Original Research Article

STRUCTURAL ELUCIDATION OF BIOACTIVE CONSTITUENTS OF *JUSTICIA CARNEA* LEAF USING GAS CHROMATOGRAPHY-MASS SPECTROSCOPY TECHNIQUE

ABSTRACT

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| **Aims:** This investigation focused on the bioactive constituents of *Justicia carnea* aqueous leaf extractusing Gas Chromatography-Mass Spectroscopy (GC-MS) analysis. **Study design:** Fresh leaves of *Justicia carnea* were collected from their natural habitat at Rumuogba, Obio/Akpor L. G. A. Rivers State. The plant materials were identified and authenticated by a plant taxonomist at the Department of Plant Science, University of Port Harcourt, Nigeria, Dr Ekeke Chimezie and given the Voucher number UPH/V/1448.**Place and Duration of Study:** The study was conducted in the Faculty of Sciences Laboratory, University of Port Harcourt, Choba, Rivers state, Nigeria. Between December 2024 to April 2025 **Methodology:** Fresh leaves *of Justicia carnea* were collected, washed thoroughly with water to remove any dirt or impurity and air dried at room temperature for 21 days. The dried leaves were then ground into a fine powder using a blender after which 1g of sample was weighed and transferred in a test tube and 15ml water and 10ml of 50% W/V potassium hydroxide was added. 0.4g of sample was weighed and transferred in a test tube and 15ml of water was added. The test tube was allowed to react in a water bath at 600C for 60mins. After the reaction time, the reaction product contained in the test tube was transferred to a separatory funnel for the GC-MS analyses of both phytochemicals and bioactive constituents.**Results:** Analysis of aqueous leaf extract of *Justicia carnea* revealed the presence of a cassette of phytochemicals that possess pharmacological benefits. Spartein, Epihedrine, Narigenin, Phytate, Kaempferol and Dihydrocytisine were observed as the most abundant secondary metabolites of *J. carnea* leaf aqueous extract. GC-MS analysis result showed 29 volatile bioactive compounds in the aqueous extract of *Justicia carnea* leaf. Among the identified compounds of aqueous extract of *J. carnea* were 1,2-Benzenedicarboxylic acid and Ethyltriethylene glycol, which has been reported to possess antioxidant, antimicrobial and positive anti-cancer activity. The compound Bis (2-ethylhexyl) phthalate, a member of the class of phthalates was identified in the extract. The compound is a plasticizer which play a role as a precursor of polyvinyl chloride. Bis(2-ethylhexyl) phthalate and its metabolites exhibit acute and chronic toxicities which includes endocrine disruption and testicular toxicity.**Conclusion:** The current investigation has identified significant bioactive compounds in the aqueous extract of *Justicia carnea* leaf.. The presence of these phytochemical and bioactive compounds could be a pointer to its applicability as a safer alternative therapy in the management of diseases. |

*Keywords: Phytochemicals, Spectroscopy, structural elucidation, Justicia carnea, Bioactive compounds*

1. INTRODUCTION

Plants are rich with many bioactive Secondary metabolites with specific biological or pharmacological activity in the human body (Altemimi *et al.,* 2017; Asiwe *et al*., 2023). These compounds have been widely studied for their potential health benefits, and physiological actions, including their ability to prevent and treat disease (Pandey and Rizvi, 2009; Ullah *et al.,* 2020). The tropical rain forest of Nigeria boasts of a vast distribution of vegetation, from which plants and plant-derived products are sourced as herbal remedies traditionally (Asiwe *et al.,* 2023). These diverse plants species are in practice, chemical factories that produce a plethora of structurally diverse organic compounds credited with therapeutic benefits and are also precursors for the synthesis of useful drugs (Patel *et al.,* 2022). Natural products of plant origin have attracted considerable attentions in modern times due to the multifaceted pharmacological properties including antioxidant and antitumour activity (Onyegeme-Okerenta *et al.,* 2021, Patel *et al.,* 2022). Numerous fruits, shrubs, spices and herbs and leafy vegetables are useful as food, food drinks and for medicinal purposes in Nigeria (Onyegeme-Okerenta *et al.,* 2023; Asiwe *et al.,* 2023). Despite these enormous benefits, elucidating the active principles is only just beginning to gain appropriate scholarly attention.. *Justicia carnea* isaflowering plant native to the Atlantic forest of eastern Brazil, it is classified among the family Acanthaceae. It also finds a niche in the rain forest belt of Nigeria. *Justicia carnea* is a perennial shrub that typically reaches a height of about 3 to 4 feet (Corrêa *et al.,* 2011; Onyegeme-Okerenta *et al.,* 2021). Common names include Brazilian plume flower, Brazilian-plume and flamingo flower**.** The plant blossoms in clusters, with conspicuous pink or red flowers. It is planted majorly as an ornamental plant, with value as vegetable and medicine (Parker and Pearson, 2011). *Justicia carnea* has gained attention from researchers due to its potential medicinal value (Asiwe *et al.,* 2023). Several species of *Justicia* are widely used in folk medicine for the treatment of inflammation, respiratory and gastrointestinal disorder. In south-east and south-south parts Nigeria, concoction of the leaf is administered for its blood-boosting potential (Onyeabo *et al.,* 2017; Akpovwehwee *et al.,* 2021; Onyegeme-Okerenta *et al., 2021*).

Studies have reported the presence of various bioactive compounds in different parts of the plant. Asiwe *et al.,* 2023 reported the presence of alkaloids and flavonoids in the ethanol extract of leaves of *Justicia carnea.*  Oloruntola *et al.,* (2022) identified the presence of alkaloids and flavonoids in the leaves of *Justicia carnea.* Oloruntola *et al.,* (2022) demonstrated the anti-inflammatory activity of extract derived from the leaves of *Justicia carnea*. Falode *et al.,* 2022 reported reduction of pro-inflammatory cytokines and increase in the anti-inflammatory cytokine by the extract. Other works by Imohiosen 2023, reported significant antimicrobial effects against several strains of bacteria by aqueous extract of *Justicia carnea* leaves. Safety of *Justicia carnea* leaves ethanol extract has been documented (Falode, *et al.,* 2023); the extract was shown to positively impact hematopoiesis and antioxidant enzymes activity (Onyegeme-Okerenta *et al.,* 2021; Falode, *et al.,* 2023). Medicinal plants remain the mainstay of drug discovery (Dias *et al.,* 2012); phytochemical screenings of plants are useful in identification of new sources of therapeutically and industrially bioactive compounds from plants (Asiwe *et al.,* 2023). The bioactive substances of note in plants are alkaloids, flavonoids, tannins, saponins and phenolic compounds (Tungmunnithum, *et al.,* 2018). Noteably research reports firmly document *J.carnea* as having potential medicinal properties*,* however, further research is needed for a comprehensive understanding of its active principles and safety although (Onyegeme-Okerenta *et al.,* 2021 posits that the leaf of Justicia carnea is relatively safe with LD50 result 5000mg/kg showing no mortality in Wistar rat. The present study is aimed at assessing the bioactive and phytochemical composition of *Justicia carnea* leaf aqueous extract.

2. material and methods

**2.1. Chemicals/reagents**

All chemicals and reagents were of analytical grade.

**2.2. Plant materials**

Fresh leaves of *Justicia carnea* were collected from their natural habitat at Rumuogba, Obio/Akpor L. G. A. Rivers State. The plant materials were identified and authenticated by a plant taxonomist at the Department of Plant Science, University of Port Harcourt, Nigeria, Dr Ekeke Chimezie and given the Voucher number UPH/V/1448.

**2.3. Preparation of plant extract**

Fresh leaves *of Justicia carnea* were collected, washed thoroughly with water to remove any dirt or impurity and air dried at room temperature for 21 days. The dried leaves were then ground into a fine powder using a blender. The powdered leaves of *Justicia carnea* (400 g) was extracted with 3litres of water using maceration method with intermittent shaking for 24 hours. The extract was then filtered using filter paper and the filtrate was collected in a clean, dry container. The filtered extract was concentrated using a water bath at 600C to remove the solvent and a concentrated to a semi-solid residue. The concentrated extract was transferred to a sterilized, amber- coloured plastic vials to protect them from light and stored in at 4-8⁰C before analysis.

**2.4. Extraction of phytochemicals for GC-MS analysis**

1g of sample was weighed and transferred in a test tube and 15ml water and 10ml of 50% W/V potassium hydroxide was added. The test tube was allowed to react in a water bath at 600C for 60mins. After the reaction time, the reaction product contained in the test tube was transferred to a separating funnel. The tube was washed successfully with 20ml of alcohol, 10ml of cold water, 10ml of hot water and 3ml of hexane, which was all transferred to the funnel. These extracts were combined and washed three times with 10ml of 10%v/v ethanol aqueous solution. The solution was dried with anhydrous sodium sulphate and the solvent was evaporated. The sample was solubilized in 1000ul of pyridine of which 200ul was transferred to a vial for analysis.

 **2.5. Gas chromatography- Mass spectrometry analysis**

0.4g of sample was weighed and transferred in a test tube and 15ml of water was added. The test tube was allowed to react in a water bath at 600C for 60mins. After the reaction time, the reaction product contained in the test tube was transferred to a separatory funnel. The tube was washed successfully with 20ml of alcohol, 10ml of cold water, 10ml of hot water and 3ml of hexane, which was all transferred to the funnel. These extracts were combined and washed three times with 10ml of 10%v/v ethanol aqueous solution. The solution was dried with anhydrous sodium sulphate and the solvent was evaporated. The sample was solubilized in 1000ul of petroleum ether of which 200ul was transferred to a vial for analysis.

**2.6. Quantification by Gas chromatography**

 The analysis of phytochemical was performed on a BUCK M910 Gas chromatography equipped with HP-5MS column (30 m in length × 250 μm in diameter × 0.25 μm in thickness of film). Spectroscopic detection by GC–MS involved an electron ionization system which utilized high energy electrons (70 eV). Pure helium gas (99.995%) was used as the carrier gas with flow rate of 1 ml/min. The initial temperature was set at 50 –150 °C with increasing rate of 3 °C/min and holding time of about 10 min. Finally, the temperature was increased to 300 °C at 10 °C/min. One microliter of the prepared 1% of the extracts diluted with respective solvents was injected in a split less mode. Relative quantity of the chemical compounds present in each of the extracts of was expressed as percentage based on peak area produced in the chromatogram.

**2.8. Identification of bioactive compounds:**

Bioactive compounds extracted from different extracts were identified by matching of the spectra of the unknown components with the known components stored in the National Institute of Standards and Technology (NIST) library.

3. results and discussion

The current investigation assessed the phytochemical components and bioactive composition of aqueous leaf extract of *Justicia carnea.* Analysis of aqueous leaf extract of *Justicia carnea* revealed the presence of a cassette of phytochemicals that possess pharmacological benefits as shown in Table a. The extract is rich in bioactive alkaloids and flavonoid which include Spartein (8.02μg/ml), Epihedrine (6.24 μg/ml), Narigenin (6.0μg/ml), Phytate (5.62 μg/ml), Kaempferol (5.22μg/ml), Dihydrocytisine (4.32μg/ml), Sapogenin (4.10μg/ml), Flavone (3.90 μg/ml), Anthocyanin (3.42μg/ml), Tannin (2.02 μg/ml) and Ribalinidine (3.25 μg/ml). Spartein, Epihedrine, Narigenin, Phytate, Kaempferol and Dihydrocytisine were observed as the most abundant secondary metabolites of *J. carnea* leaf aqueous extract. Kaempferol and anthocyanin have reportedly shown positive results from investigation as antioxidant, anti-inflammatory, antimicrobial, and cardiovascular agents (Yeon *et al.,* 2019; Yang *et al.,* 2021; Asiwe *et al*., 2023; Xue *et al.,* 2023). Sparteine which is identified as a quinolizidine alkaloid abundant in Lupinus, sparteine has been implicated in reducing locomotor activity and exert analgesic effects in the central nervous system (Villalpando-Vargas & Medina-Ceja, 2016). It also showed anticonvulsant properties in experimental animals; delaying the onset of convulsive behavior and decreasing the severity and mortality of rats treated with pilocarpine (Villalpando-Vargas & Medina-Ceja, 2016). The hypoglycaemic effect of spartein has been reported; sparteine sulphate enhances β-cell secretion, causing a fall in plasma glucose concentration (Ogunka-Nnoka *et al.,* 2018). In another study, Sparteine exerts anticancer effect on human cervical cancer cells via induction of apoptosis, inhibiting the phosphorylation of VGFR2 in a concentration-dependent manner (Tian *et al.,* 2019, Liang and Liu, 2019). Naringenin is a natural flavonoid with significant neuroprotective properties; anti-neuroinflammation*,* anti-neuroapoptosisand antioxidant properties have been reported *(*Nouri *et al.,* 2019; Kamoru *et al.,* 2023 & Asiwe *et al*., 2023)*.* Additionally, it exerts control on body lipids through hypocholesterolemic and hypolipidemic and regulates blood pressure with antagonistic activities against inflammation *(*Nouri *et al.,* 2019; Kamoru *et al.,* 2023)*.* Phytates are salts of phytic acid, they are storage form of phosphorus in all grains, certain fruits and vegetables (Asiwe *et al*., 2023). They have been shown to exhibit anti-inflammatory, metal chelating and antioxidant activities (Urbano *et al.,* 2000; Gibson *et al.,* 2010). Epihedrine is linked with antibacterial and antifungal activities (Tulgar *et al.,* 2018). Other studies have confirmed that Kaempferol possesses antioxidant, anti-inflammatory, antimicrobial, cardiovascular, and neuroprotective properties (Zhu *et al.,* 2018, Yeon *et al.,* 2019; Bangar *et al.,* 2022). Also, physiological properties of the polyphenolic compound tannin, includes antibacterial, anti-inflammatory, antioxidant, antivirus, anti-diarrheal and anti-malarial activities (Buzzini *et al.,* 2008; Koleckar *et al.,* 2008).

**Table 1. Quantitative phytochemical composition of some phytochemicals of aqueous eleaf extract of *Justicia carnea*.**

Phytochemical Composition (μg/ml)

Spartein 8.02

Epihedrine 6.24

Narigenin 6.00

Phytate 5.62

Kaempferol 5.22

Dihydrocystisine 4.32

Sapogenin 4.10

Flavone 3.90

Anthocyanin 3.42

Tannin 2.02

Ribalinidine 3.25

GC-MS analysis result (figure 1) also showed 29 volatile bioactive compounds in the aqueous extract of *Justicia carnea* leaf which includes Propanoic acid (3.05%), Bis(2-ethylhexyl) phthalate (6.24 %), 1-(chloromethoxy)-2-methoxyethane (2.11 %), 1,2-Benzenedicarboxylic acid (4.06%), Ethyltriethylene glycol (2.19%), Diethylene glycol ethyl ether (3.83%), Pentadecanoic acid (13.62%), Tridecanoic acid (1.67%), Hexadecanoic acid (1.28%), Decanoic acid (1.19%), Octadecenoic acid (4.09%), Tetradecanoic acid (11.73%), Nonadecanoic acid (0.55%), 9, 12-Octadecadienoic acid (1.21%), 11, 14-eicosadienoic acid (1.36%), 7,10-Hexadecadienoic acidicosane (0.81%), 9, 12-octadecadien-1-ol (1.14%), 13-tetradece-11-yn-1-ol (0.82%), 11- Octadecanoic acid (2.10%), Bis(2-ethylhexyl) phthalate (0.20%), 6-Octadecanoic acid (2.10%), Cyclpropaneoctanal (2.24%), 9- tetradecanal (2.30%),, Z-(13, 14-epoxy)tetradec-11-en-1-ol acetate (10.24%), Oleic acid (4.10%), Hexadecanoic acid (3.10%), Pentafluoropropionic acid (4.40%), Deceyl fluoride (2.80%) and Nonanoic acid chloride (table 2). The identified compounds in aqueous leaf extract of *Justicia carnea* include carboxylic acids, esters, aldehydes, hydrocarbons, aromatic hydrocarbons, coumarins, amines, and terpenes/terpenoids.

Among the identified compounds of aqueous extract of *J. carnea* were 1,2-Benzenedicarboxylic acid and Ethyltriethylene glycol, which has been reported to possess antioxidant, antimicrobial and positive anti-cancer activity (Zhao *et al.,* 2018; Guimarães *et al.,* 2019). The sesquiterpene *Aromadendrene* abundant in *J. carnea* leaf have been established to inhibit the proliferation of HepG2 liver and PC3 prostate cancer cells and responsible for antibacterial activity of essential oils (Mulyaningsih *et al.,* 2011; Al-Lihaibi *et al.,* 2014; Asiwe *et al*., 2023). Also, Benzamide derivatives possess varieties of pharmacological activities including antimicrobial, analgesic, anti-inflammatory, anticancer, cardiovascular, and other biological activities (Asif, 2016, Asiwe *et al*., 2023). Acidicosane functions in the release of vitamin E into the body for healthy functioning. It has been confirmed to exhibit a number of beneficial pharmacological activities (van Zyl *et al.,* 2006). Earlier studies have established that acidicosane possesses anti-inflammatory, antioxidant activity and antimicrobial activity (van Zyl *et al.,* 2006). Acidicosane -rich essential oils have shown promising activity against *Plasmodium falciparum* (Kamatou *et al.,* 2006, Asiwe *et al.,* 2023).

The compound Bis (2-ethylhexyl) phthalate, a member of the class of phthalates was also identified in the extract. The compound is a plasticizer which play a role as a precursor of polyvinyl chloride (Rowdhwal *et al.,* 2018). Reports showed that Bis(2-ethylhexyl) phthalate and its metabolites exhibit acute and chronic toxicities which includes endocrine disruption and testicular toxicity (Martinez-Arguelles *et al.,* 2011; Rowdhwal *et al.,* 2018, Asiwe *et al.,* 2023). The presence of the identified secondary metabolites puts these results in line with earlier studies that were carried out on the ethanol and Ethyl acetate extract of *J. carnea* leaf in the work of Asiwe *et al.,* 2023 and Oloruntola *et al.,* (2022).

**Table 2: B**ioactive composition of *Justicia carnea* leaf aqueous extract

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Peak time | Reteention Time |  Compound |  Molecular Formula |   |  |  |  |  |  |
| 1 |  4.13 | Propanoic acid |  C8H16O2  |  |  |  |  |  |  |
| 2 |  7. 15 | 1-(chloromethoxy)-2-methoxyethane C4H9ClO2 |  |  |  |  |  |  |  |
| 3 |  7.16 | 1,2-Benzenedicarboxylic acid |  C8H604 |  |  |  |  |  |  |
| 4 |  8.05 | Ethyltriethylene glycol |  C6H14O3 |  |  |  |  |  |  |
| 5 |  16.85 | Diethylene glycol ethyl ether |  C5H14O3 |  |  |  |  |  |  |
| 6 |  19.88 | Pentadecanoic acid |  C17H34O2  |  |  |  |  |  |  |
| 7 |  19.99 | Tridecanoic acid |  C14H28O2 |  |  |  |  |  |  |
| 8 |  20.33 | Hexadecanoic acid |  C18H36O2 |  |  |  |  |  |  |
| 9 |  21.00 | Decanoic acid |  C11H22O2 |  |  |  |  |  |  |
| 10 |  21.16 | Octadecenoic acid |  C19H38O2 |  |  |  |  |  |  |
| 11 |  21.67 | Tetradecanoic acid |  C15H30O2 |  |  |  |  |  |  |
| 12 |  21.26 | Nonadecanoic acid |  C19H38O2 |  |  |  |  |  |  |
| 13 |  22.44 | 9, 12-Octadecadienoic acid |  C19H34O2 |  |  |  |  |  |  |
| 14 |  24.25 | 11, 14-eicosadienoic acid |  C21H38O2 |  |  |  |  |  |  |
| 15 |  26.31 | 7,10-Hexadecadienoic acidicosane |  C17H30O2 |  |  |  |  |  |  |
| 16 |  26.33 | 9, 12-octadecadien-1-ol |  C18H34O |  |  |  |  |  |  |
| 17 |  28.04 | 13-tetradece-11-yn-1-ol |  C14H24O |  |  |  |  |  |  |
| 18 |  28.10 | 11- Octadecanoic acid |  C19H36O2 |  |  |  |  |  |  |
| 19 |  28.84 | Bis(2-ethylhexyl) phthalate |  C24H38O4 |  |  |  |  |  |  |
| 20 |  29.62 | Diethylene glycol monomethyl ether |  C5H12O3  |  |  |  |  |  |  |
| 21 |  31.05 | Cyclpropaneoctanal |  C19H36O |  |  |  |  |  |  |
| 22 |  31. 83 | 9- tetradecanal |  C14H26O |  |  |  |  |  |  |
| 23 |  31. 11 | Z-(13, 14-epoxy)tetradec-11-en-1-ol acetate |  C16H28O3 |  |  |  |  |  |  |
| 24 |  32.06 | Oleic acid |  C18H34O2 |  |  |  |  |  |  |
| 25 |  32.88 | 2-aminoethyl hydrogen phosphate |  C37H74NO8P |  |  |  |  |  |  |
| 26 |  34.10 | Pentafluoropropionic acid |  C16H27F5O2 |  |  |  |  |  |  |
| 27 |  34.44 | Deceyl fluoride |  C10H21F |  |  |  |  |  |  |
| 28 |  35.51 | Nonanoic acid chloride |  C9H17ClO |  |  |  |  |  |  |

4. Conclusion

The current investigation has identified significant bioactive compounds in the aqueous extract of *Justicia carnea* leaf. The phytochemicals have been shown in numerous studies to possess pharmacologically beneficial properties, which includes anti-inflammatory, anticancer, antibacterial and antioxidant effects which is elicited by their interactions with an array of biochemical pathways. The presence of these compounds could be a pointer to its applicability as an alternative therapy to orthodox medicine in the combat and management of diseases.



Figure 1: GC-MS chromatogram of aqueous leaf extract of *justicia carnea* (x-axis represent time (minutes) and y-axis represent abundance (mau).

**Disclaimer (Artificial Intelligence)**

Authors hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during the writing or editing of this manuscript.

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