extracts in animal models. Chima (2017) reported that administration of leaf extracts mitigated phenylhydrazine-induced anemia in albino rats, improving hematological parameters such as hemoglobin levels and red blood cell counts. Similarly, Anthonia *et al.* (2019) demonstrated that aqueous leaf extracts

positively influenced hematological and biochemical indices in male Wistar rats with anemia, highlighting the plant's nutritive and restorative potential. Igbinaduwa, Kabari, and Chikwue (2019) also observed that ethanol leaf extracts exhibited anti-anemic effects, reinforcing its traditional use in blood disorders.

The hepatoprotective potential of *J. carnea* has been explored, with Ukpabi-Ugo, Ndukwe, and Iwuoha (2019) reporting that methanol extracts of the leaves protected liver tissues against carbon tetrachloride-induced toxicity in albino rats. These findings suggest that the plant contains bioactive compounds capable of mitigating oxidative stress and supporting liver function. Beyond hematological and hepatic effects, Chidi *et al.* (2018) demonstrated that *J. carnea* exhibited modulatory activity in Plasmodium-infected mice, indicating potential anti-malarial effects.

Phytochemical investigations of *J. carnea* indicate the presence of secondary metabolites such as flavonoids, tannins, saponins, alkaloids, and phenolics, which are likely responsible for its observed pharmacological effects (Ijoma, Ajiwe, & Odinma, 2023; Okocha *et al.*, 2023). Amirul-Aiman *et al.* (2013) provided anatomical studies of Justicia species petals, which, while focused on morphology, support identification and standardization of plant materials used in pharmacological research. The chemical diversity of *J. carnea* underscores its potential for natural product research and the development of functional compounds in medicinal chemistry.

Collectively, these studies highlight the ethnomedicinal relevance, pharmacological efficacy, and phytochemical richness of *Justicia carnea,* yet gaps remain in the detailed characterization of specific solvent fractions, particularly the hexane:ethyl acetate oil fraction. While methanol and ethanol extracts have been studied extensively, non-polar to moderately polar fractions remain underexplored, leaving their bioactive constituents unidentified. Comprehensive chemical profiling of these fractions, such as through GC-MS analysis, is crucial to uncovering compounds that may be responsible for the plant's therapeutic properties and guiding future drug development efforts.

1.2.1 Taxonomy and Classification

Kingdom: Plantae (Plants)

Phylum: Angiosperms (Flowering Plants)

Class: Eudicots (Eudicotyledons)

Order: Lamiales (Mint Order)

Family: Acanthaceae (Acanthus Family)

Genus: Justicia L. (water-willow P)

Species: Justicia carnea Lindl. (Brazilian plume, flamingo flower)

Taxonomic studies and botanical descriptions provide further insights into the morphological characteristics and distribution of *Justicia carnea*. The species is characterized by its striking pink to reddish-pink flowers arranged in dense spikes, which contribute to its ornamental value. Its leaves are typically lanceolate and glossy green, adding to its aesthetic appeal in landscaping and garden cultivation.

1.2.2. Botanical Description of *J. carnea*

Justicia carnea Lindl., belonging to the family Acanthaceae, is commonly known as the Brazilian plume flower, Brazilian-plume, flamingo flower, or Jacobinia (Gilman, 2007). It is an evergreen perennial subshrub or shrub native to the wet tropical regions of southeastern and southern Brazil, extending to Misiones Province in northeastern Argentina and parts of Paraguay (Kew Science, 2024; World Flora Online, 2023). In its natural habitat, it thrives in the shaded, humid understory of the Atlantic Forest, along forest edges, and near stream banks in moist, humus-rich soils (Kew Science, 2024). The species is widely cultivated for its ornamental appeal due to its striking floral display and adaptability to shade (Auckland Botanic Gardens, 2025).

This upright, bushy shrub exhibits a coarse, open texture and a moderate to rapid growth rate. Mature plants typically reach heights of 1–2 meters and a spread of 0.6–1.2 meters (Gilman, 2007). The stems are erect, often multi-trunked or clumping, and soft-wooded when young, becoming woody at the base with age. Young stems are frequently squarish or grooved. The plant remains evergreen in frost-free climates (USDA hardiness zones 8B–11), but it may exhibit semi-deciduous behavior under cooler conditions (Gilman, 2007).

The leaves of *Justicia carnea* are large, simple, and arranged oppositely or suboppositely along the stems. They are elliptic to ovate, measuring 15–30 cm in length and up to 10 cm in width. The leaves feature prominent pinnate venation, acuminate tips, wavy margins, and an obtuse base often decurrent on the petiole. The upper surface is glossy dark green, whereas the underside may display purple tinges. Petioles range from 3 to 10 cm in length, and the foliage has a distinctly coarse texture (Gilman, 2007; Auckland Botanic Gardens, 2025).

The plant's most distinctive feature is its terminal, dense, plume-like inflorescences, with spikes measuring 12–15 cm long (Gilman, 2007). These plumes consist of overlapping bracts and numerous tubular, bilabiate flowers that are 3–6 cm long. The flowers are slightly fragrant and range in color from bright pink to rose-purple, crimson, or red, though cultivars may display white, yellow, apricot, or orange hues. The upper lip of each flower is erect and curved, while the lower lip is reflexed and trilobed. Flowering typically occurs from early summer through fall, often in multiple cycles per year under suitable conditions. The showy plumes are oriented upwards and attract hummingbirds and butterflies (Gilman, 2007).

The fruit of *Justicia carnea* is a small, inconspicuous dry capsule, less than 1.3 cm long, characteristic of the Acanthaceae family, containing seeds adapted for dispersal (Lindley, 1831). The specific epithet *carnea*, meaning "flesh-colored" in Latin, refers to the pinkish hue of the flowers and bracts (Gilman, 2007). The species prefers partial to full shade, moist but well-drained soils, and high

humidity. Propagation is commonly achieved through stem cuttings (Gilman, 2007). This morphological description provides a foundation for understanding the plant's medicinal applications and supports its horticultural and pharmacological study (World Flora Online, 2023).

1.2.3. Medicinal Use of *J. carnea*

Medicinal plants remain a crucial source of bioactive compounds, bridging traditional ethnomedicine and modern drug discovery (Marrelli, 2021; Salmerón-Manzano, Garrido-Cardenas, & Manzano-Agugliaro, 2020). Among these, *Justicia carnea* Lindley (family Acanthaceae), commonly known as Brazilian plume or "ewe eje"/"ogwu obara" in Nigeria, has emerged as an important ethnomedicinal shrub. Indigenous communities have traditionally used its leaves as a blood tonic for anemia and for managing inflammation, diabetes, gastrointestinal disorders, malaria, sickle cell disease, liver disorders, and other ailments (Anarado *et al.*, 2021; Jabar *et al.*, 2025). Recent scientific investigations have validated many of these traditional claims, confirming the plant's broad pharmacological potential.

Studies using chromatographic techniques such as GC-MS have revealed that *J. carnea* leaves contain a diverse array of secondary metabolites, including alkaloids (ribalinidine, spartein, epihedrine), flavonoids (naringenin, kaempferol, flavan-3-ol, anthocyanins), saponins, tannins, phytates, and sapogenins (Andrew *et al.*, 2024; Asiwe *et al.*, 2023). Proximate analyses indicate high carbohydrate, fiber, and ash content, alongside essential minerals such as iron and potassium, and vitamins including C and A (Andrew *et al.*, 2024). Volatile terpenes, including β-bisabolene and aromandendrene, further contribute to the plant's bioactivity (Asiwe *et al.*, 2023). Such a diverse phytochemical profile positions *J. carnea* as a valuable candidate for natural product research and medicinal chemistry studies.

Pharmacologically, *J. carnea* exhibits potent antioxidant properties, mitigating oxidative stress and inflammation. Anigboro *et al.* (2021) demonstrated strong free-radical scavenging activity and

enzyme inhibition by bioactive compounds, while in vivo studies reported reductions in oxidative damage and inflammatory markers in animal models (Enaohwo *et al.*, 2025; Falode *et al.*, 2023). Oloruntola *et al.* (2023) confirmed improvements in antioxidant status and reduced oxidative DNA damage in broilers supplemented with leaf powder, supporting traditional anti-inflammatory applications. Compounds in *J. carnea* have also been shown to inhibit enzymes linked to diabetes and obesity, with Anigboro *et al.* (2021) identifying molecules with strong α-amylase inhibition, and Falode *et al.* (2023) reporting hepatoprotective effects in diabetic rat models through reductions in oxidative stress, inflammation, and apoptosis.

Consistent with its traditional use as a blood tonic, *J. carnea* improves hematological parameters, with Andrew *et al.* (2024) highlighting its high iron and vitamin C content, Iyekowa *et al.* (2024) demonstrating anti-sickling potential in methanol extracts, and Oloruntola *et al.* (2023) observing improved blood indices in supplemented animals. The plant also exhibits antimicrobial activity against pathogenic strains, attributed to flavonoids and alkaloids (Asiwe *et al.*, 2023), while other studies indicate neuroprotective effects (Enaohwo *et al.*, 2025), improved intestinal motility (Ikiriko *et al.*, 2025), and general safety in hematological and histopathological assessments (Isichei-Ukah *et al.*, 2024; Peters *et al.*, 2022).

Most studies report that *J. carnea* is relatively safe at therapeutic doses. Oboma *et al.* (2024) found no adverse effects on liver or kidney function, and Andrew *et al.* (2024) confirmed no toxicity under standard preparations. Earlier studies by Igbinaduwa *et al.* (2019) also support its safety as a nutraceutical.

Collectively, literature from 2019 to 2026 consistently demonstrates that *Justicia carnea* possesses antioxidant, anti-inflammatory, antidiabetic, hematinic, antimicrobial, and anti-sickling properties, largely due to its diverse phytochemical profile. While traditional uses have been scientifically validated, knowledge gaps remain regarding detailed characterization of specific solvent fractions,

clinical trials, and standardized dosing. Comprehensive studies of fractions such as the hexane:ethyl acetate oil fraction are therefore essential to fully elucidate the bioactive compounds responsible for its pharmacological effects, providing a foundation for future medicinal chemistry research and natural product-based drug development.

1.3. Phytochemical Constituents of *J. carnea*

The leaves of Justicia carnea are rich in diverse secondary metabolites that underpin its traditional medicinal applications, particularly as a hematinic, antioxidant, anti-inflammatory, and antimicrobial agent. Qualitative and quantitative phytochemical screenings, combined with advanced analytical techniques such as GC-MS and HPLC, have consistently confirmed the presence of flavonoids, terpenoids, phenolic compounds, alkaloids, saponins, tannins, essential oils, and sterols/terpenes (Anigboro *et al.*, 2021; Asiwe *et al.*, 2023; Andrew *et al.*, 2024).

1.3.1. Flavonoids

Flavonoids are among the most abundant bioactive compounds in J. carnea leaves and contribute significantly to its antioxidant and enzyme-inhibitory properties. Quantitative analysis revealed high flavonoid content (31.08 mg catechin equivalent/g) in aqueous leaf extracts. Specific flavonoids identified include naringenin, kaempferol, flavan-3-ol, and anthocyanins. These compounds are responsible for strong free-radical scavenging activity and α-amylase inhibition, supporting the plant's traditional use in managing oxidative stress and diabetes-related conditions (Anigboro *et al.*, 2021; Andrew *et al.*, 2024).

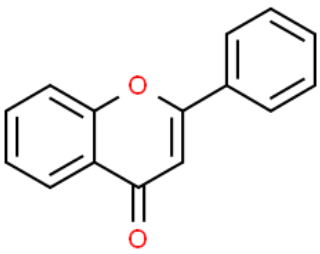

Figure 2. Flavone

1.3.2. Terpenoids

Terpenoids are well-represented in J. carnea. Quantitative screening of leaf extracts showed substantial terpenoid levels (8.55×10^{-1} mg/g in some studies). GC-MS analyses have identified various terpenoid derivatives and volatile terpenes, which contribute to the plant's anti-inflammatory and antimicrobial effects. Terpenoids also play a role in the overall bioactivity of the essential oil fraction (Asiwe *et al.*, 2023).

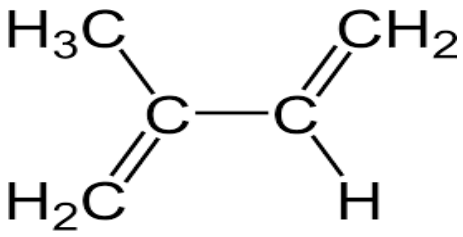

Figure 3. Isoprene

1.3.3. Phenolic Compounds

Phenolic compounds are present in high concentrations in J. carnea leaves, with total phenolic content reported as 132 mg gallic acid equivalent/g in aqueous extracts. These compounds are key contributors to the plant's potent antioxidant capacity and its ability to mitigate oxidative damage in biological systems. Phenolics work synergistically with flavonoids to enhance free-radical scavenging and reduce inflammation (Anigboro *et al.*, 2021; Andrew *et al.*, 2024).

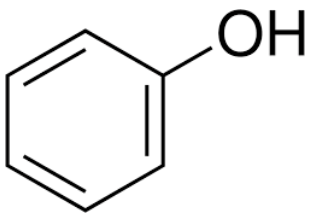

Figure 4. Phenol

1.3.4. Alkaloids

Alkaloids are consistently detected in both qualitative and quantitative screenings of J. carnea leaves. Notable levels (e.g., 1.01×10^{-1} mg/g) have been reported, with specific compounds such as ribalinidine, spartein, and epihedrine identified through GC-MS. Alkaloids are believed to contribute to the antimicrobial, anti-sickling, and neuroprotective activities of the plant (Asiwe *et al.*, 2023; Andrew *et al.*, 2024).

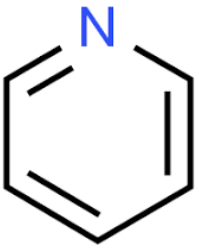

Figure 5. Pyridine

1.3.5. Saponins

Saponins occur in moderate to high amounts in J. carnea leaf extracts and are linked to its foaming properties and potential cholesterol-lowering and immune-modulatory effects. Quantitative studies have confirmed their presence alongside other glycosides, supporting traditional claims for blood purification and anti-inflammatory uses (Anarado *et al.*, 2021; Asiwe *et al.*, 2023).

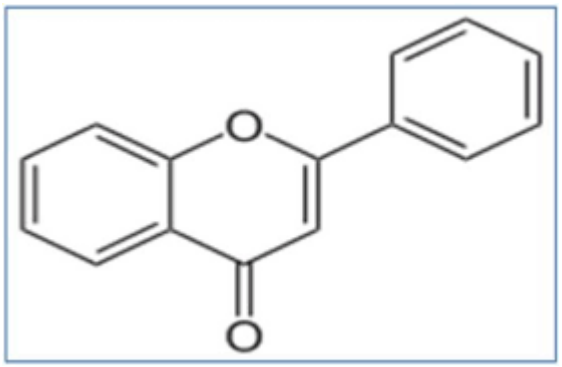

Figure 6. Sapogenin

1.3.6. Tanins

Tannins are highly detected in aqueous and ethanolic leaf extracts of J. carnea. They are associated with the plant's astringent properties and its traditional application in wound healing and gastrointestinal disorders. Tannins also contribute to antioxidant and antimicrobial activities by binding to proteins and enzymes (Asiwe *et al.*, 2023; Andrew *et al.*, 2024).

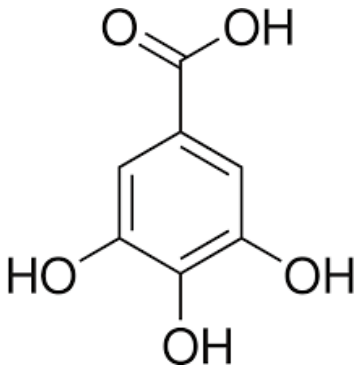

Figure 7. Gallic acid

1.3.7. Essential Oils

The essential oil and volatile fractions of J. carnea leaves have been extensively characterized using GC-MS. Analyses identified up to 86 volatile bioactive compounds, including terpenes (e.g., β-bisabolene, aromandendrene) and other aroma compounds. These volatiles are responsible for the characteristic scent and contribute to the plant's antimicrobial and anti-inflammatory properties (Asiwe *et al.*, 2023).

1.3.8. Sterols and Terpenes

Sterols and various terpenes (including phytosterols) are present in J. carnea leaves. These lipophilic compounds support membrane stability and exhibit anti-inflammatory and antioxidant effects. GC-

MS studies have confirmed the presence of steroidal structures alongside terpenoids, adding to the broad pharmacological profile of the plant (Andrew *et al.*, 2024; Asiwe *et al.*, 2023).

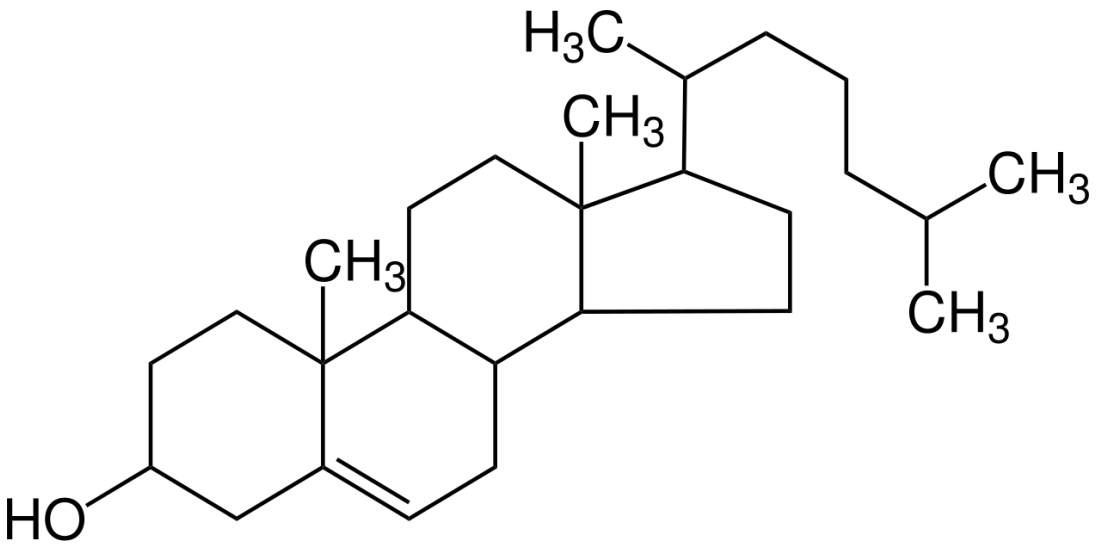

Figure 8. Cholesterol

The phytochemical diversity of Justicia carnea, dominated by polar (flavonoids, phenolics, alkaloids) and non-polar (terpenoids, sterols, essential oils) compounds, provides a strong scientific basis for its ethnomedicinal uses.

1.4. Extraction

Extraction is a critical step in isolating bioactive phytochemicals from Justicia carnea leaves for pharmacological evaluation and potential therapeutic applications. Researchers have primarily employed conventional solvent-based techniques due to their simplicity, cost-effectiveness, and efficiency in recovering polar and semi-polar compounds such as flavonoids, alkaloids, phenolics, and saponins. Advanced methods like supercritical fluid extraction remain largely unexplored for this species (Asiwe *et al.*, 2023; Oboma *et al.*, 2024; Andrew *et al.*, 2024).

1.4.1. Solvent Extraction

Solvent extraction is the most widely used method for *J. carnea* leaves. It involves maceration or Soxhlet extraction with solvents of varying polarity. Common solvents include ethanol (absolute or 80%), methanol, ethyl acetate, n-hexane, and distilled water (aqueous).

Typical procedures involve air-drying or shade-drying the leaves, pulverizing them into fine powder, and soaking in solvent (e.g., 1 kg powder in 6 L ethanol for 48 hours with occasional stirring, or cold maceration for 72 hours). The mixture is filtered (using Whatman No. 1 filter paper or muslin cloth) and concentrated under reduced pressure or in a water bath at 40–60°C to obtain a semi-solid or gummy extract (Andrew *et al.*, 2024; Asiwe *et al.*, 2023; Oboma *et al.*, 2024).

Ethanol and hydroethanolic mixtures (50:50 ethanol-water) are particularly effective, yielding higher recovery of phytochemicals such as alkaloids, flavonoids, and phenolics compared to aqueous extracts alone. Methanol extracts have also been successfully used for anti-sickling and hepatoprotective studies (Anarado *et al.*, 2021; Okorie *et al.*, 2025). Solvent extraction is simple, scalable, and suitable for both qualitative screening and bioactivity assays.

1.4.2. Steam Distillation

Steam distillation is occasionally applied for isolating volatile fractions and essential oils from *J. carnea* leaves. This hydrodistillation-based method captures aromatic compounds such as terpenes (e.g., β-bisabolene and aromandendrene) identified in the volatile profile (Asiwe *et al.*, 2023).

The process typically involves passing steam through the powdered or chopped plant material, condensing the vapor, and separating the oil layer. However, reports on steam distillation for *J. carnea* are limited compared to solvent methods, as the plant's medicinal value lies mainly in non-volatile polar constituents. Steam distillation may result in loss of heat-sensitive compounds and is less efficient for the high flavonoid and alkaloid content targeted in most studies.

1.4.3. Supercritical Fluid Extraction (SFE)

Supercritical fluid extraction, usually with carbon dioxide (SC-CO_2), has not been reported in the available literature (2019–2026) for Justicia carnea. SFE offers advantages such as solvent-free extracts, lower temperature operation, and high selectivity for lipophilic compounds. Given the presence of terpenoids and essential oil components in J. carnea, SFE could be a promising green alternative for future studies, especially for preserving thermolabile volatiles and obtaining high-purity extracts without residual solvent concerns.

1.4.4. Solid Phase Extraction (SPE)

Solid phase extraction is rarely mentioned as a primary extraction technique for J. carnea but is sometimes used as a clean-up or fractionation step after initial solvent extraction. SPE cartridges help isolate and concentrate specific classes of compounds (e.g., flavonoids or alkaloids) from crude extracts prior to chromatographic analysis (GC-MS or HPLC). Its application in J. carnea research remains minimal, with most studies relying on direct solvent extraction followed by filtration and evaporation.

1.4.5. Pressurized Liquid Extraction

Pressurized liquid extraction (also known as accelerated solvent extraction) has not been documented for Justicia carnea in recent literature. This technique uses high pressure and temperature with solvents to achieve faster and more efficient extraction. It could potentially improve yield and reduce solvent consumption for polar phytochemicals in J. carnea, but conventional maceration and Soxhlet methods continue to dominate due to equipment availability and established protocols in African research settings.

1.4.6. Parameters for Selecting Appropriate Extraction Methods

Several factors influence the selection of an appropriate extraction method for *Justicia carnea*, particularly with respect to the nature of the target compounds, efficiency, and practical considerations. The polarity of the solvent plays a critical role, as polar solvents such as ethanol, methanol, and water are more effective for extracting flavonoids, phenolics, alkaloids, and saponins, whereas less polar solvents like ethyl acetate and n-hexane are better suited for isolating terpenoids and lipid-based compounds. In terms of yield and efficiency, ethanol has been reported to provide higher phytochemical recovery and enhanced bioactivity compared to aqueous extracts (Andrew *et al.*, 2024). Practical factors such as cost and scalability also guide method selection, with techniques like maceration and Soxhlet extraction being widely used due to their affordability and suitability for laboratory and small-scale operations, especially in resource-limited settings.

Safety, environmental impact, and the stability of phytochemicals are also important considerations. Hydroethanolic or aqueous extraction methods reduce toxicity risks associated with pure organic solvents, while advanced green techniques such as supercritical fluid extraction minimize solvent residues but require specialized equipment. Additionally, thermolabile compounds are better preserved using low-temperature methods such as cold maceration rather than high-heat processes. The intended downstream application of the extract further influences solvent choice, with food-grade solvents like ethanol and water being preferred for in vivo studies and nutraceutical development due to their safety profiles (Oboma *et al.*, 2024).

1.5. Chromatography

Chromatographic techniques play a vital role in the separation, identification, and quantification of complex mixtures of bioactive phytochemicals present in *Justicia carnea* leaf extracts. These methods enable detailed profiling of compounds such as flavonoids, alkaloids, terpenoids, phenolics, and volatile constituents, thereby supporting standardization, quality control, and validation of the plant's

traditional medicinal applications (David E. Peters, O. Ahaotu, & M. O. Wegwu, 2022; I. Osamwonyi, O. Iyekowa, & A.P. Oviawe, 2024; H. Omeje, 2025).

1.5.1. Liquid Chromatography

Liquid chromatography, particularly high-performance liquid chromatography (HPLC), is widely used for the analysis of non-volatile and polar compounds in *Justicia carnea* extracts. Reverse-phase HPLC systems coupled with ultraviolet or diode-array detectors allow for the separation and quantification of important phytochemicals such as kaempferol, resveratrol, gallic acid, and justicinol. In methanol leaf extracts of *Justicia carnea*, HPLC analysis identified kaempferol as the predominant flavonoid with a peak area of 16.72%, alongside resveratrol (13.85%) and other phenolic compounds. This method offers high resolution, sensitivity, and reproducibility, making it particularly suitable for phytochemical fingerprinting and studies related to antioxidant and anti-sickling activities (I. Osamwonyi, O. Iyekowa, & A. P. Oviawe, 2024; J. Wood, S. Senthilkumar, & M. Masi, 2020).

1.5.2. Gas Chromatography

Gas chromatography, often coupled with mass spectrometry (GC-MS) or flame ionization detection (GC-FID), is one of the most extensively applied techniques in the analysis of *Justicia carnea*. It is especially effective for identifying volatile and semi-volatile compounds, including fatty acid methyl esters, phytol, and various terpenoids. GC-MS analysis of methanolic and aqueous extracts has revealed major constituents such as phytol, which may constitute up to 67.28% of the extract, as well as hexadecanoic acid methyl ester and 9,12-octadecadienoic acid methyl ester. Additionally, GC-FID has been utilized for quantitative determination of alkaloids such as ribalinidine and various flavonoids. These hyphenated techniques provide high sensitivity and enable compound identification through comparison with mass spectral libraries (David E. Peters, O. Ahaotu, & M. O. Wegwu, 2022; H. Omeje, 2025).

1.5.3. Thin Layer Chromatography

Thin-layer chromatography (TLC), including high-performance thin-layer chromatography (HPTLC), serves as a rapid, cost-effective, and efficient preliminary screening method for *Justicia carnea* extracts. It is commonly used to detect classes of phytochemicals such as flavonoids, phenolics, alkaloids, and terpenoids by comparing retention factor (Rf) values under ultraviolet light or after derivatization with specific reagents. TLC is frequently applied during fractionation processes to monitor the separation of compounds from crude methanol extracts prior to further analysis using GC-MS or HPLC, thereby aiding in preliminary phytochemical profiling (I. Osamwonyi, O. Iyekowa, & A. P. Oviawe, 2024; O. Iyekowa, 2024).

1.5.4. Paper Chromatography

Paper chromatography is a classical analytical technique that has been used for the qualitative separation of highly polar, water-soluble compounds such as flavonoids and phenolic acids in medicinal plants, including *Justicia carnea*. The technique employs filter paper as the stationary phase and solvent systems such as butanol-acetic acid-water as the mobile phase. Although largely replaced by more advanced chromatographic methods such as TLC and HPLC due to its lower resolution and longer analysis time, paper chromatography remains useful for basic screening in educational settings and resource-limited laboratories (B. Sharma, 2023).

1.5.5. Affinity Chromatography

Affinity chromatography, which is based on specific biological interactions such as ligand–receptor or antibody–antigen binding, has not been widely applied in the analysis of small-molecule phytochemicals from *Justicia carnea*. This technique is more commonly used for the purification of biomolecules such as proteins, enzymes, and glycoproteins. While its application in *Justicia carnea* research is limited, it may hold potential for the targeted isolation of specific macromolecular bioactive compounds if present. However, most current studies rely on adsorption, size-exclusion, or reversed-phase chromatographic techniques rather than affinity-based methods (O. Coskun, 2025).

Basically, chromatographic techniques such as GC-MS and HPLC remain the dominant analytical tools for the detailed phytochemical characterization of *Justicia carnea*, while TLC provides a valuable preliminary screening approach. Together, these methods offer comprehensive insights into the identity and composition of bioactive compounds responsible for the plant's medicinal properties.

1.6. Spectroscopy

Spectroscopic techniques are essential tools for the structural elucidation, functional group identification, and confirmation of bioactive phytochemicals isolated from *Justicia carnea* leaves. These techniques complement chromatographic methods by providing detailed information on molecular structure, conjugation, and compound purity. They are particularly useful in the analysis of secondary metabolites such as flavonoids, phenolics, alkaloids, and terpenoids (I. Osamwonyi, O. Iyekowa, M. I. Ebengho, M. O. Edema, A. P. Oviawe, S. M. Momoh, L. O. Eduwuirofo, R. Iyekekpolor, O. T. Michael, R. O. Oghomwen, & A. A. Umeodinka, 2024). Although studies on *Justicia carnea* have predominantly utilized ultra-violet visible spectroscopy (UV-Vis), Fourier Transform Infrared (FTIR) spectroscopy, and mass spectrometry, techniques such as Nuclear Magnetic Resonance (NMR) and fluorescence spectrometry remain underexplored but hold strong potential for advanced characterization (J. M. Jabar, 2025; A. A. Anigboro, O. J. Avwioroko, O. A. Ohwokevwo, B. Pessu, & N. J. Tonukari, 2021).

1.6.1. Ultra-violet Visible Spectroscopy

Ultra-violet visible (UV-Vis) spectroscopy is widely employed for the detection of chromophores and the assessment of conjugated systems in *Justicia carnea* extracts, particularly for identifying phenolic compounds, flavonoids, and anthocyanins due to their characteristic absorption in the ultraviolet and visible regions (J. M. Jabar, 2025). In studies involving *Justicia carnea* leaf extracts used as natural dyes, UV-Vis analysis confirmed the presence and stability of chemical constituents through distinct absorption peaks associated with flavonoid and phenolic structures (J. M. Jabar, 2025). Furthermore, UV-Vis spectroscopy has been used to monitor interactions between plant extracts and biological

targets, such as enzyme conformational changes, by observing shifts in absorption maxima (A. A. Anigboro, O. J. Avwioroko, O. A. Ohwokevwo, B. Pessu, & N. J. Tonukari, 2021).

1.6.2. Infrared Spectroscopy

Infrared spectroscopy, particularly Fourier Transform Infrared (FTIR), is extensively applied for the identification of functional groups in *Justicia carnea* extracts, revealing characteristic vibrational bands associated with hydroxyl (O–H), carbonyl (C=O), ether (C–O), and aromatic carbon-carbon (C=C) bonds typical of flavonoids and phenolics (J. M. Jabar, 2025). FTIR spectra of *Justicia carnea* leaf extracts have shown prominent absorption peaks around 3388 cm^{-1} corresponding to O–H stretching, 1708 cm^{-1} for carbonyl groups, and approximately 1060 cm^{-1} for C–O vibrations, confirming the presence and stability of bioactive compounds during extraction processes (J. M. Jabar, 2025).

1.6.3. Nuclear Magnetic Spectroscopy

Nuclear Magnetic Resonance (NMR) spectroscopy, including one-dimensional and two-dimensional techniques such as ^{1}H-NMR, ^{13}C-NMR, COSY, and HSQC, provides detailed structural information on isolated compounds from *Justicia carnea*. Although direct NMR studies on crude extracts are limited, analyses of purified fractions, particularly from n-hexane extracts, have successfully elucidated the structures of compounds such as chlorogenic acid, quinaldic acid, and oleic acid, enabling precise assignment of proton and carbon environments (Phytochemical Constituents Study, 2025). This makes NMR a gold standard for confirming the identity of both known and novel phytochemicals.

1.6.4. Mass Spectrometry

Mass spectrometry (MS), often coupled with chromatographic techniques such as gas chromatography (GC-MS) or liquid chromatography (LC-MS), is one of the most widely used analytical approaches for *Justicia carnea*, as it provides molecular weight, fragmentation patterns,

and structural information for compound identification through spectral library comparison (D. E. Peters, O. Ahaotu, & M. O. Wegwu, 2022). GC-MS analysis of methanolic, ethanolic, and aqueous leaf extracts has identified major constituents such as phytol, hexadecanoic acid methyl ester, and 9,12-octadecadienoic acid methyl ester, along with other alkaloids and flavonoids (I. Osamwonyi, O. Iyekowa, M. I. Ebengho, M. O. Edema, A. P. Oviawe, S. M. Momoh, L. O. Eduwuirofo, R. Iyekekpolor, O. T. Michael, R. O. Oghomwen, & A. A. Umeodinka, 2024). These findings support the presence of bioactive terpenoids, fatty acid derivatives, and phenolics responsible for the plant's antioxidant, anti-sickling, and anti-inflammatory properties (Structural Elucidation Study, 2025).

1.6.5. Fluorescence Spectrometry

Fluorescence spectrometry, including intrinsic and synchronous fluorescence techniques, is applied in studying molecular interactions between *Justicia carnea* extracts and biological macromolecules such as enzymes. This method detects changes in the microenvironment of aromatic amino acids, particularly tryptophan residues, through fluorescence quenching (A. A. Anigboro, O. J. Avwioroko, O. A. Ohwokevwo, B. Pessu, & N. J. Tonukari, 2021). In studies involving the interaction between *Justicia carnea* aqueous leaf extract and α-amylase, synchronous fluorescence spectroscopy revealed structural changes near the enzyme's active site, supporting its potential role in modulating enzyme activity related to metabolic disorders such as diabetes (A. A. Anigboro, O. J. Avwioroko, O. A. Ohwokevwo, B. Pessu, & N. J. Tonukari, 2021).

Summarily, spectroscopic techniques such as UV-Vis, FTIR, and mass spectrometry are central to the characterization of phytochemicals in *Justicia carnea*, providing essential insights into functional groups, molecular identity, and mechanisms of bioactivity (J. M. Jabar, 2025; I. Osamwonyi *et al.*, 2024). The integration of these techniques with chromatographic methods enhances the reliability and depth of phytochemical analysis, while advanced NMR and fluorescence applications present promising opportunities for future structural and interaction studies.

CHAPTER 2

MATERIALS AND METHODS

2.1 Materials

2.1.2 Medicinal Plants

- Plant samples of *J. carne*a (e.g., leaves)
- Herbarium specimens of *J. carnea* (for taxonomic identification)

2.1.3 Solvents and Reagents

- n- Hexane (BDH, England)
- Ethyl acetate (BDH, England)
- Diethyl ether (Aldrich Germany)
- Anhydrous sodium sulfate (Aldrich, Germany)
- Distilled water (Central Research Laboratory, UNIBEN)

2.1.4 Laboratory Equipment

- Rotary evaporator (R.E 200)
- Soxhlet extractor (EYELA N-Soxhlet Extractor)
- Electric blender (Vitamix Professional Series 750 Blender)
- Heating mantle (IKA ETS-D5 Digital Heating Mantle)
- Vacuum filtration setup
- Glassware (flasks, beakers, pipettes, burettes)

- Centrifuge (Eppendorf 5418)
- Analytical balance

2.1.5 Safety Equipment

- Lab coat
- Gloves
- Safety goggles
- Fume hood
- Fire extinguisher

2.1.6 Reference Standards and Chemicals

- Authentic standards of known compounds
- Internal standards
- Calibration standards

2.1.7 Consumables

- Syringe filters
- Vials and caps
- Solvent reservoirs
- Filter papers
- Laboratory labels

2.2 Methods

2.2.1 Collection of samples

Justicia carnea leaves were collected from their normal habitat at Ogida Quarters in Egor Local Government of Edo State and was identified by Prof. J.F. Bamidele of the department of Plant Biology and Biotechnology, University of Benin, Benin City.

2.2.2 Treatment and Extraction (Maceration)

J. carnea leaves were cleaned and washed to remove any visible dirt, debris, or contaminants from the leaves by gently washing them with clean water. After which the leaves were allowed to air dry to remove excess moisture.

The cleaned leaves were dried in the laboratory for 28days after which the dried leaves were pulverized and packed into a 1L beaker containing 500ml methanol (150g) solvent. This was allowed to stand for 48 hours with occasional stirring.

The extract was then filtered and concentrated using a rotary evaporator to afford a synergy solid mass. The crude mass was stored into two portions with one portion used for the phytochemical screening and the other isolation by VLC.

2.2.3 Isolation of methanol extract of *J. carnea*

50g of the crude methanol extract was subjected to VLC using hexane, hexane: ethyl acetate and methanol as the eluting solvents. Each eluting solvent was collected separately due to the oily nature of the hexane: ethyl acetate fraction, it was then chosen, dried and sent for GC- MS analysis.

2.3 Phytochemical screening of *j. Carnea* extracts

The phytochemical screening of *J. carnea* were performed using standard methods and procedures as prescribed by *Sofowora (1993) and Trease & Evans (1987).*

2.3.1 Test for Flavonoids

2ml of the extract was boiled in 10mlof distilled water and filtered. The filtrate was divided into two different portions of A and B of 5ml each.

(i) **To portions A:** 10% Lead Acetate solution was added in few drops. A yellowish precipitate is required for a positive result.

(ii) **To portions B:** 5ml of 20% NaOH and few drops of dilute HCl were added to the solution. Formation of a colorless solution is required for a positive result.

2.3.2 Test for Alkaloids

Dragendoff's reagent, Wagner's reagent and Picric acid were used to test for alkaloids. About 1ml of each of the plant extract was transferred into three different test tubes labelled A, B and C.

(i) **To portions A:** 2ml of Dragendoff's reagent (made of a mixture of potassium Bismuth Iodide Salt) was added. Reddish brown precipitate is required for a positive test.

(ii) **To portions B:** 2ml of Wagner's reagent was added. Reddish brown precipitate is required for a positive test.

(iii) **To portions C:** 2ml of Picric acid was added. A yellowish precipitate is required for a positive test.

2.3.3 Test for Saponins

0.5g of the plant extract was shaken with water in a test-tube and observed for frothing. Saponin rein Weiss (supplied by Merck) was used as standard.

2.3.4 Test for Tanins

To 2ml of extract, 10ml of distilled water was added and boiled for 5 minutes and then filtered into halves.

(i) To about two drops of the filtrate, ferric chloride ($FeCl_3$) solution was added; formation of a bluish precipitate is required for hydrolysable tannin.

(ii) To about five drops of the filtrate, 2ml of dilute HCl was added and boiled for 5 minutes. Red precipitate is required for a positive test.

2.3.5 Test for Steroids

2ml of acetic anhydride was added to 0.5g plant extract in 2ml of dilute H_2SO_4. A color change from violet to blue or green is required for a positive test for steroids.

2.3.6 Test for Terpenoids

5ml of extract was mixed in 2ml of chloroform and 3ml of conc. H_2SO_4 was carefully added down the side of the inner wall of the test tube to form a layer. A reddish-brown coloration of the interphase is required for the presence of terpenoids.

2.3.7 Test for Eugenols

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

2.3.8 Test for Glycosides

1ml of the extract was dissolved in 1ml of glacial acetic acid containing one drop of ferric chloride solution. This was under-layered with 1ml of conc. H_2SO_4. A brown ring is required for the presence of glycoside.

2.3.9 Test for Phenolic Compounds

1ml of the plant extract was added to 5ml of 90% ethanol. In addition, 1 drop of 10% $FeCl_3$ was added. A pale-yellow coloration is indicative of a positive test.

CHAPTER 3

RESULTS AND DISCUSSION

3.1 Result

3.1.1 Phytochemical Screening of Methanol Extract of *J. carnea*

S/N	Constituents	Tests	Methanol Extracts
1	Flavonoids	Lead Acetate	+
2	Alkaloids	Picric Acid	+
3	Saponins	Frothing	+
4	Tannins	Ferrric Chloride	-
5	Steroids	Acetic Acid / H_2s0_4	-
6	Terpenoids	Salkowiski	+
7	Eugenols	Ethanol / Ferrric Chloride	-
8	Glycosides	General	+
9	Phenolic Compounds	Ethanol / Ferrric Chloride	+

Table. 1. Phytochemical of the Methanol Extract of Justicia Carnea

+ = Present

+ = Largely Present

- = Not Seen

The obtained results indicate that the leaves of *Justicia carnea* possess a high abundance

of Flavonoid, Alkaloid, Saponin, Terpenoid, Glycoside and Phenolic Compounds**.**

3.2 Thin layer chromatography

$$R_f = \frac{distancee\ moved\ by\ compound}{distance\ moved\ by\ solvent\ f}$$

3.2.1 Colour Reaction & Retention Factor (R_f) Values of Ethyl Acetate Extract of *Justicia carnea* Using:

Solvent system: Hexane 100%

Solvent front: 8cm

Spot	Distance moved by compound (cm)	Color under white light	R_f value
Origin	0.00	Green	0.00
1	3.70	Light brown	0.4625

Table 2. Color reaction and retention factor (R_f) values of hexane extract

3.2.2 Colour Reaction & Retention Factor (R_f) Value of Ethyl Acetate Extract of *Justicia carnea* Using:

Solvent system: Ethyl Acetate 100%

Solvent front: 8cm

Spot	Distance moved by compound (cm)	Color under white light	R_f value
Origin	0.00	Green	0.00
1	5.50	Brown	0.6875

Table. 3: Color reaction and retention factor (R_f) values of ethyl acetate extract

3.3 GC-MS Analysis

The GC-MS chromatogram of oil from hexane: ethyl acetate fraction in Fig. 2 showed 10 peaks indicating from the search list of the chemical abstract services ten compounds. The chemical compounds identified in the oil are presented in the **Figure 9** and **Table 3**.

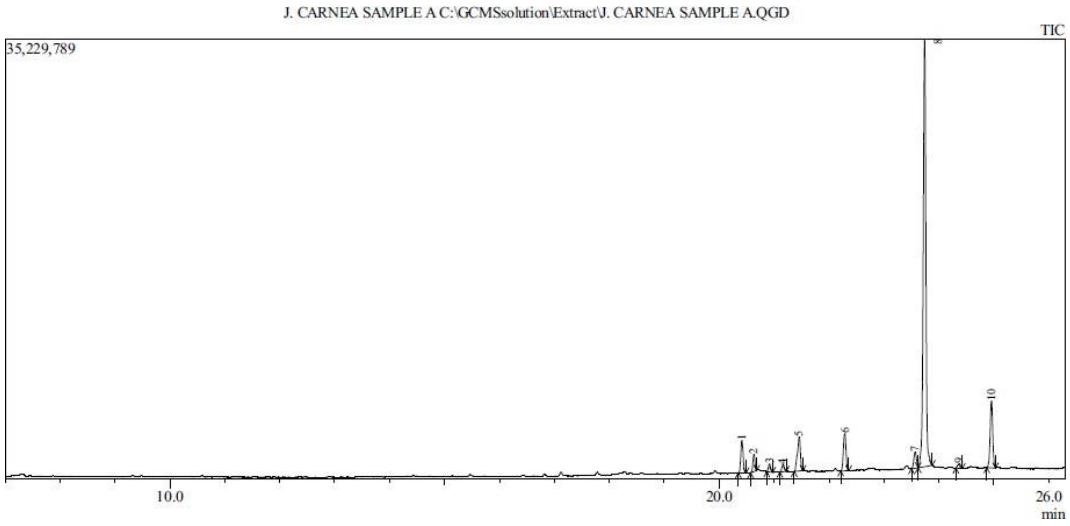

Fig 2. J. carnea GC-MS Solution Extract

Table 3. GC-MS Analysis of Isolated Oil of *J. carnea*

Peak#	R. Time	Area	Area %	Height	Height %	A/H	Name
1	20.416	7169545	4.08	2531261	4.97	2.77	Oxirane

2	20.633	3553025	2.02	1369483	2.63	2.59	2-Pentadecanone
3	20.915	1689176	0.96	653104	1.25	2.59	3,7,11,15-Tetramethyl-2-hexadecen-1-ol
4	21.164	1813593	1.03	605548	1.16	2.99	1,2-Benzadicarboxylic acid
5	21.460	10542598	5.99	2743286	5.26	3.84	Palmitic acid
6	22.288	9407008	5.35	2994833	5.74	3.14	Dibutyl phthalate
7	23.567	4548083	2.59	1301242	2.49	3.50	Linoleic acid
8	23.741	118336427	67.28	34216317	65.60	3.46	Phytol
9	24.364	1072161	0.61	333277	0.64	3.22	Palmitoyl chloride
10	24.958	17766204	10.10	5348232	10.25	3.32	Phytol, acetate
		175897820	100.00	52156583	100.00		

Sample Information

Analyzed by	: Ronald Ibia
Analyzed	: 5/28/2011 8:14:27 AM
Sample Type	: Unknown
Level #	: 1
Sample Name	: J. CARNEA SAMPLE A
Sample ID	: J. CARNEA SAMPLE A
IS Amount	: [1]=1
Sample Amount	: 1
Dilution Factor	: 1
Vial #	: 4
Injection Volume	: 0.20
Data File	: C:\GCMSsolution\Extract\J. CARNEA SAMPLE A.QGD
Org Data File	: C:\GCMSsolution\Extract\J. CARNEA SAMPLE A.QGD
Method File	: C:\GCMSsolution\Extract\Extract md.qgm
Org Method File	: C:\GCMSsolution\Extract\Extract md.qgm
Report File	:
Tuning File	: C:\GCMSsolution\System\Tune1\EXTRACT TUNNING 01-03-2024.qgt
[Comment]	
J. CARNEA SAMPLE A	
Modified by	: Admin
Modified	: 3/2/2024 2:36:01 PM

Figure 9. Characterization of sample using GC-MS

3.4 Discussion

The GC-MS analysis of the isolated oil from the hexane/ethyl ether fraction of *Justicia carnea* revealed a diverse array of compounds, including major components such as (2E,7R,11R)-3,7,11,15-Tetramethylhexadec-2-en-1-ol (Phytol) and other minor compounds such as Hexandecanoic acid (palmitic acid), 9,12-Octadecadienoic Acid (Linoleic Acid). These findings suggest that *Justicia carnea* oil contains important chemical constituents which possess physiological agents.

Several compounds identified in the oil are known for their pharmacological activities, such as antioxidant, antimicrobial, and anti-inflammatory properties, indicating potential medicinal applications.

3.5. Conclusion

The study on the extraction and analysis of *Justicia carnea* oil provides valuable insights into its chemical composition and potential medicinal properties. The GC-MS analysis revealed a complex mixture of compounds with the main compounds identified major components such as (2E,7R,11R) - 3,7,11,15-Tetramethylhexadec-2-en-1-ol (Phytol) and other minor compounds such as Hexandecanoic acid (palmitic acid), 9,12- Octadecadienoic Acid (Linoleic Acid). These findings suggest that *Justicia carnea* oil contains important chemical constituents which possess physiological agents. In addition, bioactive components with antioxidant, antimicrobial, and anti-inflammatory properties were also seen in this research.

While the research highlights the potential pharmacological benefits of Justicia carnea oil, further studies are needed to understand its specific biological activities and optimize

extraction methods for higher efficiency. Despite some limitations, this research sets the stage for future exploration of *Justicia carnea's* therapeutic potential in medicine and industry.

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