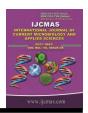


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GC-MS Identification of Stem Extract of Sida acuta (Burm.f)

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ABSTRACT

Keywords

Sida acuta, Stem, GC-MS, Antimicrobial, Antifungal, Antiinflammatory

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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. Furthermore, stem part of Sida acuta plant has less studied and hence, it is important to isolate phytoactives from stem extract of Sida acuta. Hence, the present study was designed to conduct with main objective to determine bioactive components of aqueous (aq.) stem extract of Sida acuta by GC-MS analysis. The fresh stem parts of Sida acuta collected was subjected aqueous extraction by successive extraction with water. In the present study we identified 9, 12, 15-Octadecatrienoic acid, ethylester, (Z, Z, Z), gamma-Sitpsterol, 1-(+)-Ascorbicacid2, 6-dihexadecanoate, 9, 12-Octadecadienoicacid(Z, Z), 4-Hydroxy-2-methylacetophenone, 1, 3-Propanediol, 2-(hydroxymethyl)-2-nitro, Stigmasterol and Trilinolein as predominant compounds present in the aq. stem extract of Sida acuta through GC-MS analysis and characterization. In conclusion, this is the preliminary study wherein we identified eleven bioactive compounds in aq. stem extract of Sida acuta and hence, further invitro and in-vivo research investigations are recommended to evaluate the pharmacological activities of prevailing compounds identified in aq. stem extract of Sida acuta.

Introduction

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 people used indigenous the management of some health problems. This plant is an erect, branched small perennial herb or small shrub of about1.5m height (Mohiden et al., 2002). 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 (Gamble et al., 1935; Sreedevi et al., 2009). 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 (Tcheghebe et al., 2017).

All parts of the Sida acuta are used for therapeutic purposes, but the leaves are the frequently request. Leaves most are considered to possess demulcent, diuretic, anthelmintic, wound healing properties and are used to treat rheumatic affections (Tcheghebe et al., 2017). The leaves decoction is used to treat abdominal pain, hemorrhoids, azoospermia and oligospermia (Mshana et al., 2000). The leaf juice is also used in India for vomiting and gastric disorders(Ramachandra and Nair 1981). The roots of the Sida 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 (Nadkarni et al., 1976). Root is also claimed to possess aphrodisiac, antirheumatic, stomachic. diaphoretic, diuretic, antipyretic and wound

healing properties (Mohiden et al., 2002). The root extract is taken in the case of leucorrhoea (Saraswathy et al., 1976), breathing problems and cough(Silija et al., 2008). In Papua New Guinea, the fresh root is chewed for the treatment of dysentery (Hodlsworth 1974). In Indian traditional medicine, the root of Sida acuta is extensively used as a stomachic, diaphoretic and antipyretic. It is regarded as cooling, astringent, tonic and useful in treating nervous and urinary diseases and also disorders of the blood, bile and liver (Khare et al., 2002), while the hot water extract of the dried entire plant is administered orally as a febrifuge, an abortifacient and a diuretic (Kholkute et al., 1978). It is also used to treat gonorrhea, elephantiasis, ulcers and is claimed to have aphrodisiac properties. The juice of the root is applied to wounds. The whole plant extract is often assumed to treat diseases such as fever, headache, skin diseases, diarrhea and dysentery (Ignacimuthu et al., 2006). It is used as diuretic in rheumatic infections, used as a gonorrhoea and chronic demulcent in dysentery (Nadkarni et al., 1976). Sida acuta is an ingredient in Siddha formulation indicates in rheumatism, facial paralysis, pulmonary tuberculosis, sciatica, haemorrhage, spermatorrhoea, leucorrhoea and gonorrhea (Saraswathy et al., 1976).

Sida acuta plant is credited with abortifacient, anthelmintic and antiemetic properties (Jain 1991). In Nicaragua, the decoction of the entire plant is taken orally for asthma, fever, aches, pains, ulcers and as an anti-worm medication; while a decoction of the dried entire plant is taken orally for venereal diseases (Barrett 1994; Coe and Anderson 1996). In Central America, the plant is used to treat asthma, renal inflammation, cold, fever, headache, ulcers and worms (Caceres et al., 1996). In Colombia the whole plant is known to treat snake bites and it lessened the hemorrhagic effect of Bothrops atrox venom (Otero et al., 2000; Trivedi et al., 2013). In

Cameroon, the whole plant is claimed to possess antiinflammatory, antirheumatic, diaphoretic, febrifuge, sedative, anti-ulcer and anthelmintic properties. A decoction of the whole plant is used for the treatment of fever and rheumatism and to eradicate intestinal worms. The same decoction is also used to treat malaria (Olivier *et al.*, 2016).

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 (Osunlaja 1990). 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 (Alka et al., 2012). Literature reports evidenced extracts of Sida acuta possess antifungal activites against Candida albicans (Akilandeshwari et al., 2010), Aspergillus nigerand Aspergillus flavus (Ezeabara and Egenti 2018). 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.

Literature survey revealed that stem part of *Sida acuta* plant has less studied and hence, it is important to isolate phytoactives from stem extract of *Sida acuta*. The initial steps are extraction and separation of the active phytochemicals from plants before identifying their active ingredients (Karimi and Jaafar 2011). 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 (Gomathi *et al.*, 2015). Therefore, the present research investigation was carried out to identify the active molecules present in aq. stem extract of *Sida acuta* using simple solvent extraction followed by GC-MS analysis.

Materials and Methods

Plant Material

The stem part 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.

Extraction Procedure

The fresh stem parts of Sida acuta collected was subjected aqueous extraction according to method described by Jose and Radhamany. 500 g of the fresh stem parts of Sida acuta sample was washed to remove the surface pollutants, dried at 40°C 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 60°C for drying. The dried extracts thus obtained were used for GC-MS analysis and characterization (Jose and Radhamany 2013).

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

Sample preparation

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

GC-MS instrument setup details

GC-MS analysis was performed using an Agilent 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°C with a hold of 2 mins and then 200°C at 9°C/min and a hold for 4 min and then to 300°C at 10°C/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°C, injection size 1.0 µl needle with spitless mode. Injector temperature was 250°C and ion source temperature was 230°C. The interface and MS ion source were maintained at 300°C and 230°C, 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.

Results and Discussion

GC-MS analyses of the aq.stem extract of *Sida acuta* led to the identification of 41 components(Figure 1). The 41 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.

The GC-MS examinations of the aq.stem prompted extract of Sida acuta distinguishing proof of 41 compounds. The 41peaks recognized record for 100% of the extract. Forty-one compounds were detected in the aq. stem extract of Sida acuta. Based on the RT and peak area of individual bioactive compounds, the predominant compounds were 9,12,15-Octadecatrienoic acid, ethylester, (Z,Z,Z)- (14.5%), gamma-Sitpsterol (12.14%), Ascorbicacid 2,6-dihexadecanoate 1-(+)-9,12-Octadecadienoicacid(Z,Z)-(11.24%),4-Hydroxy-2-methylacetophenone (9.65%),(6.94%), 1,3-Propanediol, 2-(hydroxymethyl)-2-nitro- (6.25%), Stigmasterol (6.00 %) and Trilinolein (4.47%). Their chemical structures were predicted using the mass spectra based on their fragmentation, which generates peaks with different mass-to-charge ratios (m/z).

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. 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 (Tacheghebe et al., 2017). Extracts of Sida acuta can be effectively employed as antimicrobial agents specifically antifungal to colonization control growth and

commercially important plants like Sorghum species since literature reports evidenced antifungal activites of *Sida acuta* extracts (Alka *et al.*, 2012; Akilandeshwari *et al.*, 2010; Ezeabara and Egenti 2018). Moreover, that stem part of Sida acuta plant has less studied. Hence, in the present study we aimed to identify the active molecules present in aq. stem extract of *Sida acuta* using simple solvent extraction followed by GC–MS analysis.

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) (Prakash et al., 1981; Karou et al., 2005), saponosides, coumarins, (ecdysterone, β-sistosterol, steroids stigmaterol, ampesterol), tannins, phenolic compounds (evofolin-A, B-scopoletin, loliolid and 4-ketopinoresinol), polyphenol, sesquiterpene and flavonoids (Konate et al., 2010). In another study, Nwankpa et al., evaluated the phytochemical and micronutrient composition of Sida acuta using analytical methods. standard 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 (Nwankpa et al., 2015).

In our study, the predominant compounds identified through GC-MS analysis were 9, 12, 15-Octadecatrienoicacid, ethylester, (Z, Z, Z), gamma-Sitpsterol, l-(+)-Ascorbicacid2, 6-dihexadecanoate, 9, 12-Octadecadienoic acid

(Z, Z), 4-Hydroxy-2-methylacetophenone, 1, 3-Propanediol, 2-(hydroxymethyl)-2-nitro, Stigmasterol and Trilinolein. In the study conducted by Muneeswari et al., the GC-MS analysis observed the presence of 35 different compounds each belonging to different classes sterols. flavonoids, such terpenes, heterocyclic aromatic compounds, phenols, acids, vitamins, alkaloids fatty 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 (Muneeswari et al., 2019).

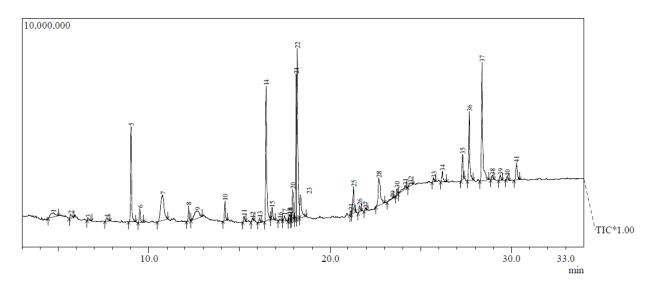
Akilandeswari et al., carried out antibacterial and antifungal activity studies of leaf extracts of Sida acuta. Antibacterial and antifungal activity studies of leaf extracts of Sida acuta were carried out. Two common solvents (Chloroform and Ethanol, 95% each) were used successively for extraction of 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 laboratory same condition (Akilandeshwari et al., 2010). The similar result was previously obtained by Oboh et al., (Obah et al., 2007).

Table.1 Chemical composition of aq. stem extract of Sida acuta

Peak No.	RT	Area (%)	Name of Compound
1	4.727	1.55	N, N-Dimethylglycine
2	5.683	0.50	1,3,5-Triazine-2,4,6-triamine
3	6.650	0.24	4H-Pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl-
4	7.672	0.41	Benzofuran,2,3-dihydro-
5	9.018	6.94	4-Hydroxy-2-methylacetophenone
6	9.507	1.09	Pyridine,3-(1-methyl-2-pyrrolidinyl)-,(S)-
7	10.761	6.25	1,3-Propanediol, 2-(hydroxymethyl)-2-nitro-
8	12.201	1.11	3',5'-Dimethoxyacetophenone
9	12.664	2.74	3-Deoxy-d-mannoiclactone
10	14.201	1.49	4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol
11	15.256	0.42	3,7,11,15-Tetramethyl-2-hexadecen-1-ol
12	15.726	0.23	Syn-Tricyclo[5.1.0.0(2,4)]oct-5-ene,3,3,5,6,8,8-hexamethyl-
13	16.110	0.29	Hexadecanoicacid, methylester
14	16.471	11.24	l-(+)-Ascorbicacid2,6-dihexadecanoate
15	16.805	0.90	Benzenemethanol,2,5-dimethoxy-,acetate
16	17.246	0.20	Z,Z-8,10-Hexadecadien-1-ol
17	17.499	0.48	1-(1-Ethyl-2,3-dimethyl-cyclopent-2-enyl)-ethanone
18	17.760	0.43	9,12-Octadecadienoicacid(Z,Z)-,methyl ester
19	17.816	0.33	9,12,15-Octadecatrienoicacid,ethyl ester, (Z,Z,Z)-
20	17.941	1.87	Phytol
21	18.131	9.65	9,12-Octadecadienoicacid(Z,Z)-
22	18.184	14.5	9,12,15-Octadecatrienoicacid,ethylester, (Z,Z,Z)-
23	18.371	2.93	Octadecanoicacid
24	21.148	0.23	Bacteriochlorophyll-c-stearyl
25	21.294	2.22	Hexadecanoicacid,2-hydroxy-1-(hydroxymethyl)ethylester
26	21.627	0.58	1,2-Benzenedicarboxylicacid,mono(2-ethylhexyl)ester
27	21.956	0.23	1H-Phenanthro[9,10-c]pyrazole
28	22.684	4.47	Trilinolein
29	23.451	0.22	Hexatriacontane
30	23.696	0.43	2,6,10,14,18,22-Tetracosahexaene,2,6,10,15,19,23-hexamethyl-, (all-
31	24.142	0.71	Tetrapentacontane
32	24.442	0.52	Tetracosamethyl-cyclododecasiloxane
33	25.705	0.32	Tetrapentacontane
34	26.190	1.01	26-Nor-5-cholesten-3.betaol-25-one
35	27.314	2.03	Ergost-5-en-3-ol, (3.beta.)-
36	27.676	6.00	Stigmasterol
37	28.385	12.14	gammaSitosterol

38	28.949	0.43	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-oc
39	29.394	0.44	Ergosta-4,22-dien-3-one
40	29.774	0.33	Vitamin E
41	30.284	1.85	Stigmast-4-en-3-one

Fig.1 GC-MS spectra of aq. stem extract of Sida acuta



In another study, antimicrobial activity of aqueous and ethanol leaves extracts of Sida acuta against 45clinical isolates of Staphylococccus aureus isolated from nasal Immunodeficiency cavity Human Virus/Acquired Immuno deficiency Syndrome (HIV/AIDS) patients from University of Nigeria teaching hospital, Enugu, evaluated using agar well diffusion method(Iroha et al., 2009). 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 appreciable extracts have 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 cardioactive 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) (Kannan and Vincent 2012).

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(Sreedevi *et al.*, 2009). 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 the ulcer index in those models (Akilandeshwari *et al.*, 2010). The antiulcer activity of ethanol extract of whole plant of *Sida acuta* was also supported by Malairajan *et al.*, (2006).

Overall, Sida acuta has been scientifically studied for its numerous pharmacological profiles such as antioxidant, antimicrobial, antibacterial. antimalarial. cardiovascular. analgesic, anti-inflammatory, antiulcer, 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 cardioprotection, 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 (Arab et al., 2016). The plant-based sterols have been reported to contain various roles in the prevention of human pathologies (Han et al., 2016).

Each year, about 20% of the sorghum that otherwise would be available for food and feed is lost due to diseases. Seed health plays an important role in the successful cultivation

and yield exploitation of a crop species. Healthy seed plays an important role not only for successful cultivation but also for increasing the yields of crops. Seed-borne pathogens of sorghum are responsible for variation in plant morphology and also reducing yield up to 15 to 90% if untreated seeds are grown in the field.

Several seed-borne pathogens are known to be associated with sorghum seed which are responsible for deteriorating seed quality during storage. Seed stored in humid and warm environments tend to absorb moisture from the surroundings, leading to increased seed moisture content until equilibrium is established. As seed moisture content increases, the rate of deterioration also increases (Jayashree et al., 2019). Therefore, in future studies we planned to evaluate the antifungal activities of phytoactives of Sida acuta against fungal species associated with Kharif Sorghum since literature reports evidenced antifungal activites of Sida acuta extracts (Alka et al., 2012; Akilandeshwari et al., 2010; Ezeabara and Egenti 2018).

To the best of our literature this is the preliminary study wherein we identified 9, 12, 15-Octadecatrienoicacid, ethylester, (Z, Z, Z), gamma- Sitpsterol, 1-(+)-Ascorbicacid2, 6-dihexadecanoate, 9, 12-Octadecadienoic acid(Z, Z), 4-Hydroxy-2-methylacetophenone, 1, 3-Propanediol, 2-(hydroxymethyl)-2-nitro, Stigmasterol and Trilinolein as predominant compounds present in the *aq*.stem 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. stem extract of *Sida acuta*.

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