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GC-MS and FTIR analyses of bioactive compounds present in ethanol leaf extract of *Sida acuta* from Imo State, Nigeria

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Abstract

Medicinal plants are vital to human and community health. This work aimed at using gas chromatography-mass spectrometry (GC-MS) and Fourier transform infrared (FTIR) to determine the phytochemical contents of ethanol leaf extract of *Sida acuta* from Imo State, Nigeria. Leaves of *Sida acuta* were collected, washed, air-dried, and pulverized. Pulverized leaves were extracted in 100% ethanol at 70°C in a water bath. Bioactive compounds from the extract were analyzed using Agilent Technologies GC equipment and Buck Scientific M530 USA FTIR. The GC-MS Screening of the plant's extract revealed the presence of seventy-one (71) phytocompounds including gamma-terpinene (1.11%), p-cymene (0.29%), linalool (2.45%), alpha-cubebene (0.11%), alpha-copaene (1.34%), aromadendrene (6.21%), humulene (1.90%), alpha-selinine, guaiol (0.58%), phytol (0.11%), butyl-2-ethylhexylphthalate (0.32%), 1,2-benzenedicarboxylic acid (0.40%), 1-docosene (0.36%), 1,4-dichlorobenzene (0.22%), (e)-.beta.-farnesene (10.87%), and piperine (0.61%), which are reported to exhibit various biological activities. The FTIR analysis of the plant extract revealed functional groups such as Primary (1°) and Secondary (2°) amines, alkanes, alkenes, alkyl halides, carboxylic acids, allenes, aromatics, aliphatic amines, esters, ethers, phenols, and aldehydes. Cross-referencing the phytocompounds with literature revealed a variety of biological functions, including antibacterial, antifungal, anti-inflammatory, anticancer, antimutagenic, antioxidant, antitumor, and others. It may be concluded that *Sida acuta* contains bioactive compounds with anti-microbial, anti-inflammatory, anti-oxidant, hepatoprotective, and anti-carcinogenic properties, with (E). beta.-farnesene being the most abundant (10.87%).

Keywords: GC-MS; FTIR; *Sida acuta;* Phytocompounds

1. Introduction

Medicinal plants have been utilised therapeutically since human civilisation began [1]. Medicinal plants are vital to human and community health. These plants have therapeutic properties due to chemical compounds that affect the body physiologically. The most important plant bioactives are alkaloids, tannins, flavonoids, terpenes, and phenolic chemicals [2]. Plants are used in traditional medicine and pharmaceuticals. Due to the paucity and cost of orthodox medication, many people worldwide use traditional medicine. Medicinal plants have given contemporary medicine several medicinal substances. Phytopharmaceuticals, found in many plants, are significant in agriculture, human, and veterinary medicine. Novel pharmacological leads for disease therapy and prevention are mostly derived from natural materials [3][4][5].

Due to their nutritional and therapeutic benefits, medicinal plants have been used throughout human history. Many modern medications come from natural sources, which have been a source of therapeutic substances for millennia.

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These phytochemicals were mostly isolated from traditional medicine. Tcheghebe, Seukep, and Tatong [6] found that indigenous people use *Sida acuta* Burm.f (Malvaceae) to treat several health issues.

The Malvaceae subfamily of Sida includes *Sida acuta* Burm. f. Sometimes called tenacious weed. It is a tiny, upright perennial shrub with hairy branches that can grow to 1 m tall and has a woody tap root [7]. It thrives in abandoned fields, wastelands, pasture habitat, and roadside ditches. *S. acuta* grows in southern Nigeria year-round. Native names include Igbo (Udo), Yoruba (Iyeye), Efik (Nsukerra), and Hausa (Tsadar lamarudu). It is often used as a weed in rural regions to cure skin, inflammatory, and liver disorders [8]. It treats malaria, diarrhoea, asthma, headaches, colds, fevers, skin conditions, urinary conditions, ulcers, snake bites, facial paralysis, and anti-fertility drugs [9][10][11].

Most phytoconstituents are determined via arduous and expensive High Performance Liquid Chromatography. However, phytocomponent detection requires simple, inexpensive, and fast testing. Since GC-MS uses both qualitative and quantitative methods to identify plant phytoconstituents, it can be utilised with or without FTIR. Muthulakshmi and Anusya [12] found that the FTIR can characterise and identify chemicals or functional groups (Chemical bonds) in an unknown plant extract mixture. Therefore, the study is aimed at using GC-MS and FTIR to evaluate the phytochemical contents of *Sida acuta* ethanol leaf extract from Imo State, Nigeria.

2. Materials and Methods.

2.1. Plant Collection and Processing

The leaves of *Sida acuta* were gathered from the premises of the Federal University of Technology, Owerri (FUTO). The sample was identified by Mr. Francis Iwueze, a taxonomist in the Department of Forestry and Wildlife Technology at the Federal University of Technology, Owerri (FUTO). The samples underwent a washing process using clean water and were air dried for a duration of 14 days. The desiccated leaves were ground into a fine powder, weighed, and stored in preparation for the extraction process.

2.2. Extraction

Over a period of 72 hours, a quantity of 200 g of the powdered substance was dissolved in 800 ml of absolute ethanol. To acquire a yield of 23.82g (11.9%) of extract, the extract was subjected to filtration using Whatman filter paper (No. 4) and subsequently evaporated to form a slurry in a water bath maintained at a temperature of 70°C [13].

Calculation of the percentage (%) yield of extract:

$$\text{Yield} = \frac{w_2}{w_1} \times 100$$

Where, w_2 = weight of extracted sample (g), w_1 = weight of powdered sample (g) before extraction.

The extract was then stored airtight in a covered container and refrigerated at 4 °C.

2.3. GC–MS Analyses of the Phytocompounds

GC-MS analysis of bioactive compounds from the extract was done using Agilent Technologies GC equipment. Specifically, the GC-7890A/MS-5975C model (Agilent Technologies, Santa Clara, CA, USA) was used with an HP-5MS column (30 m length, 250 µm diameter, 0.25 µm film thickness). The electron ionisation method used in GC-MS spectroscopic detection was 70-eV electrons. This study used 99.995% pure helium gas as a carrier. Carrier gas flowed at 1 mL/min. The temperature was first set between 50 and 150 °C and increased by 3 °C every minute. This temperature was maintained for 10 min. The temperature rose 10 °C every minute to 300 °C. An adequate solvent-diluted one microliter of the produced 1% extracts was splitless administered into the system. Each extract's chemical component % was calculated using the chromatogram peak area [14].

2.4. Identification of chemical constituents

The identification of bioactive compounds derived from the extract was performed through the analysis of their gas chromatography (GC) retention time on an HP-5MS column, as well as by comparing their spectra with computer software data of established standards from GC–MS systems, namely Replib and Mainlab data [14].

2.5. Procedure for FTIR Spectroscopy

FTIR analysis was done with the Buck Scientific M530 USA. A deuterated triglycine sulphate detector and potassium bromide beam splitter were used. Gramme A1 programme collected and altered spectra. A 1.0 g sample was well mixed with 0.5 ml of nujol and put over the salt pellet. Fourier Transform Infrared (FTIR) spectra were taken from 4,000 to 6,000 cm^{-1} during measurement. Co-addition with 32 scans and 4 cm^{-1} resolution merged these spectra. Transmitter values were used to display FTIR spectra [15].

3. Results

3.1. Extract yield

Yield (%) = $\frac{23.82\ g}{200.00\ g} \times 100$

Yield = 11.91 %

GC-MS screening of the plant's extract indicated the presence of phytocompounds like monoterpenoids, diterpenoids, alkaloids, phenols, and sesquiterpenoids with varied biological properties. Also found were benzene, alkanes, alkenes, alkanols, and esters.

FTIR analysis revealed the plant extract contains Primary (1º) and Secondary (2º) amines, alkanes, alkenes, alkyl halides, carboxylic acids, allenes, aromatics, aliphatic amines, esters, ethers, phenols, and aldehydes.

Numerous phytocompounds found in the plant by GC-MS analysis were cross-referenced with existing literature, revealing antibacterial, antifungal, anti-inflammatory, anticancer, antimutagenic, antioxidant, antitumor, and other biological activities.

Table 1 Phytocompounds Present in Ethanol Leaf Extract of *Sida acuta* by GC-MS Analyses

Pk	RT	Area %	Phytocompounds	Molecular Formula	Molecular Weight (G/Mol)	Quality
1	6.496	0.27	Oxirane, (chloromethyl)-	C_3H_5ClO	92.52	47
2	6.848	0.22	Benzene, 1,4-dichloro-	$C_6H_4Cl_2$	147.00	97
3	7.202	0.29	p-Cymene	$C_{10}H_{14}$	134.22	96
4	8.162	1.11	. Gamma. -Terpinene	$C_{10}H_{16}$	136.23	97
5	8.251	0.22	Octane, 3,4,5,6-tetramethyl-	$C_{12}H_{26}$	170.33	50
6	8.380	0.80	Decane, 2,6,7-trimethyl-	$C_{13}H_{28}$	184.36	59
7	8.534	0.43	Nonane	C_9H_{20}	128.25	86
8	8.591	0.20	Tetradecane	$C_{14}H_{30}$	198.39	86
9	8.644	0.22	Undecane, 2-methyl-	$C_{12}H_{26}$	170.33	64
10	8.957	2.14	Heptadecane, 2,6,10,14-tetramethyl-	$C_{21}H_{44}$	296.60	86
11	9.118	0.46	Octane, 4-ethyl-	$C_{10}H_{22}$	142.28	64
12	9.339	0.89	Tridecane	$C_{13}H_{28}$	184.36	80
13	9.441	2.45	Linalool	$C_{10}H_{18}O$	154.25	83
14	9.735	0.40	Oxalic acid, 2-ethylhexyl hexyl ester	$C_{16}H_{30}O_4$	286.41	64
15	9.808	0.50	Nonane, 3-methyl-	$C_{10}H_{22}$	142.28	72
16	10.027	0.11	Undecane, 5-methyl-	$C_{12}H_{26}$	170.33	81

17	10.101	0.09	Octane, 2-methyl-	C_9H_{20}	128.25	83
18	12.028	0.12	1-Dodecene	$C_{12}H_{24}$	168.32	86
19	12.262	0.42	Dodecane	$C_{12}H_{26}$	170.33	95
20	16.471	0.11	. Alpha. -Cubebene	$C_{15}H_{24}$	204.35	95
21	17.185	1.34	. Alpha. -Copaene	$C_{15}H_{24}$	204.35	97
22	17.645	2.76	. Gamma. -Elemene	$C_{15}H_{24}$	204.35	83
23	18.083	0.42	1H-Cycloprop[e]azulene, 1a,2,3,4,4a,5,6,7b-octahydro-1,1,4,7-tetramethyl-	$C_{15}H_{24}$	204.35	97
24	18.251	0.17	1,3,6,10-Dodecatetraene, 3,7,11-trimethyl-, (Z, E)-	$C_{15}H_{24}$	204.35	86
25	18.364	6.21	Aromandendrene	$C_{15}H_{24}$	204.35	55
26	18.737	0.63	Santolina triene	$C_{10}H_{16}$	136.23	74
27	18.980	0.22	(1R, 3aS, 8aS)-7-Isopropyl-1,4-dimethyl-1,2,3,3a,6,8a-hexahydroazulene,	$C_{15}H_{24}$	204.35	97
28	19.251	1.90	Humulene	$C_{15}H_{24}$	204.35	98
29	19.478	0.69	Bicyclo [7.2.0] undec-4-ene, 4,11,11-trimethyl-8-methylene-	$C_{11}H_{18}$	150.26	70
30	19.872	0.46	. Gamma. -Muurolene	$C_{15}H_{24}$	204.35	99
31	19.982	3.70	1H-Cyclopenta [1,3] cyclopropa[1,2]benzene, octahydro-7-methyl-3-methylene-4-(1-methylethyl)-	$C_{10}H_8$	128.17	92
32	20.114	2.07	Naphthalene, 1,2,3,4,4a,5,6,8a, -octahydro-4a,8-dimethyl-2-(1-methylethenyl)-	$C_{15}H_{24}$	204.35	99
33	20.481	2.92	(E, Z)-Alpha-farnesene	$C_{15}H_{24}$	204.35	89
34	20.588	0.37	8-Isopropenyl-1,5-dimethylcyclodeca-1,5-diene	$C_{15}H_{24}$	204.35	93
35	20.718	10.87	(E)-. beta. -farnesene	$C_{15}H_{24}$	204.35	93
36	20.982	1.35	2,4-di-tert-butylphenol	$C_{14}H_{22}O$	206.32	96
37	21.089	7.51	Cyclohexene, 3-(1,5-dimethyl-4-hexenyl)-6-methylene-	$C_{15}H_{24}$	204.35	96
38	21.260	0.90	(E)-1-methyl-4-(6-methylhept-5-en-2-ylidene) cyclohex-1-ene	$C_{15}H_{24}$	204.35	94
39	21.531	0.53	Cyclohexene, 4-[(1E)-1,5-dimethyl-1,4-hexadien-1-yl]-1-methyl-	$C_{15}H_{24}$	204.35	94
40	21.748	0.32	Cyclohexanemethanol, elemol	$C_{15}H_{26}O$	222.37	68
41	21.881	1.00	Alloaromandendrene	$C_{15}H_{24}$	204.35	78
42	22.078	2.59	1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl-	$C_{12}H_{20}O$	180.29	91
43	22.450	0.12	1H-Cycloprop(e)azulen-7-ol, decahydro-1,1,7-trimethyl-4-methylene	$C_{11}H_8O$	156.18	64
44	22.533	0.79	1,3, Bis-(2-cyclopropyl, 2-methylcyclopropyl) but-2-en-1-one	$C_{18}H_{26}O$	258.40	50
45	22.691	1.06	Cetene, 1-Hexadecene	$C_{16}H_{32}$	224.42	90

46	22.927	0.58	Guaiol	$C_{15}H_{26}O$	222.37	99
47	23.181	0.10	3-Cyclohexen-1-carboxaldehyde, 3,4-dimethyl-	$C_9H_{14}O$	138.21	81
48	23.598	0.16	Apiol	$C_{12}H_{14}O_4$	222.24	48
49	23.983	0.21	. Tau.-Muurolol	$C_{15}H_{26}O$	222.37	64
50	24.299	0.80	Naphthalene, 1,2,3,5,6,7,8,8a-octahydro-1,8a-dimethyl-7-(1-methylethenyl)-	$C_{15}H_{24}$	204.35	93
51	25.073	0.72	1H-Cycloprop[e]azulene, decahydro-1,1,4,7-tetramethyl-	$C_{15}H_{26}$	206.37	60
52	27.255	0.80	1-octadecene	$C_{18}H_{36}$	252.50	94
53	27.790	0.22	8-Hexadecenal, 14-methyl-	$C_{17}H_{32}O$	252.40	38
54	29.764	0.81	Ethanone, 1-(4-methyl-1H-imidazol-2-yl)-	$C_6H_8N_2O$	124.14	59
55	30.016	0.32	Butyl-2-ethylhexylphthalate	$C_{20}H_{30}O_4$	334.40	90
56	30.256	1.20	3-Heptafluorobutyroxypentadecane	$C_{19}H_{31}F_7O_2$	424.40	91
57	31.306	0.11	Phytol	$C_{20}H_{40}O$	296.50	80
58	31.628	0.23	Ethyl, 9.cis,11. trans.-octadecadienoate	$C_{20}H_{36}O_2$	308.50	99
59	31.664	0.26	9-Oxabicyclo [6.1.0] nonane	$C_8H_{14}O$	126.20	96
60	31.813	0.36	1-Docosene	$C_{22}H_{44}$	308.60	97
61	33.264	1.59	(E)-5-(Benzo [d][1,3] dioxol-5-yl)-N-isobutylpent-2-enamide	$C_{16}H_{21}NO_3$	275.34	90
62	34.004	0.42	Bis(2-ethylhexyl) phthalate	$C_{24}H_{38}O_4$	390.60	90
63	34.141	0.30	(1S,15S)-Bicyclo[13.1.0]hexadecane-2-one	C16H28O	236.39	35
64	34.213	0.49	Tetradecanoic acid, 2-hydroxy, methyl ester	$C_{15}H_{30}O_3$	258.40	32
65	34.685	3.45	1-Piperonyl-3,5-diamino-1,2,4-triazine	$C_{10}H_{11}N_5O_2$	233.23	64
66	35.655	0.41	E-1,9-hexadecadiene	$C_{16}H_{30}$	222.41	42
67	35.842	1.71	Tricyclo [4.2.0.0(2,4)] octan-5-one	$C_8H_{10}O$	122.16	25
68	36.007	0.61	Piperine	$C_{17}H_{19}NO_3$	285.34	94
69	36.183	0.94	Furo [3,4-C] pyridine-3,4(1H,5H)-dione, 6-methyl	$C_8H_7NO_3$	165.15	52
70	36.447	1.45	Benzamide, N-(4-cyanomethylphenyl)-3,4-dimethoxy-	$C_{17}H_{16}N_2O_3$	296.32	64
71	37.409	-0.15	Pyrrolidine, 1-[5-(1,3-benzodioxol-5-yl)-1-oxo-2,4-pentadienyl]-	$C_{16}H_{17}NO_3$	271.31	93

Table 2 Fourier Transform Infrared (FTIR) Analyses of The Functional Groups and Peak Details of Ethanol Leaf Extract of *Sida acuta*

S/N	Wave Number (Cm^{-1})	Bond/Mode of Vibration	Functional Group	Peak Details
1.	3410.538	O-H, stretch. H-bonded	Alcohols, Phenols	Strong, Broad.
2.	3269.905	N-H, stretch	Primary (1°), Secondary (2°) amines, amides	Medium
3.	3005.212	=C-H, stretch C-H, stretch	Alkenes, Alkanes	Medium, Strong.
4.	2756.945	H-C=O: C-H, stretch	Aldehydes	Medium
5.	2616.28	O-H, stretch	Carboxylic acids	Medium
6.	2168.433	-C≡C-, stretch	Alkynes	Weak
7.	1989.044	C=C=C	Allene	Medium
8.	1841.169	C-H, bend	Aromatics	Weak
9.	1662.897	-C=C-, stretch	Alkenes	Medium
10.	1221.974	C-N, stretch. C-O, stretch. C-H, wag($-CH_2X$)	Aliphatic amines, Alcohols, Carboxylic acids, Esters, Ethers, Alkyl halides.	Medium, Strong Medium
11.	1084.34	C-O, stretch. C-N, stretch	Alcohols, Carboxylic acids, Esters, Ethers, Aliphatic amines.	Strong Medium
12.	905.1005	=C-H, bend N-H, wag	Alkenes, Primary (1°), Secondary (2°) amines	Strong Strong broad
13.	769.9082	C-Cl, stretch. C-H N-H, wag =C-H, bend	Alkyl halides, Aromatics, Primary (1°), Secondary (2°) amines, Alkenes	Medium Strong Strong broad Strong

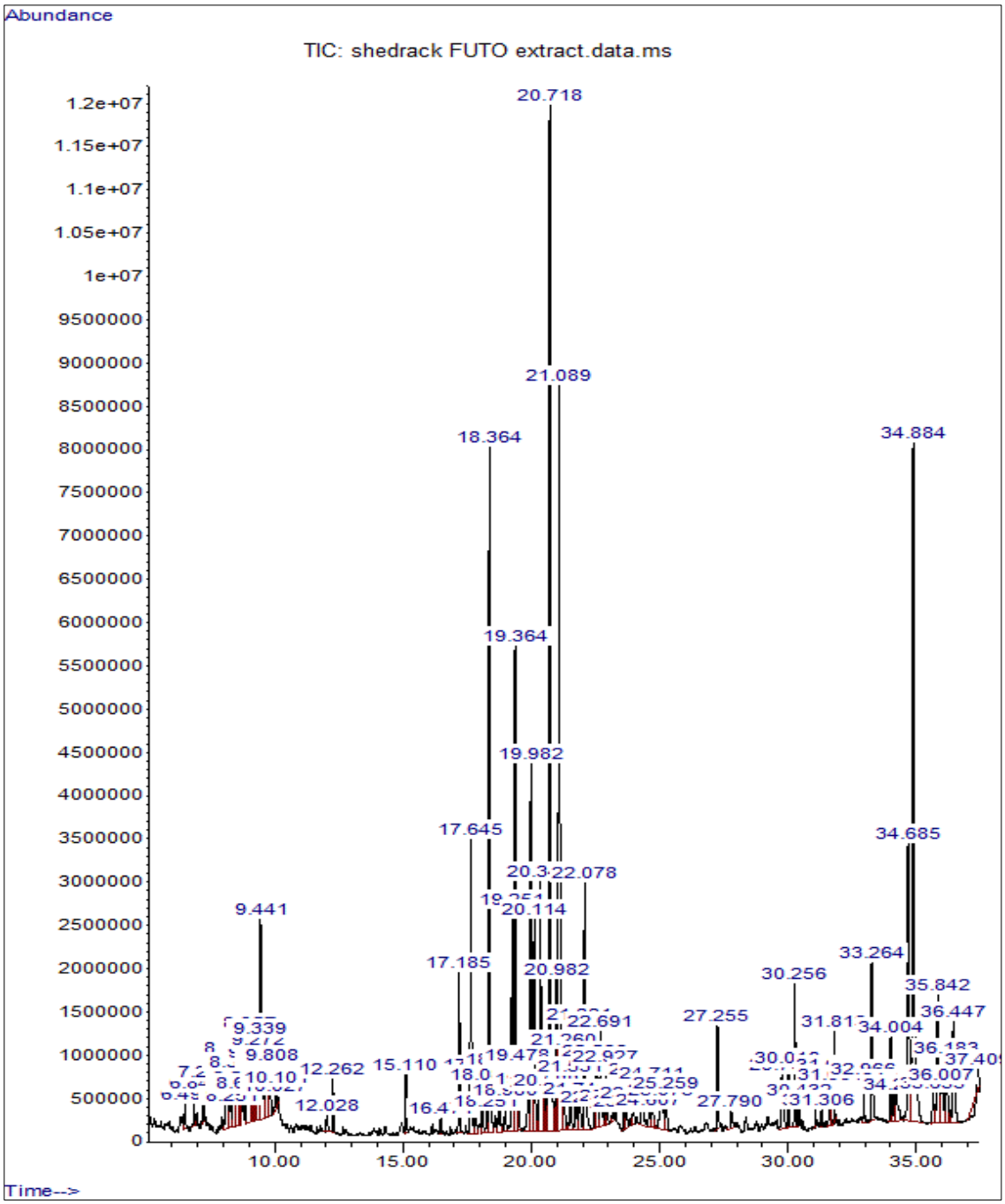
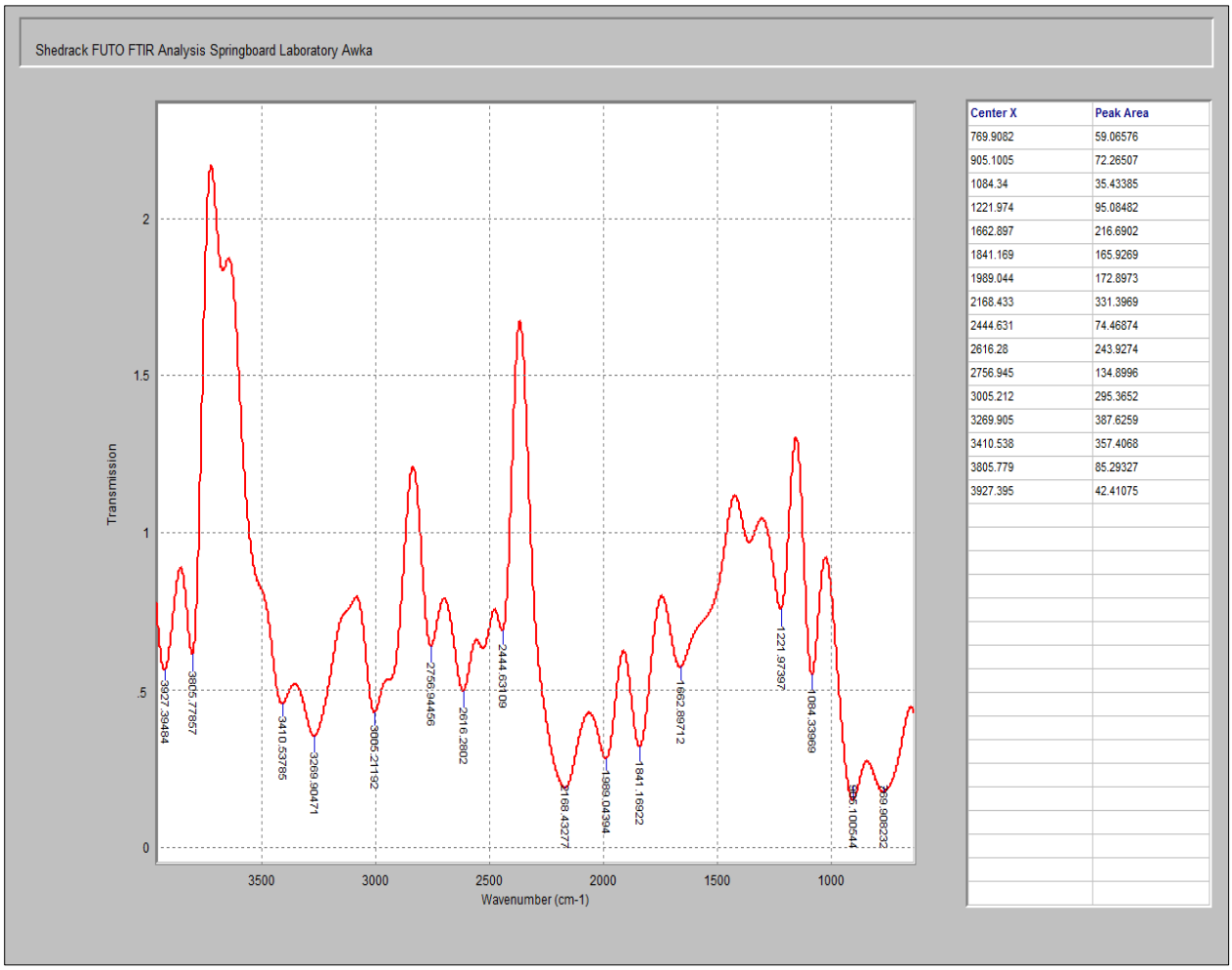


Figure 1 GC-MS Chromatogram of the *Sida acuta* Ethanol Leaf Extract



Center X	Peak Area
769.9082	59.06576
905.1005	72.26507
1084.34	35.43385
1221.974	95.08482
1662.897	216.6902
1841.169	165.9269
1989.044	172.8973
2168.433	331.3969
2444.631	74.46874
2616.28	243.9274
2756.945	134.8996
3005.212	295.3652
3269.905	387.6259
3410.538	357.4068
3805.779	85.29327
3927.395	42.41075

Figure 2 FTIR Spectrum of the *Sida acuta* Ethanol Leaf Extract

4. Discussion

The identification of active ingredients in medicinal plants and the assessment of whether a plant species contains certain compounds, or a mixture thereof begin with GC-MS analysis [16]. Analysis of the GC-MS spectra profile and retention periods confirmed the key components' presence. Peak heights show extract component concentrations. The current investigation has identified several phytochemicals in the sample being studied. These phytochemicals include gamma-terpinene (1.11%), p-cymene (0.29%), linalool (2.45%), alpha-cubebene (0.11%), alpha-copaene (1.34%), aromadendrene (6.21%), humulene (1.90%), alpha-selinine, guaiol (0.58%), phytol (0.11%), and piperine (0.61%). Previous studies have demonstrated that these phytochemicals possess anti-inflammatory properties [17][18][19][20][21][22][23]. The compounds such as gamma-terpinene, dodecane (0.42%), alpha-selinine, butyl-2-ethylhexylphthalate (0.32%), 1,2-benzenedicarboxylic acid (0.40%), butyl-2-ethylhexyl ester, phytol, and piperine have been found to exhibit antioxidant properties [17][18][19][23][24]. Moreover, previous studies have reported the antibacterial properties of tetradecane, linalool, 1-dodecene, alpha-cubebene, alpha-copaene, aromadendrene, (e,z)-alpha-farnesene (2.92%), tau-muurolol, butyl-2-ethylhexylphthalate, 1,2-benzenedicarboxylic acid, butyl-2-ethylhexyl ester, phytol, 1-docosene (0.36%), and piperine [17][20][21][25][26][27][28][29][30].

However, it is important to highlight that oxirane (0.27%), 1,4-dichlorobenzene (0.22%), and apiol (0.16%) have carcinogenic, hepatotoxic, and nephrotoxic properties [31][32][33][34]. Beta-farnesene, with a relative abundance of 10.87%, is the most prevalent compound. It possesses notable properties such as anti-carcinogenic, antibacterial, and antifungal actions, as reported by Turkez *et al*. (2014)**.** The purported anti-arthritic properties of the herb as stated by traditional practitioners, can be attributed to the presence of the anti-inflammatory compounds [36][37][38][39].

According to a study conducted by Movasaghi, Rehman, and urRehman [40], the Fourier Transform Infrared (FTIR) vibration spectroscopy technique is widely recognised for its capability to identify crucial functional groups found in

plant extracts, biological substances, and synthetic chemicals. In addition, Ashokkumar and Ramaswamy [41] asserted that FT-IR spectroscopy possesses unmatched efficacy in the identification and differentiation of diverse chemical bonds, usually known as functional groups, within molecular structures. The ethanol extract used in this investigation was analysed using FTIR spectroscopy. The spectra revealed the presence of thirteen (13) peaks, each corresponding to a separate functional group. The peaks seen in the ethanol extract are located within the spectral range of 3410.538, 3269.905, 3005.212, 2756.945, 2616.28, 2168.433, 1989.044, 1841.169, 1662.897, 1221.974, 1084.34, 905.1005, and 769.9082 cm^{-1}. The ethanol extract of the leaf showed a total of 13 functional groups. These functional groups include alcohols, phenols, primary (1º) and secondary (2º) amines, amides, alkenes, alkanes, aldehydes, carboxylic acids, alkynes, allene, aromatics, esters, and alkyl halides. Hydroxyl groups (-OH), carboxylic acids (-COOH), amines ($-NH_2$) and esters (-COOR) have been reported to be involved in anti-inflammation and are found in most conventional NSAIDs used in the treatment of arthritis and other inflammatory diseases [42]. Therefore, this may be indicative of the anti-inflammatory potential of *Sida acuta*.

5. Conclusion

Based on the findings derived from the current study, it can be inferred that *Sida acuta* harbours a diverse range of bioactive compounds exhibiting distinct biological properties, including antimicrobial, anti-inflammatory, antioxidant, hepatoprotective, and anti-carcinogenic activities. Among the identified compounds, (E)-β-farnesene emerges as the most prevalent constituent, constituting 10.87% of the total compounds detected. The utilisation of *Sida acuta* leaves by traditional healers for numerous medical conditions is supported by the presence of diverse bioactive components. Hence, it is advisable to consider *Sida acuta* as a viable source of phytopharmaceutical value.

Compliance with ethical standards

Disclosure of conflict of interest

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

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