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Evaluation of volatile and bioactive compounds of ethanol leaf extracts of *Justicia secunda* using GC-MS and GC-FID analysis.

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Abstract

The study evaluated the volatile and bioactive constituents of ethanol extracts of *Justicia secunda* using gas chromatography-mass spectrometry (GC-MS) for volatile composition while bioactive compound were assessed using gas chromatography flame ionization detector (GC-FID). The leaf extracts were prepared by being collected, air-dried, macerated in 96% ethanol, filtered and concentrated at 450C. The result obtained revealed the presences of bioactive compound such as flavonoid that include: Spartein (28.10 µg/ml), Ruti (15.6 µg/ml), Oxalate (12.70 µg/ml), Naringin (12.4 µg/ml), Steroids (11.7 µg/ml), Epinedrine (11.10 µg/ml), Cardiac glycoside (10.10 µg/ml), Flavonone (10.10 µg/ml), Ribalinidine (9.30 µg/ml), Anthocyanin (7.70%), Flavone (7.30 µg/ml), Sapogenin (7.30µg/ml), Proanthocyanin (6.90 µg/ml),Resveratol (6.70µg/ml), Epicatechin (5.70 µg/ml), Phytate (5.70µg/ml), Kaempferol (3.90 µg/ml),Catechin (2.00 µg/ml), Tannin (2.00 µg/ml). The GC-MS Screening of the plant's extract identified eighty-two (82) bioactive compounds, which include Ethyl palmitate (5.54%), 2,4-Di-tert-butylphenol (4.04%),9,17-Octadecadienal, (4.05%), Linoleic acid ethyl ester (4.05%), 9,12-Octadecadienoic acid, ethyl ester (4.00%), Decane, 2-methyl (3.82%), Hexadecane (3.82%), Heptadecane, 2,6-dimethyl (3.26%), N-(3-methylButyl) acetamide (3.02%), Sulfurous acid, butyl octyl ester (3.25%), Hexane,1-(hexyloxy)-5-(3.06%) as the major bioactive compounds. The result of the GC-MS analysis showed that the ethanol extract of *Justicia secunda* contains many pharmacologically important bioactive compounds such as antioxidant, anti-inflammatory, anti-microbial, anti-diabetic anti-cancer effects. Traditionally, *Justicia* species are used in the treatment of inflammation, rheumatism, arthritis gastrointestinal diseases, anaemia and respiratory tract infection. The proximate analysis of the ethanol extract of the leaves revealed notable levels of various components, where carbohydrates were found to be the highest, followed by moisture content, protein, fats, fiber, and ash content, respectively.

Keywords: Phytochemicals; GC-MS; GC-FID; Volatile components; *Justicia secunda*

1. Introduction

Justicia secunda Vahl, an important and largest species in the *Acanthaceae genus* comprising approximately 600 species. It is a creeping perennial plant that can grow up to 1 to 1.5 meters in height [1]. *Justicia secunda* is prevalent in tropical and subtropical zones of Madagascar, Asia, the West Indies, and Africa [2]. This plant is known as "bloodroot" in Barbados "sanguinaria" in Venezuela, and "hounsiman" in Benin[3], it is recognized for its medicinal properties, often referred to as "blood leaf" or "blood tonic" in parts of Nigeria [4]. *J. secunda* possess edible, medicinal, and economical values. Different parts of the *Justicia* plant, particularly the leaves, are commonly used in traditional remedies in the treatment of anaemia, inflammation, fever, diarrhea, liver diseases, arthritis and respiro-gastrointestinal disorder [5] [6]

Justicia secunda plant has been recognized for its anti-sickling, anti-inflammatory, immunomodulatory, nephroprotective, antitumoral, hepatoprotective, anti-platelet aggregation, antimicrobial, antiviral, superoxide anion

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radical scavenging, and hematinic [7] [8] [9]. Phytochemical analyses of *J. secunda* leaf extract has identified various bioactive compounds, contributing to its pharmacological potential, the plant play a crucial role in traditional medicine and nutrition, serving as both therapeutic remedies and dietary supplements because of the presence of phytoconstituents in the plant. [10] [11]. The most important plant bioactive are alkaloids, tannins, flavonoids, terpenes, and phenolic chemicals [12]. Due to the high cost of orthodox medication, many people worldwide use traditional medicine. Phytopharmaceuticals, found in many plants, are significant in agriculture, human, and veterinary medicine which probably increased the interest in studying and understanding the components of plants. This is also very important in identifying new sources of important compounds like flavonoids, alkaloids, saponins, steroids, phenolic compounds, triterpenoids and coumarin etc. that are therapeutically and industrially important.

The interest is focused in obtaining biologically active molecules that could be used in curing various diseases, we hereby present GC-MS analysis of ethanol extract of *J. secunda*.

2. Materials and Methods

2.1. Plant Materials

Fresh leaves of *Justicia secunda* were harvested at Obinze town in Imo State, Nigeria. The plant leaves were identified at the Taxonomy section of School of Agriculture and Agricultural Technology in the Department of Forestry and Wildlife Technology at the Federal University of Technology, Owerri (FUTO), Imo State, Nigeria.

2.2. Preparation of Plant Extract

Ethanol extraction of *Justicia secunda* was carried out using ethanol as the extracting solvents. Hundred grams (100 g) of the plant powders was macerated in 98% ethanol. The extracts were filtered using Whatman No. 1 filter paper and concentrated using a Rotary evaporator (BUCHI R 200; Labortechnik) set at 45 °C. Dried extract was sent for GC-MS analysis.

2.3. GCMS Analysis of *Justicia secunda*

The characterization of the bioactive compounds in *Justicia secunda* were done using GC-MS QP2010 Plus (Shimadzu, Japan). The identification of the phytochemicals in the sample was carried out using aQP2010 gas chromatography with Thermal Desorption System, TD 20 coupled with Mass Spectroscopy (Shimadzu). The ionization voltage used in GC-MS spectroscopic detection was 70eV electrons. This was conducted in the initial temperature of 80°C for 1min, and then increased linearly at 70 °C min-1 to 220 °C, held for 3 min. the temperature rose to 290 °C for 10 min. The temperature of the injection port was 290 °C and the GC-MS interface was maintained at 290 °C. The sample was introduced via a helium carrier gas flow at the rate of 1.2 ml min-1 through an all-glass injector working in the split mode. The identification of compounds was accomplished by comparison of retention time and fragmentation pattern, as well as with mass spectra of the GC-MS. Extract component in percentage was calculated using chromatogram peak area.

2.4. Identification of chemical components in *Justicia secunda*

The retention indices, peak area percentage and mass spectra fragmentation pattern of GC-MS chromatogram of ethanol extract of *Justicia secunda* was compared with the database of National Institute of Standards and Technology (NIST), NIST08.LIB [13], WILEY8.LIB [14] and with published literature.

2.5. GC-FID characterization

Crude extracts of *Justicia secunda* were assayed to determine the functional groups present and the results are depicted in Figure 2. The phytochemical analysis was performed on a BUCK M910 gas chromatography equipped with a flame ionization detector. A RESTEK 15 meter MXT-1 column (15 m x 250 um x 0.15 um) was used. The injector temperature was 280 ^{o}C with splitless injection of 2 ul 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 ^{o}C, it was heated to 330 ^{o}C at a rate of 30 ^{o}C min-1 and was kept at this temperature for 5 min. the detector operated at a temperature of 320 ^{o}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 [15].

2.6. 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.

2.7. Determination of Proximate Composition

The proximate analyses of *Justicia secunda* were conducted following the standard methods outlined by the Association of Official Analytical Chemists [16]. All analyses were performed in triplicate, and the results were expressed as mean ± standard deviation.

2.7.1. Moisture Content

Moisture content was determined according to AOAC method 14:004. Samples were dried to a constant weight at 100°C, and moisture was calculated as the loss in weight of the dried samples using the formula: % moisture=W2−W3W2−W1×100% moisture=W2−W1W2−W3×100 where:

- W1W1 = Initial weight of empty crucible
- W2W2 = Weight of crucible + sample before drying
- W3W3 = Final weight of crucible + sample after drying

2.7.2. Crude Ash Determination

Total ash content was determined by furnace incineration using AOAC method 14:006. Approximately 1.0 g of finely ground dried sample was ashed at 600°C, and the percentage ash content was calculated as:

% Ash=Weight of Ash×100Weight of original sample% Ash=Weight of original sampleWeight of Ash×100

2.7.3. Crude Fibre

Crude fibre was determined following AOAC method 14:020, and the percentage crude fibre was calculated as:

% crude fibre=Weight after drying×100Weight of sample% crude fibre=Weight of sample Weight after drying×100

2.7.4. Crude Fat

Total fat was determined using Soxhlet extraction for 4 hours with methanol and ethanol, respectively. The percentage fat content was calculated as:

% fat=Weight of fat×100Weight of sample% fat=Weight of sampleWeight of fat×100

2.7.5. Crude Protein Content

The crude protein content was determined using the Microkjeldah method of AOAC, and the percentage crude protein was calculated as: % crude protein=% N×F% crude protein=% N×F where FF (conversion factor) is equivalent to 6.25.

2.7.6. Total Carbohydrate

Total carbohydrate content was determined by the difference method, and the percentage was calculated as:

Total carbohydrate (%)=100−(moisture+lipids+ash+crude protein+fibre)Total carbohydrate (%)=100−(moisture+lipids+ash+crude protein+fibre)

3. Results

GC-FID analysis indicated the presence of alkaloids, flavonoids, steroids, tannins, saponins, and phenols. Also found were proanthocyanin, anthocyanin, naringenin, flavonones, flavone, rutin, kaempferol, flavones flavanones, catechin, epicatechin, triterpenoid aglycone (sapogenin), spartein, ribalinidine and cardiac glycosides, this was also reported by [17], this is shown in table 3.1. Anti-nutrients such as oxalates, phytates and tannins were found in the plant also as seen in table 3.2. The proximate components of the *Justicia secunda* leaf extracts were identified and shown in table 3.4

Table 1 Quantitative phytochemical components and biological activity of some phytocompounds of *Justicia secunda* leaf extract

Components	Quantitative values	Qualitative tests
Proanthocyanin	6.90	++
Naringin	12.43	+++
Cardiac glycoside	10.15	+++
Anthocyanin	7.70	++
Ribalinidine	9.30	+++
Naringenin	3.11	+
Spartein	28.66	+++
Rutin	15.68	+++
Flavonones	10.18	+++
Steroids	11.72	+++
Kaempferol	3.98	+
Epicatechin	5.73	++
Flavone	7.36	++
Catechin	2.06	+
Resveratrol	6.71	++
Sapogenin	7.40	++
Epihedrine	11.19	+++

+++ – present in a high amount; ++ – a moderately high amount.; + – present in a low amount; Results are expressed as mean ±standard deviation (SD) of three replicates

Table 2 Some anti-nutrients composition of *Justicia secunda* leaves

Components	Quantitative values	Qualitative tests
Phytate	5.75	++
Oxalate	12.74	+++
Tannin	2.00	+

+++ – present in a high amount; ++ – a moderately high amount; + – present in a low amount; Results are expressed as mean ±standard deviation (SD) of three replicates.

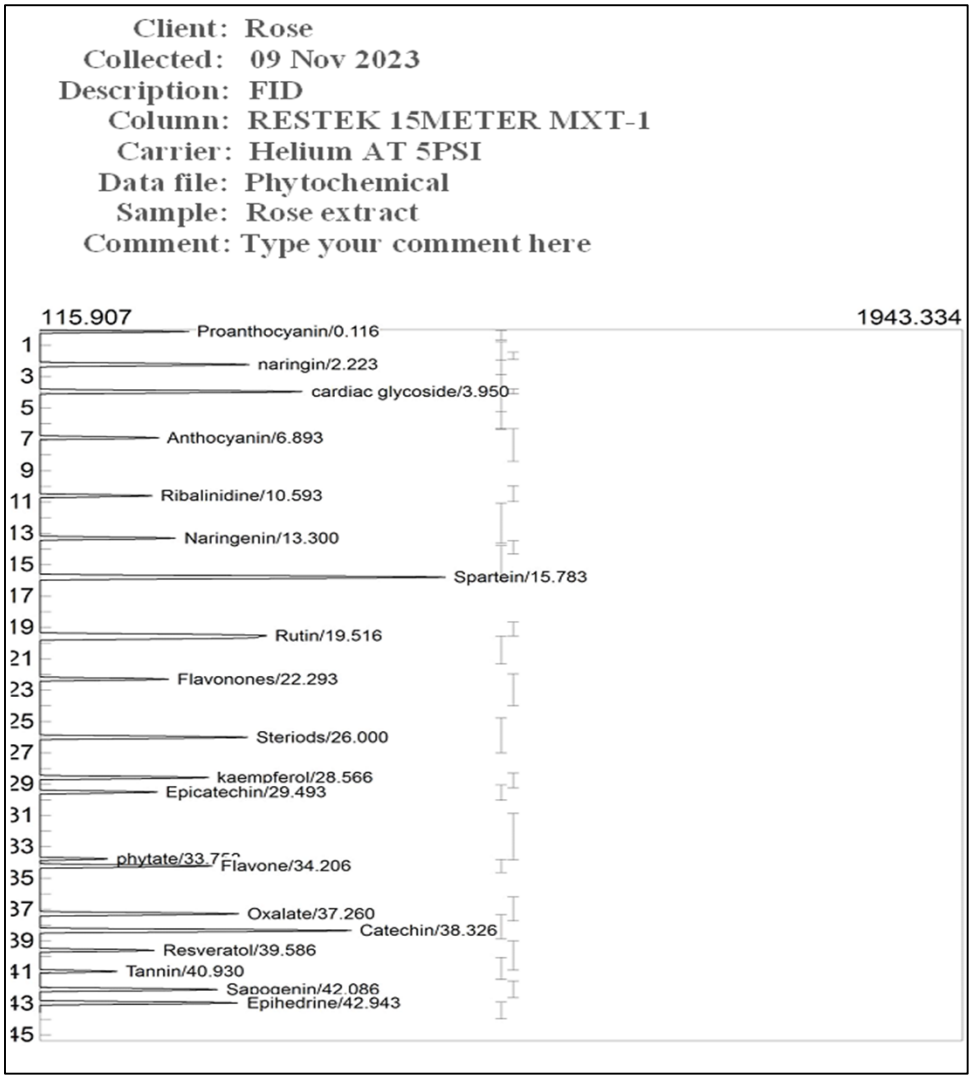

Figure 1 Chromogram of quantitative phytochemical analysis of *Justicia secunda* using GC-FID analysis

Table 3 Volatile bioactive composition Present in Ethanol Leaf Extract of *Justicia seconda* by GC-MS Analyses

Peak No	Retention Time	Area%	Library ID	Molecular Formula	Molecular Weight (g/mol)
1	5.547	0.33	Methylene chloride	CH_2Cl_2	84.93
2	5.723	0.23	Benzene,1,2,3-trimethyl	C_9H_{12}	120.192
3	6.496	0.87	Decane	$C_{10}H_{22}$	142.286
4	6.774	0.34	2-dimethyl-propionamide	$C_5H_{11}NO$	101.15
5	6.846	1.27	Benzene,1,4-dichloro	$C_6H_4Cl_2$	147.00
6	6.901	0.81	2,2-Dichloroethanol	$C_2H_4Cl_2O$	114.96
7	7.132	0.50	Acetic acid, chloro-, 1,1-dimethyl ethyl ester, N-(2,2-Dichloro-1-hydroxy-ethyl)-2,2-dimethyl-propionamide	$C_6H_{11}ClO_2$ $C_7H_{13}Cl_2NO_2$	150.601 214.093

8	7.199	0.70	p-Cymene, o-Cymene, Benzene,1-methyl-3-(1-methylethyl	$C_{10}H_{14}$ $C_{10}H_{14}S$	134.21 166.29
9	7.944	0.58	Pentane, 3-ethyl-2,4-dimethyl- Undecane,5,6-dimethyl- Octane,4-ethyl-	C_9H_{20} $C_{10}H_{22}$	128.2551 142.28
10	8.112	3.25	Sulfurous acid, butyl octyl ester	$C_{18}H_{38}O_3S$	250.40
11	8.159	1.38	gamma.-Terpinene-3-Carene	$C_{10}H_{16}$	136.23
12	8.380	2.29	Decane, 3,4-dimethyl Dodecane, 4,6-dimethyl	$C_{12}H_{26}$	170.335
13	8.527	3.02	N-3-methylButyl acetamide	$C_7H_{15}NO$	129.20
14	8.593	0.57	Oxalic acid, isobutyl nonyl ester	$C_{15}H_{28}O_4$	272.38
15	8.700	3.06	Hexane,1-hexyloxy-5-methyl	$C_{13}H_{28}O$	200.36
16	8.787	1.40	alpha-copaene		268.18
17	8.911	1.72	Undecane, 2,10-dimethyl	$C_{13}H_{28}$	184.361
18	8.959	3.26	Heptadecane, 2,6-dimethyl	$C_{15}H_{32}$	268.521
19	9.039	1.19	Carbonic acid, nonyl vinyl ester Heptane, 2,6-dimethyl	$C_{12}H_{22}O_3$ C_9H_{20}	214.30 128.255
20	9.119	1.42	Nonane, 4-methyl	$C_{10}H_{22}$	142.281
21	9.176	2.23	Dodecane Tridecane	$C_{12}H_{26}$ $C_{13}H_{28}$	170.33 184.36
22	9.270	2.31	Heptadecane, 2,6,10,14-tetramethyl, 2-Ethylhexyl mercaptoacetate Dodecane, 2,7,10-trimethyl-	$C_{21}H_{44}$ $C_{10}H_{20}O_2S$	296.5741 204.33
23		3.82	Decane,2-methyl Hexadecane	$C_{11}H_{24}$ $C_{16}H_{34}$.	156.30 226.448
24	9.541	1.06	2-Ethylhexyl mercaptoacetate	$C_{10}H_{20}O_2S$	247.347
25	9.636	1.01	1-Iodo-2-methylnonane	$C_{10}H_{21}I$	268.18
26	9.693	1.29	Carbonic acid, nonyl vinyl ester Ether, hexyl pentyl	$C_{11}H_{24}O$	214.30
27	9.737	0.88	Oxalic acid, isobutyl nonyl ester	$C_{15}H_{28}O_4$	272.38
28	9.807	2.48	Nonane, 3-methyl	$C_{16}H_{32}O_2$	142.2817
29	9.916	1.27	Heptane,2,2,3,3,5,6,6Hepta methyl- Oxalic acid	$C_{14}H_{30}$	198.39
30	10.024	1.27	2,6-Dimethyldecane	$C_{12}H_{26}$	170.33
31	10.098	2.00	Hexatriacontane	$C_{36}H_{74}$	507.0
32	10.156	1.48	Humulene	$C_{15}H_{24}$	204.35
33	11.771	0.38	Naphthalene, Azulene	$C_{10}H_8$	128.17
34	12.026	0.45	Cyclopropane	C_3H_6	42.08
35	12.261	2.19	Dodecane, 2,6,11-trimethyl	$C_{17}H_{34}$	
36	15.109	1.24	4-Tetradecene	$C_{14}H_{28}$	196.37
37	17.182	0.41	2-3,3-Dimethyl-but-1-ynyl-1,1,3-trimethyl-cyclopropane, Hexadecane,2,6,11,15-tetramethyl-Oxalic acid, allyl nonyl ester	$C_{12}H_{20}$	164.29

38	17.628	1.61	1-Hexadecanol	$C_{16}H_{34}O$	242.44
39	17.828	1.14	Tetradecane, Tridecane	$C_{14}H_{30}$	
40	18.345	1.59	Caryophyllene, Bicyclo-undec-4-ene,4,11,11-trimethyl-8-methylene- Dodecatetraene	$C_{15}H_{24}$	204.3511
41	19.243	0.27	1,3,6-Octatriene, 3,7-dimethyl-,	$C_{10}H_{16}$	136.2340
42	19.348	0.60	Beta.-Famesene	$C_{15}H_{24}$	204.35
43	19.433	0.04	Heptadecane, 2,6,dimethyl	$C_{19}H_{40}$	268.52100
44		0.49	Salpha.,beta.,beta.,alpha.- -epi-Bicyclosesquiphellandrene	$C_{15}H_{24}$ $C_{15}H_{24}$	204.35 204.35
45	20.339	0.49	Spiro2,2pentane-1-carboxylic, acid beta,alpha.-Farnesene	$C_6H_8O_2$ $C_{15}H_{24}$	112.13 204.35
46	20.409	0.81	Pentadecane	$C_{15}H_{32}$	212.41
47	20.678	2.52	Beta.-Bisabolene, Beta.-Famesene	$C_{15}H_{24}$	204.356
48	20.975	4.04	2,4-Di-tert-butylphenol Phenol, 3,5-bis1,1-dimethylethyl	$C_{14}H_{22}O$	206.32
49	21.057	2.47	Cyclohexene, 3-1,5-dimethyl-4-hex	$C_{15}H_{24}$	204.3511
50	22.073	0.55	Nerolidol	$C_{15}H_{26}O$	222.37
51	22.688	1.93	4-Heptafluorobutyryloxyhexadecane	$C_{20}H_{33}F_7O_2$	438.5
52	22.858	0.28	10-Methylnonadecane	$C_{20}H_{42}$	282.5
53	24.729	0.22	Aromandendrene Neoisolongifolene, 8-bromo-	$C_{15}H_{24}$ $C_{15}H_{23}Br$	204.35 283.25
54	27.255	2.22	E-14-Hexadecenal	$C_{16}H_{30}O$	238.41
55	28.803	0.03	2-methyl-Glutaric acid	$C_6H_{10}O_4$	146.14
56	29.148	0.31	Piperine	$C_{17}H_{19}NO_3$	
57	29.250	0.33	3-Isothiazolone	C_3H_3NOS	101.13
58	30.017	0.95	Di-sec-butyl phthalate1,	$C_{16}H_{22}O_4$	278.34
59	30.283	5.54	Ethyl palmitate	$C_{18}H_{36}O_2$	284.5
60	30.324	0.61	4-Amino-3-hydroxybutanoic acid	$C_4H_9NO_3$	119.12
61	30.432	0.40	Acetamide	CH_3CONH_2	59.07
62	30.561	2.24	Heptanoic acid, 2-acetyl-, ethyl ester	$C_{11}H_{20}O_3$	200.2747
63	31.150	0.39	Palmitoleic acid	$C_{16}H_{30}O_2$	254.41
64	31.178	4.05	9,17-Octadecadienal	$C_{18}H_{32}O$	264.4
65	31.190	0.23	9-Oxabicyclo-6.1.0,nonane,	$C_8H_{14}O$	126.1962
66	31.629	4.05	Linoleic acid ethyl ester	$C_{18}H_{32}O_2$	308.5
67	31.501	4.00	9,12-Octadecadienoic acid,ethyl ester	$C_{20}H_{36}O_2$	
68	31.662	3.35	9-Octadecenoic acid, ethyl ester Ethyl Oleate	$C_{20}H_{38}O_2$	310.5
69	31.816	1.84	Trichloroacetic acid, 3-tetradecyl Ester	$C_{15}H_{27}Cl_3O_2$	345.7
70	32.432	0.20	Cyclopentaneundecanoic acid	$C_{16}H_{30}O_2$	254.41
71	32.609	1.03	Tetrahydrofuran,2-ethyl-5-methyl	$C_8H_{14}O$	114.1855

72	32.969	0.71	4-Trifluoroacetoxytetradecane	$C_{16}H_{29}F_3O_2$	424.4
73	33.328	0.80	3-Heptafluorobutyroxypentadecane	$C_{19}H_{31}F_7O_2$	310.39
74	33.581	0.16	Hexacosanoic acid	$C_{26}H_{52}O_2$	396.7
75	34.003	1.39	Bis(2-ethylhexyl) phthalate	$C_{24}H_{38}O_4$	390.6
76	34.220	2.83	Fumaric acid,2-decyl hexadecyl ester	$C_{23}H_{42}O_4$	452.7
77	34.884	1.48	-5-Benzo,1,3,dioxol-5-yl)-1-(piperidin-1-yl)pent-2-en-1-one	$C_{17}H_{21}NO_3$	287.353
78	34.992	0.18	cis-Vaccenic acid	$C_{18}H_{34}O_2$	282.5
79	35.373	1.60	7H-Purine, 7-benzyl-2,6-dichloro-	$C_{12}H_8Cl_2N_4$	279.12
80	35.608	1.79	2,3-Dibromopentane	$C_5H_{10}Br$	229.94
81	35.764	1.79	Cycloeucalenyl acetate	$C_{32}H_{52}O_2$	468.75
82	35.880	0.49	9-Eicosenoic acid	$C_{20}H_{38}O_2$	310.5

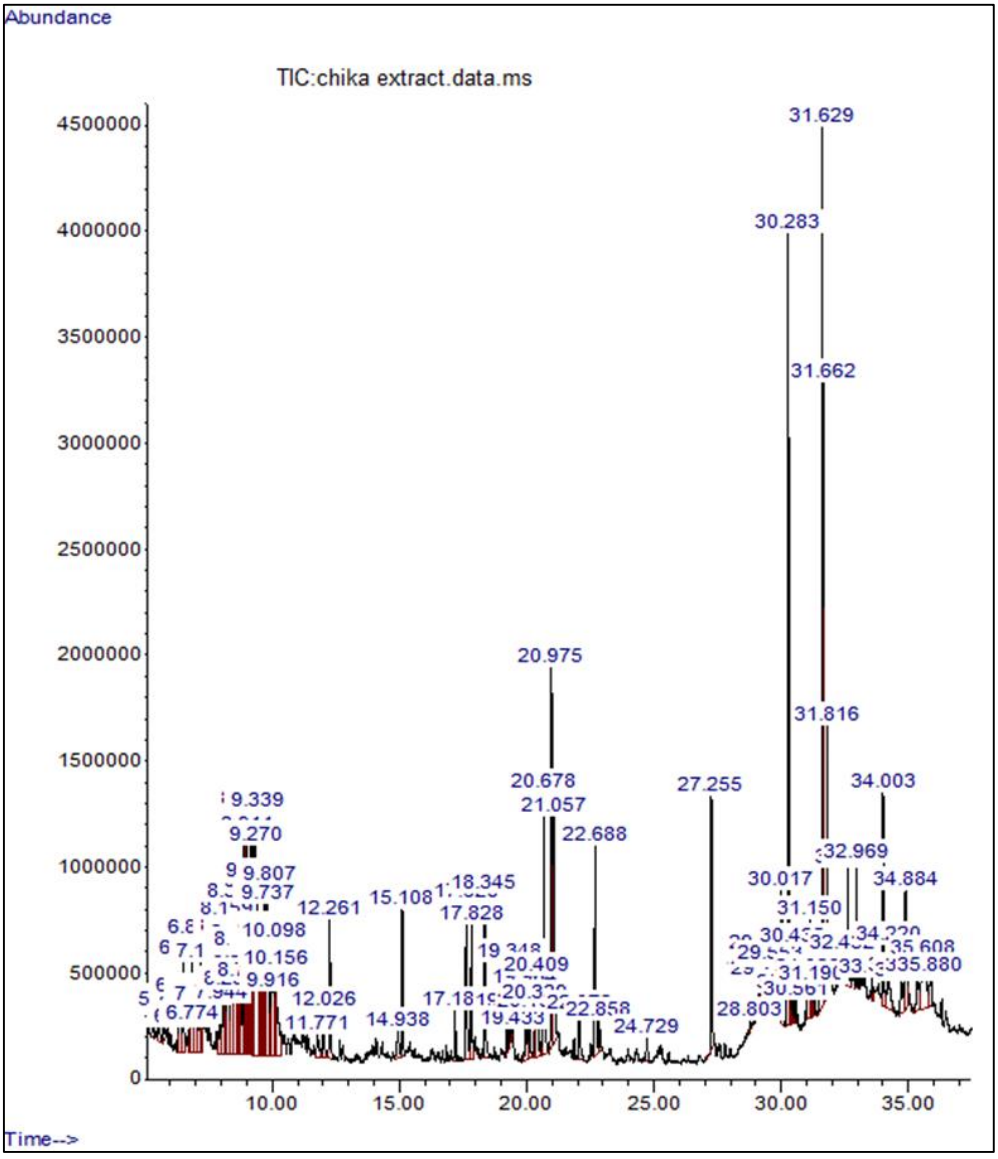

Figure 2 GC-MS Chromatogram of the *Justicia secunda* Ethanol Leaf Extract

Table 4 Proximate contents of *Justicia secunda*

Parameters	Percentages (%)
Carbohydrates	59.23±0.00
Moisture	13.92 ±0.00
Protein	11.20±0.00
Fat	6.96±0.00
Fibre	4.43±0.00
Ash	4.26±0.00

The proximate contents in Table 3.4 show that ethanol extract of *Justicia secunda* leaves contains high carbohydrate (59.23%),moisture content (13.92%) and protein contents (11.20%), Moderate amount of Fat content (6.96%), while Fibre content (4.43%)and Ash content (4.26%) were found to be low.

4. Discussion

Thus, phytochemical screening serves as the initial step in predicting the types of potentially active compounds present plant samples. These abundant phytochemicals found in the ethanolic leaf extract of *Justicia secunda* possess several pharmacological, antioxidant, immunological and antimicrobial actions, enzyme and hormone modulation, reduction of platelet aggregation and anti-cancer properties [18] [19]. Phytochemicals, mainly alkaloids, lignans, flavonoids, and terpenoids (iridoids, diterpenoids, and triterpenoids), have been reported to be found in many species of *Justicia* [17] [120]. Flavonoids have been reported to have antibacterial, antiviral actions and also possess antioxidant capacity because they neutralize free radicals, which attack most cells in the body, thereby counteracting diseases such as cancer, heart disease and even aging [21] [22] The flavonoids identified in this present study include: proanthocyanin, anthocyanin, naringenin, flavonones, flavone, rutin, and kaempferol. Rutin, (15.6 μg/ml had the highest concentration while kaempferol (3.9 μg/ml) was the lowest concentration (Table 1). Rutin being the most abundant flavonoid, is known for its antioxidant, cytoprotective, vasoprotective, cardioprotective, neuroprotective, and anti-carcinogenic properties [23]. Naringenin exhibits benefits in managing cancer, cardiovascular diseases, and osteoporosis, along with reducing oxidative stress and inflammation, flavonoids have also been reported to modulate key elements in cellular signal transduction pathways associated with apoptosis, angiogenesis, inflammation, and metastasis, thereby inhibiting cancer cell growth and angiogenesis [21]. Flavones and flavanones, two important subclasses of flavonoids, exhibit diverse biological activities. Flavones are commonly found in leaves, flowers, fruits, celery, parsley, and red peppers, while flavanones are abundant in citrus fruits like lemons, grapes, and oranges [18]. Flavones possess antimicrobial and antifungal properties and can interact with proteins like human serum albumin for easy transportation through plasma [24]. Flavanones, on the other hand, display antioxidant, antihyperlipidemic, and anti-inflammatory properties. Kaempferol, (3.9 μg/ml) was the lowest concentration (Table 1). Naringenin has been shown to be beneficial in the management of cancer, cardiovascular diseases and osteoporosis [25]. Recently, it has been shown to cause a significant reduction in the accumulation of collagen fibres in liver injury in rats [26], and it has the ability to reduce oxidative stress and possess anti-hyperlipidaemia, antioxidant, antidepressant, anti-inflammatory and anti-diabetic properties [27] [28]. Catechin and epicatechin, also found in the ethanolic extract of *Justicia secunda* leaves, offer numerous health benefits. Catechins, exhibit anti-obesity, anticancer, hepatoprotective, antidiabetic, and neuroprotective effects. Epicatechins, primarily found in green and black tea, possess cardioprotective, antioxidant, anti-diabetic, and anti-cancer activities. Epicatechin-rich green tea has been shown to inhibit platelet aggregation and enhance insulin sensitivity [29]. Saponins, a naturally occurring compounds found in plants and some marine animals, contain either a steroid or triterpenoid aglycone (sapogenin). Triterpenoid saponins are particularly common and are found in legumes like soybeans, beans, and peas, as well as oats, ginseng, yams, and tomato seeds. These compounds exhibit a range of pharmacological effects, including immunomodulatory, anti-inflammatory, antifungal, antiviral, antibacterial, hypercholesterolemic, and anticarcinogenic properties, making them important in human and animal nutrition [30]. The leaves of *Justicia secunda* were found to contain abundant amounts of sapogenin, which could serve as a natural source of these pharmacological actions. Plant alkaloids represent a diverse group of natural compounds with varied structures and biogenetic origins. They exhibit a wide range of pharmacological activities and have been utilized in numerous herbal remedies throughout history. Examples include narcotic analgesics like morphine and codeine, as well as compounds with potent antimalarial, antimicrobial, and antiprotozoal properties. The ethanolic extract of *Justicia secunda* leaves were found to contain significant amounts of spartein and ribalinidine, with spartein being the most abundant (28.6 µ/ml), followed by ribalinidine (9.3 µ/ml). Ribalinidine has been reported to possess free radical

scavenging properties, indicating potential antioxidant effects [31] Alkaloids contribute to the pharmacological properties attributed to *Justicia secunda* leaves, such as analgesic, anti-spasmodic, anti-cancer, and bactericidal effects, as reported by Saxena, *et al.,* 2013). Cardiac glycosides, classified as steroids, exert potent and specific effects on heart muscles, making them valuable in the treatment of congestive heart failure. Even in small amounts, they can promote healing in diseased hearts without increasing oxygen consumption [33]. By enhancing cardiac contraction without compromising oxygen utilization, they improve the pumping efficiency of the myocardium, enabling it to meet the demands of the cardiovascular system. In contrast to nutrients that offer health benefits, anti-nutrients hinder mineral absorption and are generally considered detrimental, although some may have health-promoting properties. Their interference with nutrient absorption can lead to various symptoms such as headaches, rashes, nausea, and nutritional deficiencies [34]. Anti-nutrients may bind to essential micronutrients, preventing their absorption, or inhibit the activity of digestive enzymes, impairing food breakdown. These compounds, often organic or synthetic, possess high reactivity and can exert toxic effects. Oxalates and phytates are two well-known anti-nutrients identified in the ethanol extract of *Justicia secunda* leaves, with concentrations of 12.7 µ/ml and 5.7 µ/ml, respectively, as shown in table 2. Phytates, present in nuts, seeds, whole grains, and roots, have been implicated in insulin secretion and suggested to inhibit plaque formation and lower serum cholesterol and triglycerides. However, ingestion of excessive phytic acid can reduce the bioavailability of essential minerals like magnesium, calcium, zinc, and iron, this was reported by Omimawo & Akubor, 2012. Oxalates, found in cruciferous vegetables, spinach, soybeans, black pepper, and chocolate, interfere with calcium absorption and utilization, leading to the formation of calcium oxalate crystals and kidney stones. Additionally, they can cause irritation and swelling in the mouth and throat, as well as arthritis-like symptoms [36]. High oxalate consumption may result in hyperoxaluria, increasing the risk of kidney stone formation and impairing calcium's essential functions in the body, such as bone health, enzyme reactions, clotting, and nerve impulse transmission [37]. Tannins, predominantly water-soluble polyphenols, are commonly found in various plant foods like tea, cocoa, vegetables, legumes, and certain unripe fruits. The ethanolic extract of *Justicia secunda* leaves was noted to contain relatively low levels of tannins (2.0 µg/ml), as indicated in Table 1. In Asian traditional medicine, tannin-containing plant extracts have been utilized for their astringent and diuretic properties, along with their applications in diarrhea, gastrointestinal ulcers, and tumors treatment. Additionally, tannins exhibit anti-inflammatory and antioxidant activities [38].

Despite their medicinal applications, tannins are classified as anti-nutrients due to their ability to bind proteins, leading to protein aggregation and the subsequent inactivation of digestive enzymes. This interaction between tannins and proteins reduces protein digestibility and interferes with the absorption of ionizable iron. [39]

GC-MS screening of *Justicia secunda* leaves showed the presence of phytocompounds like alkaloids, phenols, diterpenoids, sesquiterpenoids aromatic hydrocarbons, alkanes, alkenes, alkanols, aldehydes, carboxylic acids, allenes, coumarins, ethers, phenols, esters and terpenes/terpenoids with various biological properties were found in the plant. The GC-MS spectra profile and retention times showed 82 volatile bioactive compounds in the ethanolic leaf extract of *Justicia secuda*. Peak heights show extract component concentrations which includes: Ethyl palmitate (5.54%), 2,4-Di-tert-butylphenol (4.04%),9,17-Octadecadienal, (Z) (4.05%), Linoleic acid ethyl ester (4.05%), 9,12-Octadecadienoic acid, ethyl ester (4.00%), Decane, 2-methyl (3.82%), Hexadecane (3.82%), Heptadecane, 2,6-dimethyl (3.26%), N-(3-methylButyl) acetamide(3.02%), Sulfurous acid, butyl octyl ester (3.25%), Hexane, 1-(hexyloxy)-5-(3.06%), Fumaric acid,2-decyl hexadecyl ester (2.83%), Heptadecane, 2,6,10,14-tetramethyl (2.31%), Nonane, 4-methyl (2.48%), Cyclohexene, 3-(1,5-dimethyl-4-hex (2.47%), Heptanoic acid, 2-acetyl-, ethyl ester (2.44%),Decane, 3,4-dimethyl (2.29%), Dodecane, 4,6,-dimethyl (2.29%) Dodecane, 4,6-dimethyl (2.29%), E-14-Hexadecenal (2.22%), Hexatriacontane (2.00%), Dodecane, 2,6,11-trimethyl (2.19%), Heptane,2,2,3,3,5,6,6Hepta methyl- Oxalic acid (2.28%), as the major bioactive compounds (table 3).

The identified bioactive compounds in *Justicia secunda* ethanolic extract by GC-MS analysis reported in reference with existing literature, revealing anticancer, antimutagenic, antioxidant, antibacterial, antifungal, anti-inflammatory, antimicrobial, antitumor, hepatoprotective, antidiabetic, neuroprotective and many other biological effects. Among the identified volatile compounds of *J. secunda* extract were 1,2-Benzenedicarboxylic acid, gamma-terpinene (1.38%), dodecane (2.23%) piperine (0.31%) beta)]-(E,Z)-.alpha.-Farnesene (0.49%) butyl-2-ethylhexylphthalate (1.39%), butyl-2-ethylhexyl ester, phytol, α-Bisabolene , β-bisabolene Hexadecane-2-ol, Hexadecanoic acid methyl ester, and Tetradecane which have been reported to possess antioxidant, and positive anti-cancer activity [40][41][42][43][44]. The sesquiterpene *Aromadendrene* present in *J. secunda* leaf has been identified to inhibit the proliferation of HepG2 liver and PC3 prostate cancer cells and responsible for antibacterial activity of essential oils [45] [46]. Other studies also reported tetradecane (1.14%), e,z)-alpha-farnesene (2.52%), butyl-2-ethylhexylphthalate (1.39%), isobutyl nonyl ester(0.57%), tetradecane (1.14%), Hexadecane-2-ol, Hexadecanoic acid methyl ester, Tetradecane, 1-dodecene, alpha-copaene (1.40%), aromadendrene (0.22%), (e,z)-alpha-farnesene (2.52%), Beta-farnesene (2.52%), tau-muurolol, butyl-2-ethylhexylphthalate (1.39%), 1,2-benzenedicarboxylic acid, butyl-2-ethylhexyl ester, phytol, 1-docosene (0.36%), isobutyl nonyl ester(0.57%) and piperine to have demonstrate anti-bacterial properties. [43]

[47][48][49][50]. Previous studies demonstrated that these phytochemicals such as gamma-terpinene (1.38%), p-cymene (0.79%), alpha-copaene (1.40%), aromadendrene (0.22%%), humulene (1.48%), 9,12-Octadecatrienoic acid methyl ester and piperine (0.31%) possess anti-inflammatory properties [42][44][47][51][52][53]. The compounds such as, 1,4-dichlorobenzene (1.27%), and Beta-farnesene (2.52%) have carcinogenic, hepatotoxic, and nephrotoxic properties [54][55][56].

Furthermore, Hexadecane-2-ol, Hexadecanoic acid methyl ester, and Tetradecane possess flavoring functions. α-Bisabolene and β-bisabolene exhibit anti-tumor activities [57] [58]. 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 [59].Reports showed that Bis (2-ethylhexyl) phthalate and its metabolites exhibit acute and chronic toxicities which includes endocrine disruption and testicular toxicity [58] [60]. Also, present in *J. secunda* leaf ethanolic extract are a number of volatile organic compounds with yet to be identified biological activity.

The proximate analysis of the ethanol extract of *Justicia secunda* leaves revealed notable levels of various components, as detailed in Table 4. Carbohydrates were found to be the highest, followed by moisture content, protein, fats, fiber, and ash content, respectively.

The high carbohydrate content (59.23±0.00%) suggests that the leaves could serve as a significant source of energy, supplying the necessary energy to cells such as those in the brain, muscles, and blood [61]. Carbohydrates also play a crucial role in protecting and lubricating the respiratory, reproductive, and alimentary tracts, and they contribute to the structure of nerves, connective tissue, and hormones. The moisture content (13.92±0.00%) observed in *Justicia secunda* leaves can contribute to a longer shelf life by preventing susceptibility to microbial infection and mold degradation. Additionally, the moisture content indicates that the plant can serve as a good source of water for cellular hydration. In this present work, the moisture content 13.92±0.00% was higher than 10.48% reported by [62]. Protein, an essential nutrient for tissue formation and enzyme synthesis, was found in significant amounts (11.20±0.00%). This suggests that the leaves could aid in growth, tissue repair, and the synthesis of enzymes, hormones, and antibodies. There was a high significant difference in the crude protein (11.20±0.00) of *Justicia secunda* leaf in this study (Table 3.4) compared to some highly relished vegetables in Nigeria viz; *Hibiscus asper* leaf (0.26 ± 0.12), *Telfaria occidentalis* (0.15 ± 0.01), *Amaranthus hybridus* (1.07 ± 0.06), *Occimum gratissimum* (2.43 ± 0.04), *Talinum triangulare* (1.58 ± 0.01), *Corchorus olitorus* (0.80 ± 0.01), *Abelmoschus esculentus* (0.40 ± 0.03), *Alium cepa* (0.83 ± 0.02), *Basella alba* (0.71 ± 0.01) and *Vernonia amygdalina* (2.27 ± 0.01) as earlier reported (Omale, &. Ugwu, 2011) (62) and *T. occidentalis* 9.61±0.01 as reported by [63], this was also lower than what was reported by [64] on the leaf of *J.carnea* (26.71±0.02).

The moderate fat content (6.96±0.00%) enables the plant to store energy and act as carriers of fat-soluble vitamins such as A, D, E, and K. The fat contents was higher in the leaf of *Justicia secunda* (6.96%) when compared to 3.7% of *Amaranthus spinosus by* [65] report. It was also higher compared to 2.66% reported by [66] but lower in *Justicia secunda* leaf (7.07±0.76%) as reported by [62] and 8.3-27.0% reported in some vegetables consumed in Nigeria. The fiber content (4.43±0.00%) of the leaves may help manage constipation issues and protect against cancer and digestive disorders [67]. Adequate dietary fiber intake has been associated with various health benefits, including lower serum cholesterol levels and reduced risk of coronary heart disease, hypertension, constipation, diabetes, and certain cancers. The crude fibre content of the leaf (4.43±0.00) was lower than what was reported by [62] on *Justicia secunda* leaf (12.36±0.33%). However, it was higher than that of *Acanthus montanus*, a specie of Acanthaceae reported by [68] with a fibre content of 3.76%. The ash content (4.26±0.00%) indicates that the leaves contain appreciable amounts of mineral elements, contributing to overall nutritional value. However, this ash content was lower than that reported in previous studies on *Justicia secunda* leaves with the content of (15.99%±0.07) by [62] and *M. flagellipes* (7.80%) by [69]

5. Conclusion

In this study *Justicia secunda* leafs are very important plants with many reported biological activities. The study also reported the plant as the source of secondary metabolites i.e. alkaloids, flavonoids, tannins, reducing sugars etc. which play a vital role in treatment and prevention of various diseases. The antioxidant potential, anti-inflammatory and antimicrobial activity of *Justicia secunda* leaves can be attributed to the presence of various bioactive compounds, which collectively contribute to its medicinal and pharmacological values. This also suggests that *Justicia secunda* supports the traditional use as medicinal plant for the treatment and prevention of diseases. This plant could be beneficial in the management of inflammatory conditions such as arthritis, asthma, inflammatory bowel diseases and could also be used in the development of natural antimicrobial agents for the treatment of infections caused by pathogenic microorganisms.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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