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GC-MS identification of bioactive components of leaf extract of *Sida acuta* (BURM. F)

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Abstract
Present study was designed to conduct with main purpose to determine bioactive components of aqueous (*aq.*) leaf extract of *Sida acuta* by GC-MS analysis and characterization. In the present study identified 9,12,15-Octadecatrienoic acid ethyl ester (Z, Z, Z), 4-hydrocy-e-methylacetophenone, 1,3-Propanediol, 2-(hydroxymethyl)-2-nitro, 9,12,15-Octadecatrienoic acid ethyl ester (Z, Z, Z), alpha-tocopherol & beta-D-mannoside, l-(+)-Ascorbic acid 2,6-dihexadecanoate, 3-Deoxy-d-mannoic lactone, Hexadecanoic acid and 2-hydroxy-1-(hydroxymethyl) ethyl ester as predominant compounds present in the aq. leaf extract of *Sida acuta* through GC-MS analysis and characterization. In conclusion, all parts of the *Sida acuta* are used for therapeutic purposes, but the leaves are the most widely used and hence, further *in-vitro* and *in-vivo* research investigations are recommended to evaluate the pharmacological activites prevailing compounds identified in aq. leaf extract of *Sida acuta*.

Keywords: *Sida acuta*, Leaf, GC-MS, Antimicrobial, Anti-cancer

1. Introduction
Aspects of the modern lifestyle, such as smoking, overconsumption of alcohol and fast foods with excessive colorants and chemical preservatives place severe oxidative stress on cells and body systems leading to the production of free radicals. These free radicals cause oxidative damage to lipids, proteins and nucleic acids which leads to diseases such as atherosclerosis, cancer, diabetes, inflammation, Alzheimer's and other degenerative diseases [1]. Many plant secondary metabolites are potential free radical scavengers, including flavonoids, anthocyanins, carotenoids, dietary glutathione, polyphenols, vitamins and endogenous metabolites. Free radical scavengers are antioxidants that accept electrons from the free radicals produced *in-vivo* or *in-vitro*. Rutin, morin, quercetin (flavonoids), naringenin (flavone), catechin (flavonol), retinol, tocopherol (vitamins), and curcumin (polyphenol) are well-studied plant derived secondary metabolites that possess anti-cancer, free radical scavenging, anti-ulcer and antimicrobial activities. Flavanols are related to catechins, quercetin, kaempferol, and their glycosides are found in beverages such as green and black teas and red wines. Quercetin occurs in onions and apples, while berries contain myricetin and quercetin. These dietary compounds protect against oxidative stress. Many active pharmaceuticals have been derived from plant secondary metabolites, such as vinca alkaloids and taxol, which effectively treat cancers [2]. Since the beginning of human civilization, medicinal plants have been used by mankind for their nutritional and therapeutic values. Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources. Many of these isolations were based on the uses of these agents in traditional medicine. *Sida acuta* Burm. f (Malvaceae) is one of those plants currently used by indigenous people for the management of some health problems. This plant is an erect, branched small perennial herb or small shrub of about1.5m height [3]. The bark is smooth, greenish, the root is thin, long, cylindrical and very rough; leaves are lanceolate, nearly glabrous, peduncles equal to the petioles, the flowers are yellow, solitary or in pairs; seeds are smooth and black [4, 5]. It grows abundantly on cultivated fields, waste areas and roadsides in Cameroon, where it is called "sengh" in the Western part of the country. It common name is Sida. Once the plant becomes established, it is very competitive, holding and denying sites to other plants. The plant can be propagated both by seed and stem cuttings. All parts of this tree, including leaves, bark, root, seeds and flower are used in folkloric medicine. *Sida acuta* is regarded as astringent, tonic, useful in urinary diseases treatment (diuretic) and also blood disorders (stops bleeding), bile, liver and nervous diseases treatment (sedative) in Indian traditional medicine [5, 6]. In Mexico, smoked as marihuana substitute, and it is also used to treat asthma, renal inflammation, colds, gonorrhea,

fever, bronchitis, malaria, diarrhea, headache, dysentery, abortion, breast cancer, skin diseases, hemorrhoids, insects' bites, erectile dysfunction, elephantiasis, rheumatism and ulcers [7, 8]. It is claimed to have aphrodisiac properties [6]. The root's juice is applied to wounds and the barks are used for measles [9, 10]. In Nigeria, *S. acuta* is one of the plants most commonly used for the treatment of hypertension, using its leaves, seeds and stems in different preparations [11]. All parts of the plant are used for therapeutic purposes, but the leaves are the most frequently request. Leaves are considered to possess demulcent, diuretic, anthelmintic and wound healing properties, and are used to treat rheumatic affections [12]. The leaves decoction is used to treat abdominal pain, hemorrhoids, azoospermia and oligospermia [13]. The leaf juice is also used in India for vomiting and gastric disorders [14]. The roots of the *Sida acuta* species are considered excellent adaptogenic and immunomodulator, general nutritive tonic and prolonged life; useful in tuberculosis and in diseases associated with injury, heart diseases, cough and respiratory diseases [15].

Furthermore, In Sorghum (*Sorghum bicolour*), covered smut (*Sphacelotheca sorghi*), lead smut (*Sphacelotheca sorghi*), and long smut (*Tolyposporium ehrenbergii*) have been reported to be the most destructive pathogens, causing heavy losses in third world countries [16]. Sorghum has been found to be associated with seed-brone pathogen *viz. Fusarium moniliforme* which causes seed rot, *Gloecercospora sorgi* which causes zonate leaf spot, *Sphacelotheca sp.,* which causes smut, *Ascochyta sorghina* causal agent of rough leaf spot. *Fusarium moniliforme* is reported to be serious in sorghum as in rice and maize. This pathogen reduces sorghum stands causing stalk rot, top rot and moldy ears. It may depreciate yield to a great extent [17]. Literature reports evidenced extracts of *Sida acuta* possess antifungal activites against *Candida albicans* [18]. *Aspergillus niger* [19], *Aspergillus flavus* [20]. Hence, extracts of *Sida acuta* can be effectively employed as antimicrobial agents specifically antifungal to control growth and colonization on commercially important plants like Sorghum species. Hence, it is important to isolate phytoactives from plants. The initial steps are extraction and separation of the active phytochemicals from plants before identifying their active ingredients [21]. Methods for identifying such compounds should be simple and repeatable. One of the best methods for identifying these compounds is gas chromatography–mass spectrometry (GC–MS), which can isolate and analyze compounds in a single step using a mass detector and available GC–MS libraries [22]. Therefore, in the present we aimed to identify the active molecules present in aq. leaf extract of *Sida acuta*using simple solvent extraction followed by GC–MS analysis and characterization.

2. Materials and Methods

2.1 Plant material

The leaves of *Sida acuta* were collected from natural habitat at Shivamogga District, Karnataka, India. The plant was identified by Dr. H. N. Ramesh Babu, Associate Professor, Department of Botany and Seed Technology, Sahyadri Science College, Kuvempu University, Shivamogga, Karnataka, India.

2.2 Extraction procedure

The fresh leaves of *Sida acuta* collected was subjected aqueous extraction according to method described by Jose and Radhamany. 500 g of the fresh leaves of *Sida acuta* sample was washed to remove the surface pollutants, dried at 40ºC until complete dry and powdered. These samples were subjected for the successive extraction with water. 25 g of powdered sample was filled in a Whatmann filter paper and kept inside tumble. 200 ml of the water was added in tumble. The tumble was fit into a round bottom flask containing 700 ml of the solvent and run for 6-8 hours at the temperature based on the boiling point of the respective solvent using Soxhlet apparatus. Later the extract was subjected for the distillation for 2-3 hours. These extracts were kept in hot air over at 60ºC for drying. The dried extracts thus obtained were used for GC-MS analysis and characterization [23].

2.3 Gas Chromatography-Mass Spectrometry (GC-MS) Analysis:

Sample preparation: Sample was grinded with GC grade methanol, centrifuged and the supernatant was collected and injected into the GC-MS system.

GC-MS instrument setup details: GC-MS analysis was performed using anAgilent make 5977B GC/MSD System. GC/MS system equipped with an TG 5MS silica Capillary column (30m×0.25mm ID) ×MDF composed of 5% diphenyl/95% dimethyl polysiloxane with 0.25 µm film thickness. For GC/MS detection, an electron ionization system with ionization energy of 70 eV was used. The oven temperature was programmed from 80^0C with a hold of 2 mins and then 200^0C at 9^0C/min and a hold for 4 min and then to 300^0C at 10 ºC/min and a hold for 5 min. Helium was used as carrier gas at flow at the flow rate of 1.5 ml/min. The injector temperature was 250 ºC, injection size 1.0 µlneedle with spitless mode. Injector temperature was 250ºC and ion source temperature was 230ºC. The interface and MS ion source were maintained at 300ºC and 230ºC, respectively. Mass spectra were taken at 70 eV; a scan interval of 0.2 seconds and fragments from with a mass scan range of 50-550 amu. Total GC running time was 35 min. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. Data handling was done using Xcaliber software. The identification of compounds was based on comparison of their mass spectra with those of NIST Libraries. Software adopted to NIST 2014 (2.2.0.0) with AMDIS v.2.72 Version.

3 Results

GC-MS analyses of the aq. extract of *Sida acuta* led to the identification of 45 components (Figure 1). The 45 peaks identified account for 100% of the extractand listed along with respective retention time and the percentage of compound in the extract in Table 1.

Table1: Chemical composition of aqueous extract of *Sida acuta*

Peak No.	RT	Area (%)	Name of Compound
1	3.360	0.18	dl-Glyceraldehyde dimer
2	5.689	0.52	Maltol
3	6.644	0.17	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-
4	7.657	0.97	Benzofuran, 2,3-dihydro-
5	9.020	9.12	4-Hydroxy-2-methylacetophenone

6	9.496	0.17	Phenol, 2,6-dimethoxy-
7	10.188	0.26	2H-Indeno[1,2-b]furan-2-one, 3,3a,4,5,6,7,8,8b-octahydro-8,8-dimethyl
8	10.870	9.52	1,3-Propanediol, 2-(hydroxymethyl)-2-nitro
9	11.246	0.37	4-(2,4,4-Trimethyl-cyclohexa-1,5-dienyl)-but-3-en-2-one
10	12.200	1.47	3',5'-Dimethoxyacetophenone
11	12.783	3.82	3-Deoxy-d-mannoic lactone
12	14.198	1.24	4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol
13	15.253	2.04	3,7,11,15-Tetramethyl-2-hexadecen-1-ol
14	15.506	0.24	3,7,11,15-Tetramethyl-2-hexadecen-1-ol
15	15.705	0.72	3,7,11,15-Tetramethyl-2-hexadecen-1-ol
16	16.108	0.21	Hexadecanoic acid, methyl ester
17	16.463	6.24	l-(+)-Ascorbic acid 2,6-dihexadecanoate
18	16.802	0.60	Benzenemethanol, 2,5-dimethoxy-, acetate
19	17.228	0.15	cis,cis,cis-7,10,13-Hexadecatrienal
20	17.498	0.24	1-(1-Ethyl-2,3-dimethyl-cyclopent-2-enyl)-ethanone
21	17.941	0.34	9,12-Octadecadienoic acid (Z,Z)-, methyl ester
22	17.819	0.79	9,12,15-Octadecatrienoic acid, ethyl ester, (Z,Z,Z)-
23	17.941	0.34	Phytol
24	18.192	22.12	9,12,15-Octadecatrienoic acid, ethyl ester, (Z,Z,Z)
25	18.365	0.22	Octadecanoic acid
26	19.240	0.15	9-Hexadecenoic acid, phenylmethyl ester, (Z)-
27	19.865	0.15	9,12,15-Octadecatrienoic acid, ethyl ester, (Z,Z,Z)-
28	20.092	0.39	Benzyl. beta.-d-glucoside
29	20.937	0.32	Ethanamine, 2,2'-oxybis[N,N-dimethyl-
30	21.160	0.15	(Z)-14-Tricosenyl formate
31	21.292	3.20	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester
32	21.647	1.46	1H-Benzimidazole, 1-(1H-inden-2-yl)-
33	21.936	0.66	Benzo[b]naphtho[2,3-d]furan
34	22.743	7.68	9,12,15-Octadecatrienoic acid, ethyl ester, (Z,Z,Z)
35	23.694	1.05	2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl-, (all-E)
36	24.377	0.35	Cyclohexane, 1,2,3,5-tetraisopropyl-
37	24.624	0.17	Docosanedioic acid, dimethyl ester
38	25.437	0.41	gamma.-Tocopherol
39	26.182	5.49	alpha.-Tocopherol-.beta.-D-mannoside
40	27.313	0.55	Ergost-5-en-3-ol, (3.beta.)-
41	27.674	3.48	Stigmasterol
42	28.380	5.76	gamma.-Sitosterol
43	28.582	0.84	Fucosterol
44	28.938	0.77	4,4,6a,6b,8a,11,11,14b-Octamethyl-1,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,14,14a,14b-octadecahydro
45	30.308	0.96	11 (alpha), 17 (alpha)-Dihydroxyprogesterone

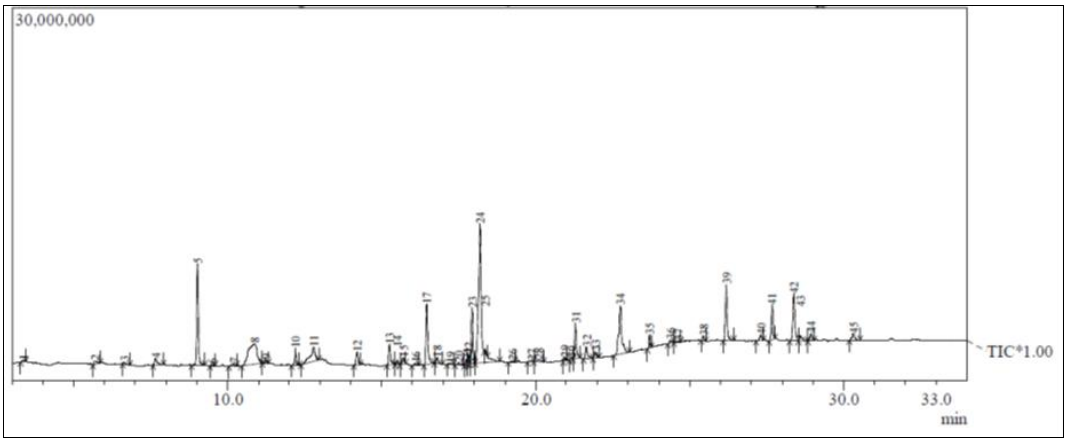

Fig 1: GC-MS spectra of aqueous extract of *Sida acuta*

The GC-MS examinations of the aq. extract of *Sida acuta* prompted the distinguishing proof of 45 compounds. The 45 peaks recognized record for 100% of the extract. Forty-five compounds were detected in the aq. extract of *Sida acuta*. Based on the RT and peak area of individual bioactive compounds, the predominant compounds were 9,12,15-Octadecatrienoic acid ethyl ester (Z,Z,Z) (22.12%), 4-hydrocy-e-methylacetophenone (9.12%), 1,3-Propanediol, 2-(hydroxymethyl)-2-nitro (9.52%), 9,12,15-Octadecatrienoic acid, ethyl ester, (Z,Z,Z) (7.68%), alpha.-Tocopherol-.beta.-D-mannoside (5.76%), l-(+)-Ascorbic acid 2,6-dihexadecanoate (6.24%), 3-Deoxy-d-mannoic lactone (3.82%), Hexadecanoic acid and 2-hydroxy-1-(hydroxymethyl) ethyl ester (3.2%). Their chemical structures were predicted using the mass spectra based on their fragmentation, which generates peaks with different mass-to-charge ratios (m/z).

4 Discussion

For thousands of years, plants and herbs have been a

tremendous source of food and medicine. Various parts of *Sida acuta* have been reported in many studies to be used by indigenous people from tropical countries to manage some health problems: rheumatic affections, azoospermia, oligospermia and spermatorrhea, leucorrhoea, wounds, sciatica, nervous, heart diseases, cold, cough, asthma, tuberculosis and respiratory diseases, disorders of the blood, bile and liver, elephantiasis, hemorrhoids, ulcers, gastric disorders, abdominal pain, headache, fever, malaria, skin diseases, worms, diarrhea, dysentery, venereal diseases, renal inflammation, toothache and snake bites. *Sida acuta* has been scientifically studied for its numerous pharmacological profiles such as: antioxidant, antimicrobial, antibacterial, antimalarial, cardiovascular, antiulcer, analgesic, anti-inflammatory, antipyretic, hepatoprotective, hypoglycemic, insecticidal and anticancer. Bioactive constituents such as alkaloids, saponins, coumarins, steroids, tannins, phenolic compounds, cardiac glycosides, sesquiterpene and flavonoids, significantly present in the plant extract of *Sida acuta* account for its multiple properties and uses in traditional medicine [12].Hence, the present study was carried out to identify the active molecules present in aq. leaf extract of *Sida acuta* using simple solvent extraction followed by GC–MS analysis and characterization. Many scientific researchers have been carried out in order to determine the chemical composition of *Sida acuta*. Almost all parts of the plant are concerned by these researches, but leaves and root are the most studied. The phytochemical screening of *Sida acuta* species revealed the presence of alkaloids such as vasicine, ephedrine and cryptolepine (the main alkaloid in the plant) [24, 25], saponosides, coumarins, steroids (ecdysterone, β-sistosterol, stigmaterol, ampesterol), tannins, phenolic compounds (evofolin-A, and B, scopoletin, loliolid and 4-ketopinoresinol), polyphenol, sesquiterpene and flavonoids [26].In another study, Nwankpa *et al.* evaluated the phytochemical and micronutrient composition of *Sida acuta* using standard analytical methods. The phytoconstituents includes tannins, alkaloids, saponins, flavonoids, steroids, terpenoids, and cardiac glycosides. The vitamin composition was thiamin, niacin, ascorbic acid, tocopherol, riboflavin, while mineral composition was found to be calcium, magnesium and zinc, respectively [27]. In our study, the predominant compounds identified through GC-MS analysis were 9,12,15-Octadecatrienoic acid ethyl ester (Z,Z,Z), 4-hydrocy-e-methylacetophenone, 1,3-Propanediol, 2-(hydroxymethyl)-2-nitro, 9,12,15-Octadecatrienoic acid ethyl ester (Z, Z,Z), alpha-tocopherol & beta-D-mannoside, l-(+)-Ascorbic acid 2,6-dihexadecanoate, 3-Deoxy-d-mannoic lactone, Hexadecanoic acid and 2-hydroxy-1-(hydroxymethyl) ethyl ester. In the study conducted by Muneeswari *et al.*, the GC-MS analysis observed the presence of 35 different compounds each belonging to different classes such as sterols, flavonoids, terpenes, heterocyclic aromatic compounds, phenols, fatty acids, vitamins, alkaloids, and sesquiterpenoids. The results indicate that the ethanolic extract of *Sida acuta* leaves collected from the Tuticorin District of Tamil Nadu is an effective scavenger of free radicals and has the potential to be used as a natural antioxidant which is attributable to the rich presence of its secondary metabolites [28].

Akilandeswari *et al.* carried out antibacterial and antifungal activity studies of leaf extracts of *Sida acuta*. Antibacterial and antifungal activity studies of leaf extracts of *Sida acuta* were carried out. Two common solvents (Chloroform and Ethanol, 95% each) were used successively for extraction of active principles from the dried powdered leaves. The antimicrobial screening was done with two Gram +ve (*Staphylococcus aureus* NCIM 2079, *Bacillus subtilis* NCIM 2063) and two Gram–ve (*Escherichia coli* NCIM 2065 *Pseudomonas aeruginosa* NCIM 2036) bacteria and fungi (*Candida albicans* NCIM 3102, *Aspergillus niger* NCIM 1054) as test microorganisms. All the three microorganisms were markedly affected by both the extracts under study, with the maximum activity recorded against gram positive *Staphylococcus aureus* and gram negative *Escherichia coli* respectively. These effects were similar to that of commercially available antibiotics under the same laboratory condition [19]. The similar result was previously obtained by Oboh *et al.* [29]. In another study, antimicrobial activity ofaqueous and ethanol leaves extracts of Sida acuta against 45clinical isolates of *Staphylococccus aureus* isolated fromnasal cavity of Human Immunodeficiency Virus/Acquired Immunodeficiency Syndrome (HIV/AIDS) patients from University of Nigeria teaching hospital, Enugu, was evaluatedusing agar well diffusion method [30]. The minimum inhibitory concentration (MIC) of the extracts was also determined using agar well diffusion technique and the killing rate of each extract was also determined at different time intervals of 0-90 min. The results of the agar well diffusion study revealed that ethanol extracts produced the highest antimicrobial activity (86%), followed by hot water (61%) and cold-water extracts (48%). The MIC obtained ranged from 0.9625-1.8125 µg /mL for ethanol extracts, 7.8125-31.25 µg/mL for hot water and 15.625-31.25 µg/mL for cold water extracts. The result of killing rate studies showed that the test organisms were killed within 0-10 min for ethanol and hot water extracts and 5-60 min for cold water extracts. The overall results indicated that *Sida acuta* extracts have appreciable antimicrobial activity against *Staphylococccus aureus* isolated from HIV/AIDS patients. In addition to authenticating the folkloric use of *Sida acuta* in the treatment of common diseases, the finding of these studies highlights the possible usefulness of this plant material in the treatment of opportunistic infections caused by *Staphylococccus aureus* in HIV/AIDS patients. Kannan *et al.* screened cardio active herbs from Western Ghats of India. The heart beat rate (HBR) and blood flow during systole and diastole were tested in Zebrafish embryos. The methanol extract of *Sida acuta* led to decrease in the HBR in Zebrafish embryos, which was greater than that caused by Nebivolol (used as reference drug) [31]. Hepatoprotective effects of methanol extract of *Sida acuta* were obtained against liver damage induced by paracetamol overdose as evident from decreased serum levels of glutamate pyruvate transaminase, glutamate oxaloacetate transaminase, alkaline phosphatase and bilirubin in the *Sida acuta* treated groups compared to the intoxicated controls [5]. Akilandeswari *et al.* reported that wounds contracting ability of the methanol extract of *Sida acuta* ointment produced significantly greater response in wound types when compared with control. Furthermore, Akilandeswari *et al.* reported the significant antiulcer activity against all the three-ulcer inducing experimental models, by reducing theulcer index in those models [19]. The antiulcer activity of ethanol extract of whole plant of *Sida acuta* was also supported by Malairajan *et al.* [32]. Overall, *Sida acuta* has been scientifically studied for its numerous pharmacological profiles such as antioxidant, antimicrobial, antibacterial, antimalarial, cardiovascular, antiulcer, analgesic, anti-inflammatory, antipyretic, hepatoprotective, hypoglycemic, insecticidal and anticancer. Earlier studies

evidenced that a variety of naturally occurring fatty acids was operational in the promotion of ideal health. In addition to its major role in cardio protection, these fatty acids possessed anti-cancer and free-radical scavenging effects, and hence the extract might be used as a promising natural source of anticancer substance [33]. The plant-based sterols have been reported to contain various roles in the prevention of human pathologies [34]. Further studies need to examine molecules that are present at high concentrations and have potential biological activity. In the future, we plan to isolate compounds from different parts of *Sida acuta* and evaluate their pharmacological activities. Also we planned to evaluate the antifungal activities of phytoactives of *Sida acuta* against fungal species associated with Kharif Sorghum since literature reports evidenced antifungal activates of *Sida acuta* extracts [18-20].

5 Conclusions

All parts of the *Sida acuta* are used for therapeutic purposes, but the leaves are the most frequently requested. In our study we identified 9,12,15-Octadecatrienoic acid ethyl ester (Z, Z, Z), 4-hydrocy-e-methylacetophenone, 1,3-Propanediol, 2-(hydroxymethyl)-2-nitro, 9,12,15-Octadecatrienoic acid ethyl ester (Z, Z, Z), alpha-tocopherol &Beta-D-mannoside, l-(+)-Ascorbic acid 2,6-dihexadecanoate, 3-Deoxy-d-mannoic lactone, Hexadecanoic acid and 2-hydroxy-1-(hydroxymethyl) ethyl ester as predominant compounds present in the *aq*. leaf extract of *Sida acuta* through GC-MS analysis and characterization. Hence, further *in-vitro* and *in-vivo* research investigations are recommended to evaluate the pharmacological activites especially antifungal activities of compounds identified in aq. leaf extract of *Sida acuta*.

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