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Phytochemicals and volatile composition of *Justicia carnea* leaf ethanol extract

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Abstract

The present study assessed the bioactive and volatile composition of *Justicia carnea* leaf ethanol extract. The quantitative phytochemical composition of the extracts was determined using gas chromatography flame ionization detector (GC-FID) while volatile components were determined using gas chromatography- mass spectrometry (GC-MS). The results obtained from the study showed that the extract is rich in a battery of pharmacologically beneficial alkaloids and flavonoid which include Spartein (9.17 µg/ml), Epihedrine (7.35 µg/ml), Narigenin (7.20 µg/ml), Phytate (6.86 µg/ml), Kaempferol (6.43 µg/ml), Dihydrocytisine (5.73 µg/ml), Sapogenin(4.42 µg/ml), Flavone (3.9 µg/ml), Anthocyanin (3.86 µg/ml), and Ribalinidine (3.37 µg/ml). Spartein, Epihedrine, Narigenin, Phytate, Kaempferol and Dihydrocytisine were the most abundant secondary metabolites of *J. carnea* leaf ethanol extract. GC-MS analysis identified 86 volatile bioactive compounds in the ethanolic extract of *Justicia carnea* leaf; these includes 1-Piperonyl-3,5-diamino-1,2,4-triazole, 1,2-Dipiperonyl-3-imino-1,2,4-triazine (3.70%), 1H-Cyclopenta[1,3]cyclopropa[1,2]benzene,octahydro-7-methyl-3-methylene-4-(1-methylethyl)-(3.74%), (E)-.beta.-Famesene,1,6,10-Dodecatriene (4.34%), Aromandendrene Bicyclo [7.2.0]undec-4-ene (6.27%), Cyclohexene, 3-(1,5-dimethyl-4-hexenyl)-6-methylene- (7.59%), 1-Formyl-1-piperonylhydrazine Benzene, isothiocyanato- (8.32%) and (E)-.beta.-Famesene, .beta.-Bisabolene (10.87%) as the major bioactive compounds. These compounds may contribute to the documented pharmacological activities of the plants, including antioxidant, antimicrobial, anti-inflammatory, and anticancer properties.

Keywords: *Justicia carnea*; Phytochemicals; GC-MS; GC-FID; Bioactive compounds

1. Introduction

Plants contain many bioactive Secondary metabolites with definite pharmacological or biological activity in the human body (Altemimi *et al.,* 2017; Newman and Cragg, 2020). 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 houses a vast distribution of vegetation, from which plants and plant-derived products are sourced as herbal remedies traditionally. These diverse plants species are de facto chemical factories producing a multitude of structurally diverse organic compounds found to contain therapeutic benefits and are also precursors for the synthesis of useful drugs (Patel *et al.,* 2022). Plant derived natural products have received considerable attentions in recent years due to the diverse pharmacological properties including antioxidant and anti-tumour activity (Patel *et al.,* 2022). In Nigeria, many fruits, shrubs, spices and herbs and leafy vegetables are useful as

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food, food drinks and for medicinal purposes; despite these enormous benefits adequate scholarly attention has not been given to elucidating active principles. *Justicia carnea* is a flowering 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). 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. Several species of *Justicia* are widely used in folk medicine for the treatment of inflammation, respiratory and gastrointestinal disorder. In south-eastern Nigeria, decoction of the leaf is administered for its blood-boosting potential (Onyeabo *et al.,* 2017; Akpovwehwee *et al.,* 2021).

Studies have reported the presence of various bioactive compounds in different parts of the plant. 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 (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. The bioactive substances of note in plants are alkaloids, flavonoids, tannins, saponins and phenolic compounds (Tungmunnithum, *et al.,* 2018); these active substances, alongside the vitamins and minerals have been widely reported to contribute to their various physiologic and protective effects (Shabab *et al.,* 2021; Shomali, *et al,* 2022). It is important to note that while these studies indicate potential medicinal properties of *Justicia carnea,* further research is required to fully understand its active principles and safety. The present study is aimed at assessing the bioactive and volatile composition of *Justicia carnea* leaf ethanolic extract.

2. Materials 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 Akokwa, Ideato North L. G. A. Imo state. The plant materials were authenticated and identified using google lens scan and further by a plant taxonomist at the Department of Plant Science and Biotechnology, Kingsley Ozumba Mbadiwe University, Ogboko, Nigeria.

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 14 days. The dried leaves were then ground into a fine powder using a blender. The powdered leaves of *Justicia carnea* (100 g) was extracted with 500ml of 80% ethanol using maceration method with intermittent shaking for 72 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 waterbath at 45^0C to remove the solvent and a concentrated extract was obtained. The concentrated extract was transferred to a sterilized, amber- coloured plastic vials to protect them from light and stored in at 4-8^0C before analysis.

2.4. Extraction of phytochemicals for GC-FID analysis

1g of sample was weighed and transferred in a test tube and 15ml ethanol and 10ml of 50% W/V potassium hydroxide was added. The test tube was allowed to react in a water bath at 60^0C 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 ethanol, 10ml of cold water, 10ml of hot water and 3ml of hexane, which was all transferred to the funnel. This extracts were combined and washed three times with 10ml of 10%v/v ethanol aqueous solution. The solution as dried with anhydrous sodium sulfate 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. Quantification by GC-FID

The analysis of phytochemical was performed on a BUCK M910 gas chromatography equipped with a flame ionization detector. A RESTEK 15 meter MXT-1 column (15m x 250um x 0.15um) was used. The injector temperature was 280^{o}C with splitless injection of 2ul of sample and a linear velocity of 30cms^{-1}, Helium 5.0pa.s was the carrier gas with a flow rate of 40 ml min^{-1}. The oven operated initially at 200^{0}C, it was heated to 330^{0}C at a rate of 3^{0}C min^{-1} and was kept at this temperature for 5min. the detector operated at a temperature of 320^{0}C. Phytochemicals were determined by the ratio between the area and mass of internal standard and the area of the identified phytochemicals. The concentration of the different phytochemicals was expressed in ug/g (Ichihara and Fukubayashi, 2010).

2.6. Gas chromatography- Mass spectrometry analysis

0.2g of sample was weighed and transferred in a test tube and 15ml of ethanol was added. The test tube was allowed to react in a water bath at 60^{0}C 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 ethanol, 10ml of cold water, 10ml of hot water and 3ml of hexane, which was all transferred to the funnel. This extracts were combined and washed three times with 10ml of 10%v/v ethanol aqueous solution. The solution as dried with anhydrous sodium sulfate and the solvent was evaporated. The sample was solubilized in 1000ul of petroleum ether of which 200ul was transferred to a vial for analysis.

2.7. 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 an splitless 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 present study assessed bioactive composition and volatile organic compounds of *Justicia carnea* leaf ethanol extract. Analysis of *Justicia carnea* leaf ethanolic extract revealed the presence of a battery of pharmacologically beneficial phytochemicals as shown in Table 1. The extract is rich in bioactive alkaloids and flavonoid which include Spartein (9.17 µg/ml), Epihedrine (7.35 µg/ml), Narigenin (7.20 µg/ml), Phytate (6.86 µg/ml), Kaempferol (6.43 µg/ml), Dihydrocytisine (5.73 µg/ml), Sapogenin(4.42 µg/ml), Flavone (3.9 µg/ml), Anthocyanin (3.86 µg/ml), and Ribalinidine (3.37 µg/ml).

Spartein, Epihedrine, Narigenin, Phytate, Kaempferol and Dihydrocytisine were the most abundant secondary metabolites of *J. carnea* leaf ethanol 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; Xue *et al.,* 2023). Sparteine is a quinolizidine alkaloid abundant in Lupinus, sparteine has been reported to reduce 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 PTZ and 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 (Paolisso *et al.,* 1988). 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-neuroapoptosis and antioxidant properties have been reported *(*Nouri *et al.,* 2019; Kamoru *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. 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 (Yeon *et al.,* 2019; Zhu *et al.,* 2018, 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 and biological activity of some phytochemicals of *Justicia carnea* leaf extract

Phytochemical	**Composition (µg/ml)**	**Biological Activity**	**References**
Kaempferol	6.43	Antioxidant, anti-inflammatory, antimicrobial, cardiovascular, and neuroprotective properties.	Yeon *et al.,* 2019, Zhu *et al.,* 2018, Bangar *et al.,* 2022
Steroid	3.53		
Anthocyanin	3.86	Antioxidant, anti-inflammatory, cardiovascular health, and anti-tumor qualities	Kong *et al.,* 2003; Yang *et al.,* 2021; Xue *et al.,* 2023
Cyanogenic glycoside	2.34		
Narigenin	7.20	Antioxidant, anti-inflammatory, anticancer, neuroprotective and cardiovascular effects, antimicrobial and antiviral properties	Salehi *et al.,* 2019; Zhao *et al.,* 2021
Aphyllidine	0.46	Antimicrobial, antioxidant, anti-inflammatory,antitumor	Ruiz-López *et al.,* 2019
Ammodendrine	3.19		
Cardiac glycoside	5.22		
Flavone	3.90	Antioxidant, anti-inflammatory, antimicrobial, anticancer and neuroprotective effects	Verma & Pratap, 2010; Kumar & Pandey, 2013
Dihydrocytisine	5.73		
Spartein	9.17	hypoglycemic; anti-convulsant	Vargas and Ceja (2016)
Ribalinidine	3.37	Anesthesia, hepatotoxicity, cardiorespiratory effects, muscle relaxation	Duru, (2020)
Phytate	6.86	Antioxidant, anti-inflammatory	Urbano *et al.,* 2000; Gibson *et al.,* 2010
Oxalate	3.04		
Flavonone	0.01	Antioxidant, anti-inflammatory cardiovascular protection, antimicrobial, anticancer, neuroprotective effects	Kumar & Pandey, 2013; Dias *et al.,* 2021
Epihedrine	7.35	Antibacterial and antifungal effects	Tulgar et al, 2018
Sapogenin	4.42	Hypocholesterolemic, immunodulatory; anti-inflammatory, cytotoxic	Sun et al. (2014); Lin et al. (2016)

GC-MS analysis result (figure 1) also showed 86 volatile bioactive compounds in the ethanolic extract of *Justicia carnea* leaf which includes 1-Piperonyl-3,5-diamino-1,2,4-triazole, 1,2-Dipiperonyl-3-imino-1,2,4-triazine (3.70%), 1H-Cyclopenta[1,3]cyclopropa[1,2]benzene, octahydro-7-methyl-3-methylene-4-(1-methylethyl)-(3.74%), (E)-.beta.-Famesene, 1,6,10-Dodecatriene (4.34%), Aromandendrene Bicyclo[7.2.0]undec-4-ene (6.27%), Cyclohexene, 3-(1,5-dimethyl-4-hexenyl)-6-methylene- (7.59%), 1-Formyl-1-piperonylhydrazine Benzene, isothiocyanato- (8.32%) and

(E)-.beta.-Famesene, .beta.-Bisabolene (10.87%) as the major bioactive compounds (table 2.0). The identified compounds in *Justicia carnea* leaf ethanolic extract include alkanes, carboxylic acids, esters, aldehydes, hydrocarbons, aromatic hydrocarbons, coumarins, amines, and terpenes/terpenoids.

Among the identified volatile compounds of *J. carnea* leaf ethanol extract were 1,2-Benzenedicarboxylic acid and gamma.-Terpinene, 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). Also, Benzamide derivatives possess varieties of pharmacological activities including antimicrobial, analgesic, anti-inflammatory, anticancer, cardiovascular, and other biological activities (Asif, 2016). Linalool 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 linalool possesses anti-inflammatory, antioxidant activity and antimicrobial activity (van Zyl *et al.,* 2006). Linalool-rich essential oils have shown promising activity against *Plasmodium falciparum* (Nyiligira *et al.,* 3004; Kamatou *et al.,* 2006).

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). 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 Okocha *et al.,* 2023 and Oloruntola *et al.,* (2022). Also, present in *J. carnea* leaf ethanolic extract are a number of volatile organic compounds with yet to be identified biological activity.

Table 2 Volatile bioactive composition of *Justicia carnea* leaf ethanol extract

Peak No	Retention Time	Area (%)	Compound Name	Molecular Formula
1	6.50	0.23	Oxirane, (chloromethyl)-Decane	C_3H_5ClO
2	6.85	0.28	Benzene, 1,4-dichloro- Benzene, 1,3-dichloro-	$C_6H_4Cl_2$
3	7.20	0.26	p-Cymene o-Cymene Benzene, 1-methyl-3-(1-methylethyl)-	$C_{10}H_{14}$
4	8.16	1.07	gamma.-Terpinene	$C_{10}H_{16}$
5	8.25	0.27	Octane, 3,4,5,6-tetramethyl- Heptadecane, 2,6,10,14-tetramethyl Tridecane, 2-methyl-	$C_{12}H_{26}$ $C_{14}H_{30}$ $C_{14}H_{30}$
6	8.30	0.82	Decane, 2,6,7-trimethyl- Undecane, 3,5-dimethyl- Decane, 3-methyl-	$C_{13}H_{28}$ $C_{11}H_{24}$
7	8.53	0.55	Nonane, Decane , Tridecane, 5-methyl-	C_9H_{20} $C_{10}H_{22}$ $C_{14}H_{30}$
8	8.59	0.19	Tetradecane Decane Undecane	$C_{14}H_{30}$ $C_{10}H_{22}$ $C_{11}H_{24}$
9	8.64	0.23	Undecane, 2-methyl- Hexadecane Tetradecane, 1-iodo-	$C_{12}H_{26}$ $C_{16}H_{34}$ $C_{14}H_{29}I$
10	8.96	2.14	Heptadecane, 2,6,10,14-tetramethyl Dodecane, 2,6,11-trimethyl- Octane, 3-ethyl-2,7-dimethyl-	$C_{21}H_{44}$

				$C_{15}H_{32}$ $C_{12}H_{26}$
11	9.12	0.46	Octane, 4-ethyl- Carbonic acid, nonyl vinyl ester Pentane, 2,2,3,3-tetramethyl-	$C_{10}H_{22}$ $C_{12}H_{22}O_3$ C_9H_{20}
12	9.18	0.72	Tetradecane Undecane, 4-methyl- Tridecane	$C_{14}H_{30}$ $C_{12}H_{26}$ $C_{13}H_{28}$
13	9.27	0.78	Heptadecane, 2,6,10,14-tetramethyl Tetradecane Undecane, 2,8-dimethyl-	$C_{21}H_{44}$ $C_{14}H_{30}$ $C_{13}H_{28}$
14	9.34	0.89	Tridecane Tetradecane Tetratetracontane	$C_{13}H_{28}$ $C_{14}H_{30}$ $C_{44}H_{90}$
15	9.44	2.52	Linalool	$C_{10}H_{18}O$
16	9.69	0.67	Heptadecane, 2,6,10,14-tetramethyl Octane, 2,3,7-trimethyl- Carbonic acid, nonyl vinyl ester	$C_{21}H_{44}$ $C_{11}H_{24}$ $C12H_{22}O_3$
17	9.74	0.42	Oxalic acid, 2-ethylhexyl hexyl ester Undecane, 6-ethyl- Decane, 2-methyl-	$C_{16}H_{30}O_4$ $C_{13}H_{28}$
18	9.81	0.50	Nonane, 3-methyl- Undecane, 3,6-dimethyl- Nonane, 3,7-dimethyl-	$C_{10}H_{22}$ $C_{13}H_{28}$ $C_{11}H_{24}$
19	10.03	0.11	Undecane, 5-methyl- Octane, 3,4,5,6-tetramethyl- Undecane, 3-methyl-	$C_{12}H_{26}$
20	10.10	0.09	Octane, 2-methyl- 2,6-Dimethyldecane Undecane, 5-methyl-	C_9H_{20} $C_{12}H_{26}$
21	12.03	0.15	1-Dodecene 4-Dodecene, (Z)- 5-Dodecene, (Z)-	$C_{12}H_{24}$
22	12.26	0.44	Dodecane	$C_{12}H_{26}$
23	15.11	0.49	Tridecane Dodecane	$C_{13}H_{28}$ $C_{12}H_{26}$
24	16.47	0.13	.alpha.-Cubebene cis-muurola-3,5-diene	$C_{15}H_{24}$
25	17.19	1.34	.alfa.-Copaene Copaene	$C_{15}H_{24}$
26	17.65	2.76	.gamma.-Elemene 2,5-Octadiene 1,3-Pentadiene	$C_{15}H_{24}$ $C_{10}H_{14}$ C_5H_8
27	17.83	1.34	Tetradecane, Decane, 2,3,5-trimethyl- Tridecane	$C_{14}H_{30}$ $C_{13}H_{28}$
28	18.08	0.46	1H-Cycloprop[e]azulene, 1a,2,3,4,4 a,5,6,7b-octahydro-1,1,4,7-tetramethyl-, [1aR-(1a.alpha.,4.alpha.,4a .beta.,7b.alpha.)]- 3H-3a,7-Methanoazulene, 2,4,5,6,7, 8-hexahydro-1,4,9,9-tetramethyl-, [3aR-(3a.alpha.,4.beta.,7.alpha.)] isoledene	$C_{15}H_{24}$

29	18.25	0.17	1,3,6,10-Dodecatetraene, 3,7,11-trimethyl-, (Z,E)- (Z,Z)-.alpha.-Farnesene trans-.alpha.-Bergamotene	$C_{15}H_{24}$
30	18.36	6.27	Aromandendrene Bicyclo[7.2.0]undec-4-ene, 4,11,11 -trimethyl-8-methylene-[1R-(1R*,4 Z,9S*)]-Farnesene epoxide, E-	$C_{15}H_{24}$ $C_{15}H_{24}O$
31	18.74	0.63	Santolina triene 1,3-Cyclopentadiene, 1,2,3,4,5-pentamethyl- 1,4-Hexadiene, 5-methyl-3-(1-methylethylidene)-	$C_{10}H_{16}$
32	18.98	0.28	(1R,3aS,8aS)-7-Isopropyl-1,4-dimethyl-1,2,3,3a,6,8a-hexahydroazulene isoledene Alloaromadendrene	$C_{15}H_{24}$
33	19.25	1.90	Humulene Cyclohexene, 4-[(1E)-1,5-dimethyl-1,4-hexadien-1-yl]-1-methyl-	$C_{15}H_{24}$
34	19.36	4.34	(E)-.beta.-Famesene 1,6,10-Dodecatriene, 7,11-dimethyl -3-methylene- (E)-.beta.-Famesene	$C_{15}H_{24}$ $C_{15}H_{26}O$
35	19.48	0.68	Bicyclo[7.2.0]undec-4-ene, 4,11,11 -trimethyl-8-methylene-, [1R-(1R*,4 Z,9S*)]-Alloaromadendrene 1H-Benzocycloheptene, 2,4a,5,6,7,8,9,9a-octahydro-3,5,5-trimethyl-9- methylene-, (4aS-cis)-	$C_{15}H_{24}$
36	19.87	0.46	.gamma.-Muurolene Naphthalene, 1,2,4a,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)- 1H-Cyclopenta[1,3]cyclopropa[1,2]benzene, octahydro-7-methyl-3-methylene-4-(1-methylethyl)- [3aS-(3a.alpha.,3b.beta.,4.beta.,7.alpha.,7 aS*)]-	$C_{15}H_{24}$
37	19.98	3.74	1H-Cyclopenta[1,3]cyclopropa[1,2]benzene, octahydro-7-methyl-3-methylene-4-(1-methylethyl)-, [3aS-(3a. alpha.,3b.beta.,4.beta.,7.alpha.,7 aS*)]- Germacrene D+)-epi-Bicyclosesquiphellandrene	$C_{15}H_{24}$
38	20.11	2.07	Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-4a,8-dimethyl-2-(1-methylet henyl)-, [2R-(2.alpha.,4a.alpha.,8 a.beta.)]-cis-.alpha.-Bisabolene Aromandendrene	$C_{15}H_{24}$ $C_{15}H_{12}O_6$
39	20.35	2.97	(E,Z)-.alpha.-Farnesene Bicyclo[3.1.1]hept-2-ene, 2,6-dimethyl-6-(4-methyl-3-pentenyl)- 1,3-Cyclohexadiene, 5-(1,5-dimethy l-4-hexenyl)-2-methyl-,[S-(R*,S*)]-	$C_{15}H_{24}$ $C_{15}H_{26}$ $C_{18}H_{28}$
40	20.48	0.37	.alpha.-Muurolene Alloaromadendrene .alpha.-Muurolene	$C_{15}H_{24}$
41	20.59	0.37	8-Isopropenyl-1,5-dimethyl-cyclode ca-1,5-diene Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-4a,8-dimethyl-2-(1-methylet henyl)-, [2R-(2.alpha.,4a.alpha.,8 a.beta.)]-1,4-Pentadiene	$C_{15}H_{24}$ C_5H_8
42	20.72	10.87	(E)-.beta.-Famesene .beta.-Bisabolene (E,Z)-.alpha.-Farnesene	$C_{15}H_{24}$ $C_{15}H_{24}$

43	20.98	1.45	2,4-Di-tert-butylphenol Phenol, 3,5-bis(1,1-dimethylethyl)	$C_{14}H_{22}O$
44	21.09	7.59	Cyclohexene, 3-(1,5-dimethyl-4-hexenyl)-6-methylene-, [S-(R*,S*)]- Cyclohexene, 3-(1,5-dimethyl-4-hexenyl)-6-methylene-, [S-(R*,S*)]-	$C_{14}H_{24}$
45	21.26	0.90	(E)-1-Methyl-4-(6-methylhept-5-en- 2-ylidene)cyclohex-1-ene (E,Z)-.alpha.-Farnesene	$C_{15}H_{24}$
46	21.53	0.53	Cyclohexene, 4-[(1E)-1,5-dimethyl-1,4-hexadien-1-yl]-1-methyl- 1,3,6-Octatriene, 3,7-dimethyl-, (Z)- 1,3,6-Octatriene, 3,7-dimethyl-, (Z)-	$C_{15}H_{24}$ $C_{10}H_{16}$
47	21.75	0.37	Cyclohexanemethanol, 4-ethenyl-.alpha.,.alpha.,4-trimethyl-3-(1-methylethenyl)-, [1R-(1.alpha.,3.alpha.,4.beta.)]- Cyclohexanemethanol, 4-ethenyl-.alpha.,.alpha.,4-trimethyl-3-(1-methylethenyl)-, [1R-(1.alpha.,3.alpha.,4.beta.)]-.alpha.-Terpineol	$C_{15}H_{26}O$ $C_{10}H_{18}O$
48	21.88	1.00	Alloaromadendrene Azulene, 1,2,3,3a,4,5,6,7-octahydro-1,4-dimethyl-7-(1-methylethenyl) -, [1R-(1.alpha.,3a.beta.,4.alpha.,7.beta.)]-.gamma.-Elemene	$C_{15}H_{24}$
49	22.08	2.59	1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl- 1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl-, (E)- Nerolidol	$C_{15}H_{26}O$
50	22.45	0.19	1H-Cycloprop[e]azulen-7-ol, decahydro-1,1,7-trimethyl-4-methylene-, [1ar-(1a.alpha.,4a.alpha.,7.beta., 7a.beta.,7b.alpha.)]- 1,3-Bis-(2-cyclopropyl,2-methylcyclopropyl)-but-2-en-1-one 1-(3,3-Dimethyl-but-1-ynyl)-1,2-dimethyl-3-methylene-cyclopropane	$C_{15}H_{24}O$ $C_{18}H_{26}O$ $C_{12}H_{18.}$
51	22.53	0.79	1,3-Bis-(2-cyclopropyl,2-methylcyclopropyl)-but-2-en-1-one 3-Tetradecen-5-yne, (Z)-Cyclohexene, 3-methyl-6-(1-methylethenyl)-, (3R-trans)-	$C_{18}H_{26}O$ $C_{14}H_{24}$ $C_{10}H_{16}$
52	22.69	1.06	Cetene 1-Pentadecene Oxalic acid, allyl hexadecyl ester	$C_{16}H_{32}$ $C_{15}H_{30}$ $C_{21}H_{38}O_4$
53	22.93	0.58	Guaiol	$C_{15}H_{26}O$
54	23.18	0.58	3-Cyclohexen-1-carboxaldehyde, 3,4-dimethyl- 3,5-Dimethylcyclohex-1-ene-4-carboxaldehyde Cyclopentene, 1-pentyl-	$C_7H_{10}O$ $C_9H_{14}O$ $C_{10}H_{18}$
55	23.60	0.16	Apiol 1,3,6-Cyclooctatriene Tricyclo[3.2.1.0<2,4>]oct-6-ene,(1.alpha.,2.alpha.,4.alpha.,5.alpha.)-	$C_{12}H_{14}O_4$ C_8H_{10}
56	23.68	0.29	Aromandendrene Naphthalene, 1,2,3,5,6,7,8,8a-octahydro-1,8a-dimethyl-7-(1-methylethenyl)-, [1R-(1.alpha.,7.beta.,8a.alpha.)]-(E,Z)-.alpha.-Farnesene	$C_{15}H_{12}O_6$ $C_{15}H_{24}$

57	23.98	0.21	.tau.-Muurolol cis-.beta.-Farnesene 1H-Benzocycloheptene, 2,4a,5,6,7,8, 9,9a-octahydro-3,5,5-trimethyl-9-methylene-, (4aS-cis)-	$C_{15}H_{26}O$ $C_{15}H_{24}$
58	24.30	0.80	Naphthalene, 1,2,3,5,6,7,8,8a-octahydro-1,8a-dimethyl-7-(1-methyleth enyl)-, [1R-(1.alpha.,7.beta.,8a.a lpha.)]-Aromandendrene cis-.beta.-Farnesene	$C_{15}H_{24}$
59	24.61	0.21	Alloaromadendrene .beta.-Bisabolene 1.alpha., 4a.beta., 8a.alpha.-Decahydro-1-naphthalenol	$C_{15}H_{24}$ $C_{10}H_{18}O$
60	24.71	0.46	Naphthalene, 1,2,3,5,6,7,8,8a-octahydro-1,8a-dimethyl-7-(1-methyleth enyl)-, [1R-(1.alpha.,7.beta.,8a.alpha.)]- Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-4a,8-dimethyl-2-(1-methylethenyl)-, [2R-(2.alpha.,4a.alpha.,8a.beta.)]- Naphthalene, 2,3,4,4a,5,6-hexahydro-1,4a-dimethyl-7-(1-methylethyl)-	$C_{15}H_{24}$
61	25.07	0.79	1H-Cycloprop[e]azulene, decahydro- 1,1,4,7-tetramethyl-, [1aR-(1a.alpha.,4.beta.,4a.beta.,7.beta.,7a.beta.,7b.alpha.)]-Cyclohexanol, 2-methyl-5-(1-methylethenyl)-, (1.alpha.,2.alpha.,5.beta.)- 1,5,7-Octatrien-3-ol, 2,6-dimethyl	$C_{15}H_{26}O$ $C_{10}H_{20}O$ $C_{10}H_{16}O$
62	25.26	0.42	Naphthalene, 1,2,3,5,6,7,8,8a-octahydro-1,8a-dimethyl-7-(1-methylethenyl)-, [1R-(1.alpha.,7.beta.,8a.alpha.)]- 1H-3a,7-Methanoazulene, octahydro- 1,9,9-trimethyl-4-methylene-, (1.a lpha.,3a.alpha.,7.alpha.,8a.beta.) Alloaromadendrene	$C_{15}H_{24}$
63	27.26	0.86	1-Octadecene, Z-8-Hexadecene, 1-Nonadecene	$C_{18}H_{36}$ $C_{16}H_{32}$ $C_{19}H_{38}$
64	27.79	0.22	8-Hexadecenal, 14-methyl-, (Z)- 1,12-Tridecadiene 11-Tetradecyn-1-ol acetate	$C_{17}H_{32}O$ $C_{13}H_{24}$ $C_{16}H_{30}O_2$
65	29.76	0.22	Ethanone, 1-(4-methyl-1H-imidazol-2-yl)- 3-Hexyne, 2-methyl- 1,3-Pentadiene, 2,3-dimethyl-	$C_6H_8N_2O$ C_7H_{12} C_7H_{12}
66	30.02	0.36	1,2-Benzenedicarboxylic acid, buty l 2-ethylhexyl ester Dibutyl phthalate 1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	$C_{20}H_{30}O$ $C_{16}H_{22}O_4$ $C_{16}H_{22}O$
67	30.26	1.20	3-Heptafluorobutyroxypentadecane 1-Nonadecene E-15-Heptadecenal	$C_{17}H_{17}F_7O$ $C_{19}H_{38}$ $C_{17}H_{32}O$
68	30.43	0.12	6-(Trifluoromethoxy)-N-(trimethylsilyl)-1,3-benzothiazol-2-amine	$C_8H_5F_3N_2OS$ $C_{18}H_{18}N_4O$

			Indazol-4-one, 3,6,6-trimethyl-1-phthalazin-1-yl-1,5,6,7-tetrahydro-1H-Indole, 3-(5-methyl-2-(pyridin- 3-yl)thiazol-4-yl-	$C_{17}H_{13}N_3S$
69	31.08	0.08	1-Octadecene Z-8-Hexadecene	$C_{18}H_{36}$ $C_{16}H_{32}$
70	31.31	0.11	Phytol	$C_{20}H_{40}O$
71	31.63	0.26	Ethyl 9.cis.,11.trans.-octadecadienoate Linoleic acid ethyl ester	$C_{20}H_{36}O_2$ $C_{20}H_{36}O_2$
72	31.66	0.26	9-Oxabicyclo[6.1.0] nonane, 9,12-Octadecadienal 9-Oxabicyclo[6.1.0]nonane, cis-	$C_8H_{14}O$ $C_{18}H_{32}O$
73	31.81	0.36	1-Docosene 1-Eicosene	$C_{22}H_{44}$ $C_{20}H_{40}$
74	32.97	0.23	1-Docosene 1-Octadecene Trifluoroacetic acid, pentadecyl ester	$C_{22}H_{44}$ $C_{18}H_{36}$ $C_{17}H_{31}F_3O_2$
75	33.26	1.59	(E)-5-(Benzo[d][1,3]dioxol-5-yl)-N-isobutylpent-2-enamide 5-Piperonyl-2,4-thiazolidinedione, 1-Piperonyl-3,5-diamino-1,2,4-triazole	$C_{16}H_{21}NO_3$ $C_{13}H_{15}NO_3S.$ $C_8H_{11}N_5O.$
76	34.00	0.49	Bis(2-ethylhexyl) phthalate Diisooctyl phthalate Bis-(3,5,5-trimethylhexyl) phthalate	$C_{24}H_{38}O_4$
77	34.14	0.30	(1S,15S)-Bicyclo[13.1.0]hexadecan-2-one cis-11-Hexadecenal 2(1H)-Naphthalenone, octahydro-, trans-	$C_{16}H_{28}O_2$ $C_{16}H_{30}O$
78	34.21	0.49	Tetradecanoic acid, 2-hydroxy-, methyl ester Piperidine-4-carbonitrile Fumaric acid, 2-decyl pentadecyl ester	$C_{15}H_{30}O$ $C_6H_{10}N_2$ $C_{29}H_{54}O$
79	34.69	3.70	1-Piperonyl-3,5-diamino-1,2,4-triazole 1,2-Dipiperonyl-3-imino-1,2,4-triazine 3,4-Methylenedioxyphenyl acetone	$C_{10}H_{11}N_5O_2$ $C_{14}H_{18}N_4O_2$ $C_{10}H_{10}O_3$
80	34.88	8.32	1-Piperonyl-3,5-diamino-1,2,4-triazole 1-Formyl-1-piperonylhydrazine Benzene, isothiocyanato-	$C_{10}H_{12}N_4O$ $C_{11}H_{14}N_2O$ C_6H_5NCS
81	35.66	0.41	E-1,9-Hexadecadiene Tricyclo[3.3.1.1(3,7)]decane-2,6-dione, 4-acetyl-Z-5-Octadecen-1-ol acetate	$C_{16}H_{30}O$ $C_{12}H_{14}O$ $C_{20}H_{38}O_2$
82	35.84	1.79	Tricyclo[4.2.0.0(2,4)]octan-5-one, (1.alpha.,2.beta.,4.beta.,6a)- Ethyl n-propyl sulfone Methallyl cyanide	$C_8H_{10}O$ $C_5H_{12}O_2S$ C_5H_7N
83	36.01	0.68	Piperine 9-Chloro-bicyclo[6.1.0]nona-1(8),2 ,2,4,6-tetraene	$C_{17}H_{19}NO_3$ C_9H_9Cl
84	36.18	0.94	Furo[3,4-c]pyridine-3,4(1H,5H)-dione, 6-methyl-	$C_{11}H_7NO_2.$

			Benzamide, 3,4-dimethoxy-N-[2-(3,4 -dihydroxybenzylidenhydrazino)-2-o xoethyl]- 2-{2-Cyano-3-[(3,4-dimethoxyphenyl) carbonyl]oxiran-2-yl}propanedinitrile	$C_{19}H_{21}N_3O_5$. $C_{12}H_{11}N_3O_3$
85	36.45	1.45	Benzamide, N-(4-cyanomethylphenyl) -3,4-dimethoxy- Butan-1-one, 1-(3,4-dimethoxypheny l)- 3,4-Dimethoxybenzoyl chloride	$C_{17}H_{18}N_2O_3$. $C_{13}H_{18}O_3$. $C_{10}H_{11}ClO_3$
86	37.41	-0.15	Pyrrolidine, 1-[5-(1,3-benzodioxol-5-yl)-1-oxo-2,4-pentadienyl]-, (E,E)- 9-Chloro-bicyclo[6.1.0]nona-1(8),2,4,6-tetraene Glutaric acid, hexyl 2-methylbutyl ester	$C_{17}H_{15}NO_3$. C_9H_9Cl $C_{16}H_{30}O_4$

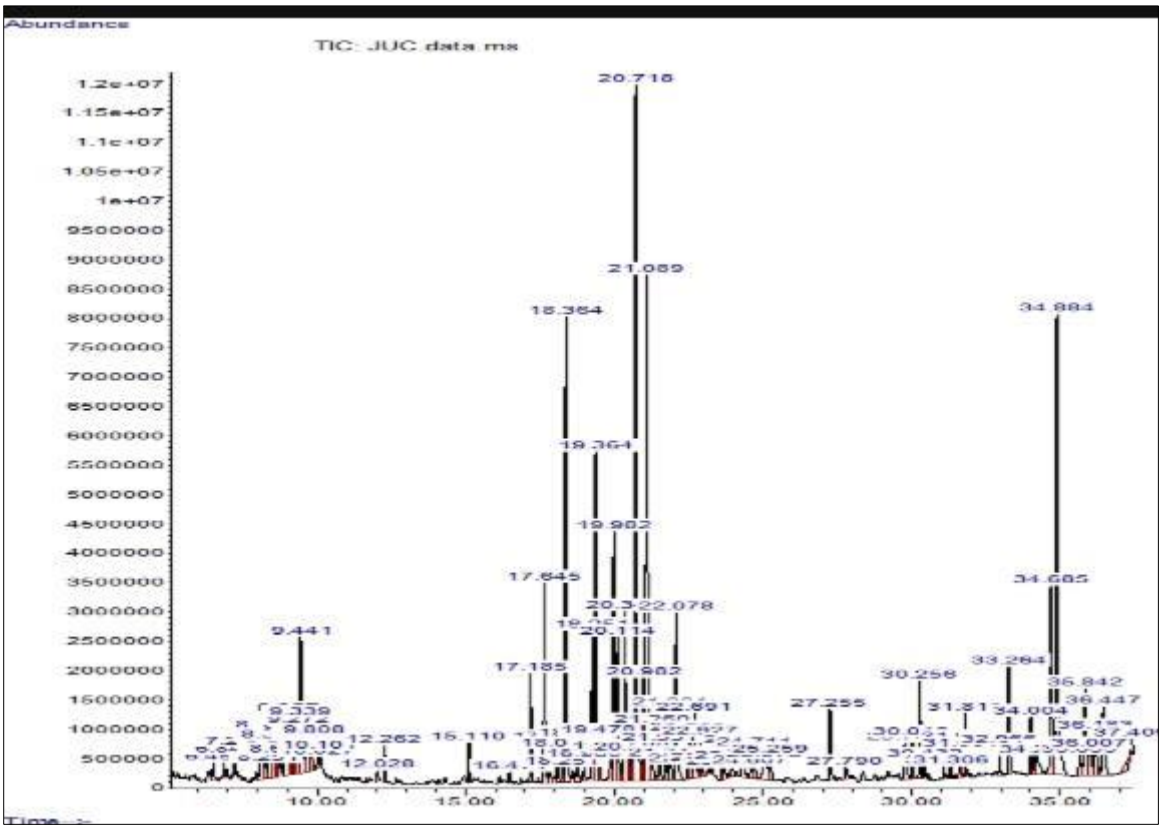

Figure 1 GC-MS chromatogram of *Justicia carnea* leaf ethanol extract (X-axis represent time (minutes) and Y-axis represent abundance (mAU).

4. **Conclusion**

The present study has identified valuable volatile bioactive compounds in the ethanol extract of *Justicia carnea* leaf. The phytochemicals have been demonstrated in various studies to mediate beneficial pharmacological properties, such as antimicrobial, anti-inflammatory, anticancer and antioxidant effect through their interactions with various biochemical pathways. The presence of this compound may be an indicator of its usefulness in traditional medicine regimen to treat a number of diseases. However, care must be taken as excessive consumption of the plant may pose health risk, due to the presence of toxic chemical groups in the extract.

Compliance with ethical standards

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Disclosure of conflict of interest

Authors have declared that no conflict of interest exists in the work.

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