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In vitro antioxidant properties and GC-MS analysis of solvent extracts of *Sida acuta* leaf

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ABSTRACT

Background & Aim: Active search for plants with diverse antioxidant and pharmacological properties capable of counteracting the deleterious effects of free radical-mediated pathologies, is still evolving. The current study investigates the phytochemical screening, antioxidant mechanisms and gas-chromatography-mass spectrometer (GC-MS) analysis of leaf extract of *Sida acuta*.

Experimental: Powdered leaves of the plant was extracted in ethanol, methanol and water. Total phenolic and flavonoids contents of the extracts were measured using established protocols. Also, free radical scavenging activity against 2, 2-diphenyl-1-picryl hydrazyl (DPPH) and nitric oxide radicals (NO•) as well as the ferric reducing antioxidant power (FRAP) assay were also evaluated. Furthermore, the structure and relative abundance of bioactive compounds in the methanolic leaf extract of *Sida acuta* was determined on GC-MS.

Results: Results indicated that the total phenol and flavonoids content is significantly ($P<0.05$) higher in methanolic extract when compare with extracts from other solvents. Additionally, DPPH radical scavenging potency and FRAP of the extract is in the order; methanolic> aqueous> ethanolic, while the aqueous extract elicits significant NO• radical scavenging properties when compared with other solvents. The GC-MS chromatogram of the methanolic leaf extract of the plant reveals the presence of 23 bioactive compounds. Taking together, the relative abundance of phytochemical contents and antioxidant capacity of *Sida acuta* leaves is partly contingent on the choice of extraction solvent.

Recommended applications/industries: The presence of various bioactive compounds justifies the use of the plant for diverse remedies in traditional medicine and the need for further studies.

1. Introduction

Wide varieties of medicinal plants are available in nature to combat myriad of heath challenges threatening human existence. In fact, WHO report indicates that more than 80% of world's population rely on plant-based products to meet their health needs (Palaksha and Ravishankar, 2012). The plant *Sida acuta Burm* is a weed belonging to the mallow family, commonly known as broom weed. This plant propagates very competitively, by seed or stem cuttings and grows abundantly on cultivated grounds, waste areas, as well as, waysides (Tcheghebe *et al.*, 2016). It is perennially used by indigenous people in parts of African, for the management of a wide range of health related problems (Tcheghebe *et al.*, 2016). The whole plant crude extract of *S. acuta* is often used in traditional medicine to remedy diseases such as fever, headache, skin diseases, diarrhea and dysentery (Ignacimuthu *et al.*, 2006). Also, the bark, root, seeds,

flower and leaves are used as aphrodisiac, ant-rheumatic, anti-inflammatory, diaphoretic, diuretic, antipyretic, stomachic, antioxidant and as wound healing agent (Mann *et al.*, 2003; Karou *et al.*, 2007; Ekpo and Etim, 2009; Nakkliang *et al.*, 2020). In addition, chewing of the leaves of *S. acuta* is used in the treatment of gonococcal infection in parts of Nigeria (Okafor *et al.*, 2013).

Essentially, the screening of extracts for individual or synergistic therapeutically active compounds in various plant species, either as medicaments or a useful start up materials for novel drugs, is of perpetual research interest (Gopalakrishnan and Udayakumar, 2014; Tcheghebe *et al.*, 2016; Ogunmoyole *et al.*, 2022). Therefore, *Sida acuta* has been reported in literature to possess compounds such as alkaloids, saponosides, coumarins, steroids, phenolic compounds and flavonoids (Karou *et al.*, 2007). These phytochemical substances have been established to have diverse pharmacological potentials such as antiplasmodial, antimicrobial, cytotoxic and antioxidant properties (Karou *et al.*, 2007; Nakkliang *et al.*, 2020).

In vitro techniques using 2, 2-diphenyl-1-picryl hydrazyl (DPPH) assay, Nitric oxide (NO) scavenging and ferric reducing antioxidant powers are some of the procedures generally used to confirm the antioxidant activity of plant samples within particular reaction systems (Alam *et al.*, 2013). Therefore, medicinal ranking of plant species based on these *in vitro* metrics is a veritable elements linked to their potentials, as good source of antioxidants either in food or herbal products. These exogenous antioxidants enhances the capacities of the innate antioxidant system to ameliorate or obliterate oxidative stress related onsets, progression and complications in several pathologies (Krishnaiah *et al.*, 2011; Deepak *et al.*, 2015; Kumari *et al.*, 2018).

Gas chromatography-mass spectroscopy (GC-MS), is one of the vital analytical methods in use to provide, not only the qualitative and quantitative purification of compounds even at trace level, but also provides lead information related to structure and composition of active principles in plants of interest (Kaushik *et al.*, 2002; Chaman and Verma, 2006; De-Fatima *et al.*, 2006; Andrew, 2007; Paranthaman *et al.*, 2012; Shareef *et al.*, 2016; Ogunmoyole *et al.*, 2022). Hence, this study is designed to investigating the in-vitro antioxidant activity and GC-MS profile of leaf extract of *S. acuta*.

2. Materials and Methods

2.1. Preparation of plant materials

Fresh leaves of *S. acuta* was collected from the Botanical garden, Ekiri State University, Ado, Ekiti, Nigeria, on the 28th of June 2022. The leaves were dried at room temperature (27± 2 ^{oC}) and pulverized to a fine powder using an electric blender. The plant materials were soaked in methanol (80%), distilled water and 100 % ethanol at a ratio of 1:10 (250 mL for 25 g of powdered sample) over a period of 72 hours. The extraction solvents were allowed to evaporate completely, which was confirmed by constant weight of the extract. The stock solution of extracts was prepared using dimethyl sulfoxide (DMSO) of 99.9 % purity (Sigma, D8418), at a concentration of 20 mg/mL, and used for further experimental analysis.

2.2. Chemicals

2,2-diphenyl-1-picrylhydrazyl (DPPH.) radical was purchased from Fluka Chemicals, phosphate buffer saline (PBS), phosphoric acid, potassium dihydrogen phosphate (KH_2PO_4), potassium hydroxide (KOH), ferric chloride ($FeCl^{3+}$), naphthylethylenediamine dihydrochloride, ethylenediaminetetraacetic acid (EDTA), sodium carbonate (Na_2CO_3), aluminium trichloride, perchloric acid ($HClO_4$), ferrous sulphate, dimethyl sulfoxide, Folin-ciocalteu reagent (FCR) and trichloroacetic acid (TCA) were all purchased from Sigma Chemical Co. (St. Louis, MO). All other reagents were of high analytical grade, obtained from standard commercial suppliers.

2.3. Phytochemical analysis

2.3.1. Determination of total phenolic content

The method of Singleton *et al.* (1999) was followed in the determination of total phenolic contents of the leaf extracts of *S. acuta*. Appropriate volume of leaf extract containing 0.2, 0.4 and 0.8 mg/mL of the extract was mixed with equal volume of distilled water. Thereafter, 2.5 mL of Folin-Ciocalteau's reagent (FCR) and 2 mL of 7.5% sodium carbonate were added to the reaction mixture. The resulting mixture was incubated at 45°C for 40 min. The mixture was allowed to cool and its absorbance read at 765 nm. Quantification was done on the basis of a standard curve of extinction against gallic acid concentration was prepared (Lin and Tang, 2007). All measurements were performed in

triplicate and the results were averaged. The results were expressed as percentage (w/w) and calculated using the formula:

Total phenolic content (% w/w) = $(GAE \times V \times D \times 10^{-6} \times 100)/W$,

GAE: Gallic acid equivalent (µg/mL), V: Total volume of sample (mL), D: Dilution factor, W: Sample weight (g).

2.3.2. Determination of total Flavonoid Content

Total flavonoids content of the extracts was determined as described by Meda *et al.* (2005) with slight modifications. One hundred microliters (100 µl) each containing 0.2, 0.4 and 0.8 mg/mL of the extracts was mixed separately with 100 µl of 20% aluminium trichloride (w/v). The mixture was acidified with 100 µl of acetic acid and made up to 5 ml with distilled water and left to stand for 40 min at 25 °C. Absorbance at the resulting mixture was then read at 415 nm against reagent blank. Quantification was done on the basis of a standard curve of quercetin (12.5−400 mg/L) and expressed as quercetin equivalent (QE) per g of dry weight (DW) sample (mg QE/gDW). The concentration of total flavonoids was calculated using the following equation:

X=c*V/m.

Where: X is the total flavonoids concentration, c is the concentration of quercetin standard established from the calibration curve, V is the volume of the extracts, and m is the weight of the dried powdered plant. All measurements were performed in triplicate and the results were averaged.

2.4. In vitro antioxidant activities of the leaf extracts

2.4.1. Nitric oxide radical (NO•) scavenging assay

Amount of NO· produced was determined following the method of Marcocci *et al.* (1994). The reaction mixture containing 5 mM SNP in phosphate buffered saline (pH 7.3), with or without the plant extracts at 0.2, 0.4 and 0.8 mg/ml was incubated for 180 min at 25°C. The NO· radical released reacts with oxygen to generate nitrite ion (NO•) which was measured at 30 min intervals by mixing 1.0 ml of incubation mixture with equivalent volume of Griess reagent (1% sulfanilamide in 5% phosphoric acid and 0.1% naphthyl ethylenediamine dihydrochloride). Absorbance of the chromophore formed as a result of diazotization of nitrite ions with sulfanilamide and subsequent coupling with napthyl ethylenediamine dihydrochloride was read at 546 nm. The amount of nitrite produced in the presence or absence of the plant extracts was estimated from a standard curve of sodium nitrite. The amount of nitric oxide radical inhibition is calculated following this equation:

% Inhibition of NO• radical = $[(A_0 - A_1)/ A_0] \times 100$.

Where: A_0 is the absorbance before reaction and A_1 is the absorbance after reaction with Griess reagent.

2.4.2. DPPH Free radical scavenging ability

DPPH radical scavenging activity of the extracts was determined following the method of Awah *et al.* (2010). Fifty microliters (50 µl) of each extract was mixed separately with 1.0 ml of 0.4 mM DPPH and made to 5ml with methanol. The reaction mixture was vortexed for 1 min and incubated in the dark for 20 min at 25 °C. Absorbance of the resulting solution was then read at 517 nm against blank containing DPPH and methanol. Percentage DPPH radical scavenged was determined with reference to the blank using the formula:

Antioxidant capacity (%) = [(Absorbance blank-Absorbance sample)/Absorbance blank] x 100

2.4.3. Ferric reducing antioxidant power

The ferric reducing potential of the extracts was determined according to the method of Pulido *et al.* (2000). Varying volumes of extracts containing 0.2, 0.4 and 0.8 mg/mL was mixed with 250 µl of 0.2 M sodium phosphate buffer pH 6.6 and 250 µl of 1% potassium ferrocyanide (w/v). The reaction mixture was incubated at 50 °C for 20 min. Two hundred and fifty (250 µl) of 10% trichloroacetic acid (TCA) was added to the mixture and centrifuged at 650 rpm for 10 min. One milliliter (1 ml) of the supernatant was mixed with equal volume of water and 100 µl of 0.1% (w/v) ferric chloride. Absorbance of the resulting mixture was measured at 700 nm against the reagent blank containing all assay components except the extracts. FRAP values was obtained by comparing the absorption change in the test mixture with those obtained from increasing concentrations of Fe^{3+} and expressed as mM of Fe^{2+} equivalents/ L of sample. All assays were carried out in triplicates and averaged.

2.5. GC-MS analysis

Chromatographic separation was achieved with the Vocol column (J & W, Agilent Technologies, CA, USA), which has dimensions of 1.8 µm× 60 m× 0.32 mm film thickness. Nitrogen was used as the carrier gas at a constant flow rate of 0.8 mL/min. The column temperature was initially held at 35 °C for 3 minutes, then, the temperature was increased to 40 °C with a heating rate of 3 °C/min and the temperature was held for 1 min. The second ramp was programmed from 40 °C to 210 °C with a heating rate of 5 °C/min. A post-run of 16 min at 210 °C was established. The mass spectrometer was used in electron ionization mode; all spectra were acquired using a mass range of m/z 30–800. The transfer line temperature was set at 210 °C, the ion source temperature at 200 °C, detector voltage 0.8 kV. The identification of VOCs was achieved by using the National Institute of Standards and Technology (NIST) reference library diluted samples (1:10 chloroform, v/v) of 0.2 µl of the mixtures were always injected automatically in the split less mode. Mass spectra were obtained by electron ionization at 70 eV, using a spectral range of m/z 30–1000. Most of the compounds were identified using the analytical method: mass spectra (authentic chemicals, Wiley spectral library collection and NSIT library; Wiley 9 and NIST 08), the comparison of the retention times (tR) and mass spectra of authentic standards. In addition, an in-house dedicated mass spectral library was built by using the mass spectra of authentic compounds to confirm the identities of detected VOCs. The following standards were

used: acetoin, ethyl acetate, cis-grandisol [(1R, 2S)-cis-2-isopropenyl-1- methylcyclobutaneethanol], isoamyl alcohol, isobutanol, 2-methyl-1-butanol, phenethyl acetate, and phenethyl alcohol. Each individual compound was quantified using standard curves calculated from three serial dilutions of analytical standards. The internal standard (tridecane) was used to improve the precision of quantitative analysis with the calibration curve by plotting the signal from analyze with the signal from the internal standard as a function of the analyte concentration of the standards. All the measurements were replicated three times for each assay and the results are presented as mean values.

All the samples and replicates were continuously injected as one batch in random order to discriminate technical from biological variations. Additionally, the prepared pooled samples were used as quality controls (QCs), which were injected at regular intervals throughout the analytical run to provide a set of data from which the repeatability can be assessed.

2.6. Statistical analysis

Data were expressed as mean ± standard deviation (SD) of an experiment performed in triplicate and were analyzed by appropriate analysis of variance (ANOVA) followed by Duncan's multiple range test. Differences were considered significant at $P<0.05$.

3. Results and discussion

The search for pharmacological strategies with potentials to counteract and/or ameliorate the deleterious effects of free radical-mediated pathologies is perpetually intense. Medicinal plants possessing bioactive compounds with diverse antioxidant and therapeutic properties continue to attract the attention of scientists around the globe. In this study, total phenolic and flavonoids content of *S. acuta* leaf extract obtained from three different solvents (water, 80% methanol and absolute ethanol) were determined. The results, as represented in Figure 1 and Figure 2, respectively, indicates that methanolic extracts showed significantly ($P<0.05$) total phenolic and flavonoids content than the aqueous and ethanolic extract (methanol> water> ethanol), in a concentration dependent manner. In previous works, methanol demonstrated more effective solvation in recovering higher amounts of phenolic compounds from rice bran and *Moringa oleifera* leaves (Chatha *et al.*, 2006). It has been previously reported that asides temperature, sonication extraction time and method applied, the nature and polarity differentials of the extraction solvents affect significantly, the phytochemical compositions in the extract, the total yield of the polyphenolic compounds and ultimately the antioxidant metrics obtained from plants materials (Ksouri *et al.*, 2009; Falleh *et al.*, 2012; Thamizhiniyan *et al.*, 2019). In fact, choice of extraction solvent maybe contingent upon different plant materials for maximum extraction of phenolic compounds and other phytoconstituents (Thamizhiniyan *et al.*, 2019).

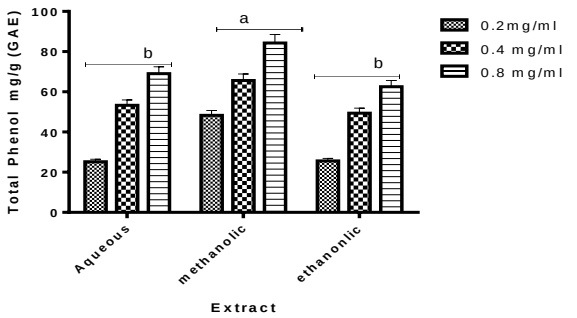

Figure 1. Total phenolic content of *S. acuta* leaf extract. Data represent mean ± SD of an experiment performed in triplicate. (a) indicates a significant difference at P< 0.05 compared to (b).

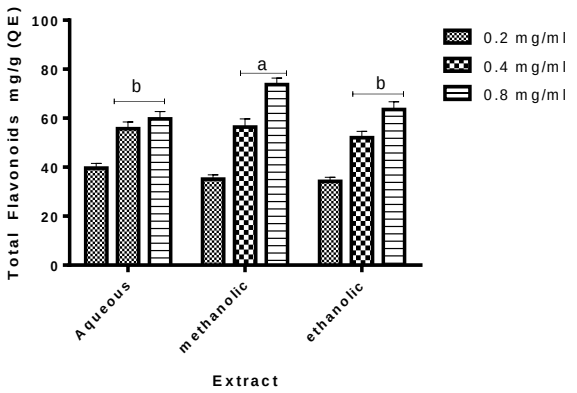

Figure 2. Total flavonoids content of *S. acuta* leaf extracts. Data represent mean ± SD of an experiment performed in triplicate. (a) indicates a significant difference at P< 0.05 compared to (b).

Several *in vitro* chemical assays with different reaction principles have been frequently used to determine the antioxidant potential of plant extracts (Thamizhiniyan *et al.,* 2019). In this study, it was observed that the leaf extracts of *S. acuta* exhibits concentration-dependent, differentials DPPH radical scavenging properties with methanolic extract displaying significant (P<0.05) radical scavenging capacity when compared with others (Figure 3). DPPH radical scavenging have involve the ability of antioxidants to donate protons (via reduction) to unstable DPPH radicals thereby attaining conformational stability. The reductive ability is physically noticeable by a change in the purple color of DPPH radicals to golden yellow depending on the strength of the antioxidant (Ogunmoyole *et al.,* 2009; Deepak *et al.,* 2015).

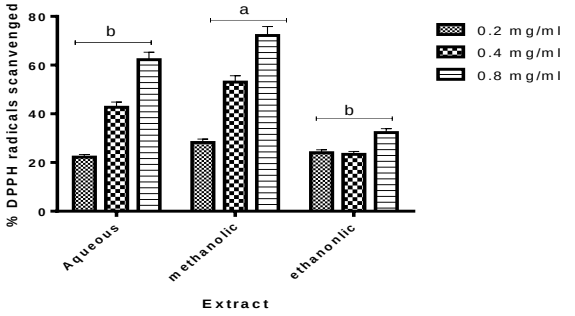

Figure 3. DPPH radical scavenging ability of *S. acuta* leaf extracts. Data represent mean ± SD of an experiment performed in triplicate. (a) indicates a significant difference at P<0.05 compared to (b).

Concentration of phenolic and flavonoid compounds have positive correlations with DPPH radical scavenging activities because of hydrogen and electron contribution from the hydroxyl groups of phenolic compounds (Rubab *et al.,* 2020). Therefore, as all extracts of *S. acuta* scavenged DPPH radicals, though at various degrees, it suggests that flavonoids and phenols in the leaf could produce excellent nucleophilic tendencies, donating electron to unstable DPPH radical and stabilizing the molecule via reduction, which could be a demonstration of its strong antioxidant potentials (Omololu *et al.,* 2009).

Figure 4 represents the result of ferric reducing antioxidant power (FRAP) of various extracts of *S. acuta* leaf. It is observed also that methanolic extract exert more significant ferric reducing antioxidant potency than the two other solvents. It has been reported that an antioxidant molecule may act differently and/or synergistically in the scavenging of different types of radicals in biological milieu (Omololu *et al.,* 2009). Therefore, apart from bond dissociation via hydrogen donation (as in DPPH reaction mechanism), possession of ionization potential to transfer an electron with resultant reduction of any compound, carbonyls, free radicals and metals is another major factor that determine the mechanism and efficiency of an antioxidant compound (Prior *et al.,* 2005; Karadag *et al.,* 2009). Therefore, given the additionally ferric reducing antioxidant properties observed, leaf of *S. acuta* could be said to possess joint arsenals for both hydrogen atom and electron transfer contraptions for its antioxidant defense mechanisms.

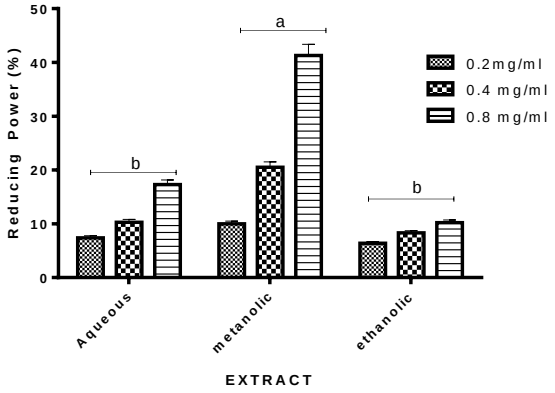

Figure 4. Ferric reducing antioxidant power of S. *acuta* leaf extracts. Data represent mean ± SD of an experiment performed in triplicate. (a) indicates a significant difference at P< 0.05 compared to (b).

In an earlier report by Ogunmoyole *et al.* (2022), treatment with methanolic leaf extract of *S. acuta* significantly improved the CCL_4 and rifampicin-induced hepto-renal damage in rat model, as measured by the capacity to positively modulate the depleted activity of antioxidant enzymes (superoxide dismutase and catalase), as well as, the concentration of both protein and non-protein thiols and reversal of lipid peroxidation in the liver, kidney and serum.

Furthermore, as previously observed with other antioxidant parameters, all the extracts of *S. acuta* leaf, demonstrated NO• scavenging properties. However, it was remarkably observed that the aqueous extract showed significance (P<0.05) scavenging ability than the alcoholic extracts, as shown in Figure 5.

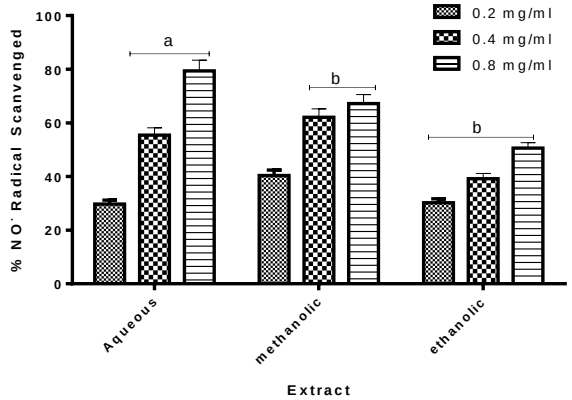

Figure 5. NO radical scavenging ability of *S. acuta* leaf extracts. Data represent mean ± SD of an experiment performed in triplicate. (a) indicates a significant difference at P< 0.05 compared to (b).

Water, with stronger dielectric constant than methanol and ethanol (Wang and Weller, 2006), may preferentially enhance the aqueous extraction of certain polyphenolic compounds-in the extract-with higher potentials to scavenge NO• radicals. At physiological concentration, NO• plays critical roles in neurotransmission and vasodilation (Andrew, 2007; Bhaskar and Balakrishnan, 2009), but when there is a perpetual-concentration-surge beyond the tolerable thresholds, nitric oxide can elicit inflammatory responses and other associated pathologies. Therefore, ability to quench NO• radical in vitro, is one of the routine methods for measuring the potential antioxidant activity of pharmacological agents (Lakhanpal and Rai, 2009; Lucian *et al.*, 2014).

Consequently, since free radicals are the major molecular felons in the onset, progression and complications of several diseases such as cancer, diabetes, metabolic disorders, atherosclerosis, and cardiovascular diseases (Kumari *et al.*, 2018), evaluation of phytochemical constituents (flavonoids and phenolic) and antioxidant potentials remain relevant to the claims that *S. acuta* poses medicinal values.

Furthermore, the GC-MS chromatogram of the methanolic extract (the extract with relative abundance of phytochemical constituents) shows the presence of 23 major peaks with the retention time range between 10.08 and 68.98 (Figure 6).

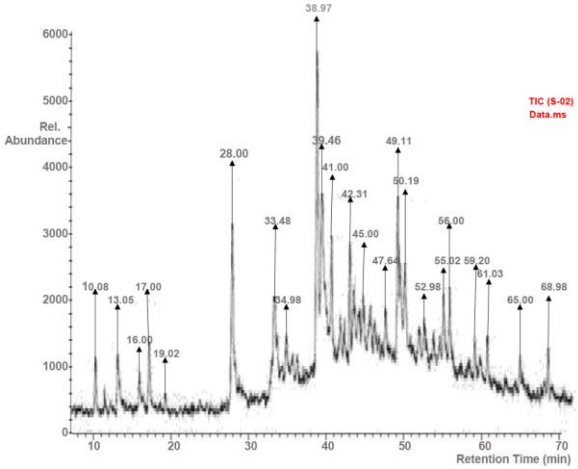

Figure 6. GC-MS chromatogram of methanolic leaf extract of *Sida acuta.*

The active principles with their retention time (RT), molecular weight (MW) and the concentration (peak area percentage) are presented in Table 1.

Table 1. Bioactive compound identified in the methanolic leaf extract of *S. acuta*.

Peak	RT	Name of Compounds	Mol. Formula	MW	Peak Area %	Comp. (% wt)	m/z
1	10.08	Quinoline, 4-methyl-	$C_{10}H_9N$	143	2.49	2.56	77, 115, 143
2	13.05	2-Methoxy-4-vinylphenol	$C_9H_{10}O_2$	150	2.52	3.77	77, 107,150
3	16.00	2',6'-Dimethoxyacetophenone	$C_{10}H_{12}O_3$	180	1.68	1.21	107, 150, 180
4	17.00	2-Octenoic acid, 4-isopropylidene-7-methyl-6-methylene-, methyl ester	$C_{14}H_{22}O_2$	222	2.94	3.01	43, 119, 222
5	19.02	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284	1.25	1.08	88, 101, 284
6	28.00	3,7,11,15-Tetramethylhexadec-2-en-1-yl acetate	$C_{22}H_{42}O_2$	338	6.75	7.32	43, 87, 338
7	33.48	Quinoxaline, 2-methyl-	$C_9H_8N_2$	144	4.22	2.01	76, 117, 144
8	34.98	Neophytadiene	$C_{20}H_{38}$	278	2.97	0.85	45, 87, 278
9	38.97	13-Tetradecen-1-ol acetate	$C_{16}H_{30}O_2$	254	12.03	14.43	43, 83, 254
10	39.46	9,12-Octadecadienoic acid, ethyl ester	C20H36O2	308	7.57	6.95	67, 81, 308
11	41.00	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	C19H32O2	292	6.39	4.39	79, 95, 292
12	42.31	Methyl stearate	C19H38O2	298	6.31	3.22	74, 87, 298
13	45.00	2,3-Pentanedione	C5H8O2	100	4.21	2.05	43, 57, 100
14	47.64	Methanone, bis(4-methylphenyl)-	C15H14O	210	4.11	3.87	65, 119, 210
15	49.11	2,5-Cyclohexadiene-1,4-dione,5-[(2-hydroxyethyl)methylamino]-2,3-dimethyl-	C11H15NO3	209	7.55	2.64	81, 152, 209
16	50.19	Thianaphthene-2-carboxylic acid	C9H6O2S	178	7.47	2.91	89. 161, 178
17	52.98	4,4'-Dimethoxybenzophenone	$C_{15}H_{14}O_3$	242	3.47	2.84	77, 138, 242
18	55.02	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	$C_{18}H_{30}O_2$	278	4.20	4.18	67, 71, 278
19	56.00	Benzoic acid, 2-(dimethylamino)ethyl ester	$C_{11}H_{15}NO_2$	193	4.21	2.11	58, 71, 193
20	59.20	Carbonic acid, neopentyl 2-ethylhexyl ester	244	244	2.54	2.66	87, 119, 144
21	61.03	Ethyl 9,12,15-octadecatrienoate	$C_{20}H_{34}O_2$	306	2.68	2.35	57, 87, 306
22	65.00	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	$C_{19}H_{38}O_4$	330	2.10	2.11	43, 98, 330
23	68.98	Campesterol	$C_{28}H_{48}O$	400	2.33	3.31	43, 81, 400

Among the bioactive compounds detected, 13-Tetradecen-1-ol acetate (12.0 3%), 9,12-Octadecadienoic acid, ethyl ester (7.57%), 2,5-Cyclohexadiene-1,4-dione,5-[(2-hydroxyethyl) methylamino]-2,3-dimethyl- (7.55 %), Thianaphthene-2-carboxylic acid (7. 47 %) 3,7,11,15-Tetramethylhexadec-2-en-1-yl acetate (6.75 %), methyl ester, (Z,Z,Z)-(7.57 %), 9,12,15-Octade-catrienoic acid, (Z,Z,Z)-(6.39 %), Methyl stearate (6.31 %) are the first seven compounds with higher concertation peaks. Some of these compounds belong alkaloids, phenolic compound, fatty acids and others with broad spectrum of biological activities. For instance, thianaphthene-2-carboxylic acid, is organosulfur compounds known to produce multiple biological effects, such as antioxidant, anti-inflammatory, and fungicides, as well as, other pharmacological and therapeutic properties (Aziz and Karboune, 2018). Other compounds of intermediate peaks are Quinoxaline, 2-methyl-(4.22 %), 2,3-Pentanedione (4.21 %), Dimethoxybenzophenone (3.74 %), Benzoic acid, 2-(dimethylamino) ethyl ester (4.21 %), 9,12,15-Octadecatrienoic acid (4.20 %), Methanone, bis (4-methylphenyl)-(4.11 %), Campesterol (3.31%), methyl ester (3.01%). Campesterol is a plant sterol known to have LDLs and cholesterol lowering effect, ant-carcinogenic agent and boosts progesterone level in luteal phase insufficiency, threatened abortion, recurrent pregnancy loss and subfertility conditions. (Heggen *et al*., 2010; Choudhary and Tran, 2011). In addition, some detected bioactive compounds with less prominent peaks are 4,4'- Carbonic acid, neopentyl 2-ethylhexyl ester (2.54 %), Ethyl 9,12,15-octadecatrien (2.66%), 2,5-Cyclohexadiene-1,4-dione,5-[(2-hydroxyethyl)methylamino]-2,3-dimethyl-(2.64%), Quinoline, 2-Methoxy-4-vinylphenol (2.52 %), 4-methyl- (2.49%) and others are shown in Table 1. Quinolone (an alkaloid) and methoxy-4-vinylphenol (a naturally occurring phenolic compound) are known to produce several pharmacological activities such as antibacterial, antifungal, antimalarial, anthelmintic, anticonvulsant, cardiotonic, anti-inflammatory, and analgesic. (Sermakkani and Thangapandian, 2012; Hameed *et al.*, 2015). Therefore, further investigations on the identified bio-active compounds and screening for its pharmacological activity will be needed for further studies.

4. Conclusion

In view of the presence of quite a number of bioactive compounds belonging to alkoids, phytosterol, polyphenols and the likes in the ethanolic leaf extract of *Sida acuta* plant, the antioxidant properties observed

in this study can be linked to these phytocomponents. This could supports the opinion that the plant is a potential therapeutic agent against oxidative stress-mediated pathologies.

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6. References

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