IJPSR (2019), Volume 10, Issue 3 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 20 June 2018; received in revised form, 03 October 2018; accepted, 05 October 2018; published 01 March 2019

IDENTIFICATION OF ACTIVE PHARMACEUTICALS OF *SIDA ACUTA* BURM. F. LEAVES USING GC-MS AND HPTLC FINGERPRINTING

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Keywords:

Sida acuta, Sesquiterpenoids, Terpenes, Glutathione

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ABSTRACT: *Sida acuta* is one of the medically active plants used for the treatment of multifaceted diseases. However, an elaborated investigation on the phytochemical composition of the ethanolic extract of the leaves of this plant is yet to be deciphered. So, in this quantification of phytochemicals, *in-vitro* free radical scavenging activity, enzymatic and non-enzymatic antioxidant levels in the fresh leaves, HPTLC fingerprinting and GC-MS analysis in the ethanolic extract of *Sida acuta* leaves were done. *In-vitro* antioxidant activities were assayed using DPPH, ABTS, nitric oxide, hydroxyl radical and ferric ions, while ascorbic acid is used as the standard. The results indicated the presence of flavonoids, tannins, phenols, and alkaloids in a reasonably good amount which has substantiated the results of HPTLC. All the tested antioxidants were present prominently in the leaves, specifically catalase and glutathione peroxidase, which may be responsible for the prominent radical scavenging tendency of the extract against the tested free radicals. 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.

INTRODUCTION: The importance of herbal extracts and phytochemical formulation in the treatment of various ailments are gaining much attention due to their various pharmacological effects as well as their affordability to common people in many parts of the world.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.10(3).1194-07

The article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.10(3).1194-07

Though plenty of pharmaceutical companies manufacture a wide range of allopathic medicines, due to their nature of causing increasing side effects, the public who suffer from chronic diseases tends to opt for alternative/traditional medicines [1].

It is an inevitable fact that increasing research on ethnomedicine could pave the way for the discovery of novel therapeutic agents against plenty of outstanding diseases in the current scenario. Interestingly, WHO has recognized the significance of traditional medicine in the health-care sector and has assessed that approximately eighty percent of the population living in the developing countries

depend on herbal medicines for their primary health care requirement [2]. Phytochemicals are the predominant substances present in the phytomedicine responsible for any observed physiological action. These phytochemicals are present in the form of alkaloids, steroids, tannins, glycosides, volatile oils, phenols, and flavonoids, which are non-specifically confined to various parts of a plant such as leaves, flowers, bark, seeds, fruits or roots. Malvaceae is one of the plant families encompassing tentatively 4225 species of plants including herbs, shrubs, and trees. *Sida acuta* belongs to this family and has been used as traditional medicine since ancient times. Fascinatingly, different parts of this plant have been used by the tribes located in India to treat the nervous disorder, reproductive problems, renal problems and rheumatism [3, 4]. Furthermore, the plant also possesses antimalarial, antibacterial, anti-inflammatory, analgesic and hepatoprotective properties [5, 6]. Even in countries outside India like Nigeria, the extract prepared from leaves, seeds, and stems of *Sida acuta* is used as an anti-hypertensive agent in different solvent combinations [7]. It is to be highlighted that the whole plant extract has been reported to be used as ointments or external bath against helminths and snake bite [8].

The *Sida acuta* present in the Kalugumalai, Tuticorin District of Tamil Nadu has not been screened extensively for the identification of phytochemical composition. Hence, the present work intends to investigate the phytochemical composition of *Sida acuta* leaves using quantitative analysis, HPTLC, GC-MS analysis and to assay the antioxidant potential using *in-vitro* free radical scavenging activities.

MATERIALS AND METHODS:

Plant Collection: The plant leaves of *Sida acuta* was collected in and around the area of K. Vengadeshwarapuram, Kalugumalai, Tuticorin District, Tamil Nadu and it was authenticated by Dr. GVS Moorthy, Scientist G, Botanical Survey of India, TNAU Campus, Coimbatore, Tamil Nadu India. The voucher number is BSI/SRC/5/23/2016/ Tech./348. Collected whole plant material was washed under running tap water, air-dried, powdered and stored in airtight container for further studies.

Preparation of Extract: 50 g of powdered leaf materials were weighed and extracted with 250 ml of ethanol for 72 h with occasional shaking. The supernatant was collected and concentrated at 40°C. It was stored at 4°C in airtight bottles for further studies.

Quantification of Phytochemicals:

Total Phenolic Content: Total phenolic content was determined using the Folin-Ciocalteau reagent. Folin-Ciocalteau colorimetry is based on a chemical reduction of the reagent, a mixture of tungsten and molybdenum oxides. The products of the metal oxide reduction have a blue absorption with a maximum at 765 nm. By using standard Gallic acid calibration curve, measure the concentration of phenolic content is measured and expressed as gallic acid, total equivalents using unit's mg/g (GAE) [9].

Total Flavonoid Content: Flavonoids reacted with aluminum chloride in ethanolic solution forming a yellow color which was read calorimetrically at 420 nm. A volume of 0.5 ml of 2% of $AlCl_3$ in ethanol solution was added to 0.5 ml of the sample solution. After an hour's incubation at room temperature, yellow color was formed. This was measured at 420 nm with UV-Visible spectrophotometer. A standard graph was prepared using the quercetin, and the total flavonoid content was expressed as quercetin equivalent (mg/g) [10].

Total Tannin Content: Tannins are widespread in nature and probably in all plant materials. The polyphenolic compounds are divided into 2 main groups, hydrolyzable and condensed. The estimation of tannin is based on the stoichiometric oxidation of molecules containing a phenolic hydroxyl group. Tannin reduces phosphomolybdic acid in an alkaline condition to lower oxides of molybdenum producing a colored complex, the absorbance of which is measured at 700 nm [11].

Enzymatic and Non-Enzymatic Antioxidant Assay:

Fresh Plant Sample Extraction: The fresh samples were prepared by grinding one gram of *Sida acuta* Burm. F. leaves in 2 ml of 50% ethanol, separately, in a pre-chilled mortar and pestle and the extracts were centrifuged at 10,000 g at 4 ºC for 10 min. The supernatants thus obtained were used

within four hours for various enzymatic and non-enzymatic antioxidants assays, such as SOD, catalase, glutathione peroxidase, glutathione reductase, glutathione S-transferase, total reduced glutathione, vitamin C and vitamin E. These assays were determined by the standard methods [12-19]. All assays were performed in triplicate.

Free Radical Scavenging Activities: The plant was used for free radical scavenging assays such as DPPH radical scavenging assay, nitric oxide and hydroxyl radical scavenging assays, ABTS assay, H_2O_2, and FRAP assay. These assays were determined by the standard methods [20-25]. All these tests were performed in triplicate.

HPTLC Fingerprinting Analysis:

Procedure: 50 mg of the given plant extract, weighed in an electronic balance (Afcoset) was dissolved with 500 µl of ethanol and centrifuged at 3000 rpm for 5 min. This solution was used as test solution for HPTLC analysis. 2 µl of the test solution and 2 µl of standard solution were loaded as 5 mm band length in the 3 × 10 silica gel $60F_{254}$ TLC plate using Hamilton syringe and CAMAG LINOMAT 5 instrument. The sample-loaded plate was kept in TLC twin trough developing chamber (after being saturated with Solvent vapor) with respective mobile phase and ran up to 90 mm.

The developed plate was dried by hot air to evaporate solvents from the plate. The plate was kept in Photo-documentation chamber (CAMAG REPRO-STAR 3) and the images were captured at visible light, UV 254 nm and UV 366 nm. The developed plate was sprayed with respective spray reagents and dried at 100 °C in a hot air oven. The plate was photo-documented at visible light and UV 366 nm using Photo-documentation chamber.

After derivatization, the plate was fixed in scanner stage (CAMAG TLC SCANNER 3) and scanning was done at UV 366 nm. The peak table, peak display, and peak densitogram were noted. The following mobile phases such as ethyl acetate-methanol-water, ethyl acetate-butanone-formic acid-water, toluene-ethyl acetate - formic acid-methanol, ethyl acetate-ethanol-water, and toluene-acetone were used for the separation of alkaloids, flavonoids, tannins, glycosides, and steroids, respectively.

GC-MS Analysis: GC-MS analysis of the ethanolic extract of *Sida acuta* leaves was performed using the equipment Thermo GC-Trace Ultra Version: 5.0, Thermo MS DSQ II. The equipment has a DB 35 - MS Capillary Standard Non-polar column with dimensions of 30 min 0.25 mm ID × 0.25 µm Film. The carrier gas used is Helium with a flow of 1.0 ml/minute. The injector was operated at 250 ºC and the oven temperature was programmed as follows; 60 ºC for 15 min, then gradually increased to 280 ºC for 3 min. The identification of components was based on the comparison of their mass spectra with those of Wiley and NBS libraries as well as a comparison of their retention indices. The constituents were identified after comparison with those available in the computer library (NIST) attached to the GC-MS instrument and the results obtained have been tabulated.

Statistical Analysis: The data are expressed as mean ± SD from three independent experiments. The statistical analysis and IC_{50} values were calculated using Microsoft excel (2007) of the Windows operating system.

RESULTS:

Quantitative Phytochemical Analysis in Ethanolic Extract of *Sida acuta* Leaves: The quantitative results are indicated in **Table 1**. The total phenol, flavonoid, tannin and alkaloid content in ethanolic extract was found to be 31 ± 0.15 (mg/g gallic acid equivalent), 40 ± 0.25 (mg/g quercetin equivalent) and, 50 ± 0.2 (mg/g catechin equivalent) and 0.025 ± 0.1 (mg/10 mg of plant extract) respectively. The results indicate a higher concentration of polyphenols, whereas, alkaloids in a minimal quantity.

TABLE 1: QUANTITATIVE PHYTOCHEMICAL ANALYSIS IN ETHANOLIC EXTRACT OF *SIDA ACUTA* LEAVES

Phytochemicals present in the ethanolic extract of *Sida acuta*	Results
Total phenol content (mg/g gallic acid equivalent)	31±0.15
Total flavonoid content (mg/g quercetin equivalent)	40±0.25
Total tannin content (mg/g catechin equivalent)	50±0.2
Total alkaloid content (mg/10mg of plant extract)	0.025±0.1

Values are expressed as mean ± SD for triplicate

Enzymatic Antioxidants Present in Fresh Leaves Homogenate of *Sida acuta*: The activities of the enzymatic antioxidants present in fresh leaf sample are shown in **Table 2**. Among the tested enzymes, the catalase had shown maximum activity followed by the glutathione-dependent enzymes. The glutathione-dependent enzymes such as GPx, GST, and GR were documented in decreasing order of activities. Nevertheless, the SOD enzyme has the lowest activity among the tested enzymes in the fresh leaf homogenate of *Sida acuta*.

TABLE 2: ENZYMATIC ANTIOXIDANTS PRESENT IN FRESH LEAVES HOMOGENATE OF *SIDA ACUTA*

Enzymatic antioxidants	Conc.
Superoxide dismutase (Units/mg protein)	42.2±0.25
Catalase (µmol of H_2O_2 consumed/min/mg protein)	150±0.15
Glutathione peroxidize (µmol/g sample)	121±0.75
Glutathione reductase (µg/mg protein)	45±0.12
Glutathione S transferase (µg/mg protein)	71±0.15

Values are expressed as mean ± SD for triplicate

Non-Enzymatic Antioxidants Present in Fresh Leaves Homogenate of *Sida acuta*: The concentration of the non-enzymatic antioxidants present in the fresh leaf of *Sida acuta* is shown in **Table 3**. The results revealed that the total reduced glutathione, vitamin-C, and vitamin-E content were found to be 52.3 ± 0.1 µg/g, 195 ± 0.29 µg/g and 58.3 ± 0.15 µg/g respectively. This suggested that *Sida acuta* was a substantial source of non-enzymatic antioxidants.

TABLE 3: NON-ENZYMATIC ANTIOXIDANTS PRESENT IN FRESH LEAVES OF *SIDA ACUTA*

Non-enzymatic antioxidants	Conc.
Total reduced glutathione (µg/g of fresh leaves)	52.3±0.1
Vitamin C (µg/g of fresh leaves)	195±0.29
Vitamin E (µg/g of fresh leaves)	58.3±0.15

Values are expressed as mean ± SD for triplicate

***In-vitro* Free Radical Scavenging Activities of Ethanolic Extract of *Sida Acuta* Leaves:**

DPPH Radical Scavenging Activity: The DPPH free radical scavenging activity results are shown in **Fig. 1**. DPPH radical scavenging assay is the standard method to measure the antioxidant ability of the plant extracts. The IC_{50} value of the ethanolic extract of *Sida acuta* and ascorbic acid was 61 ± 0.15 µg and 60 ± 0.25 µg, respectively. It is to be noted that the radical quenching activities of the extract and ascorbic acid increased in a dose-dependent manner and intercepts at 70 µg. This suggested that at this concentration the scavenging activities of the extract as well as the standard were similar.

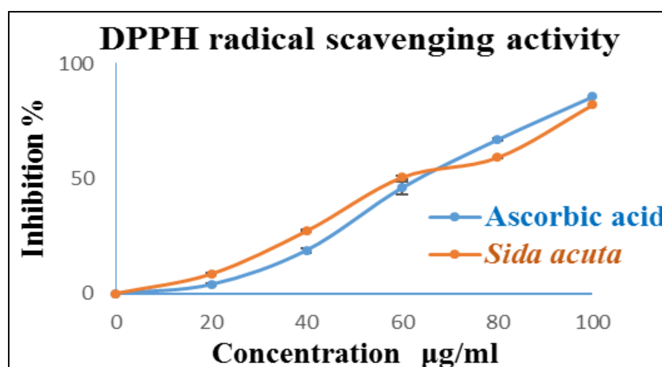

FIG. 1: DPPH RADICAL SCAVENGING ACTIVITY OF ETHANOLIC EXTRACT OF *SIDA ACUTA* LEAVES. Values are expressed as mean ± SD for triplicate

FRAP Assay: The FRAP assay results are shown in **Fig. 2**. The results demonstrate the reducing power of the ethanolic extract of *Sida acuta* as well as the standard which increases with the increase in amount of sample and standard concentrations. This is reflective of a better radical scavenging tendency of the *Sida acuta* leaf extract than the standard ascorbic acid.

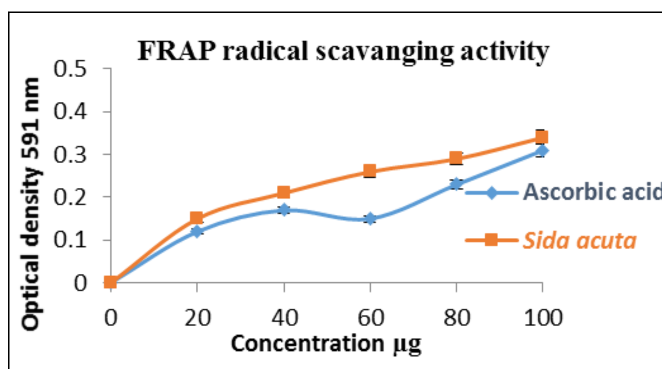

FIG. 2: FRAP ASSAY OF ETHANOLIC EXTRACT OF *SIDA ACUTA* LEAVES. Values are expressed as mean ± SD for triplicate

Nitric Oxide Radical Scavenging Activity: In the present study, the crude ethanolic extract of *Sida acuta* leaves were investigated for its inhibitory effect on nitric oxide radicals. The results displayed that the extract, as well as the standard, had moderate activity in scavenging nitric oxide radical and it is shown in **Fig. 3**. It is also emphasized that the scavenging activity of the sample and standard intercepts at two different concentrations ranged between 20 and 40 µg. However, after 40 µg concentration, the activity of the plant extract was higher than the standard ascorbic acid. In concordance with the previous assays, radical scavenging power of the sample and standard increased dose-dependently. The IC_{50} values were 71 ± 0.51 µg and 67 ± 0.31 µg for standard ascorbic acid and *Sida acuta*, respectively.

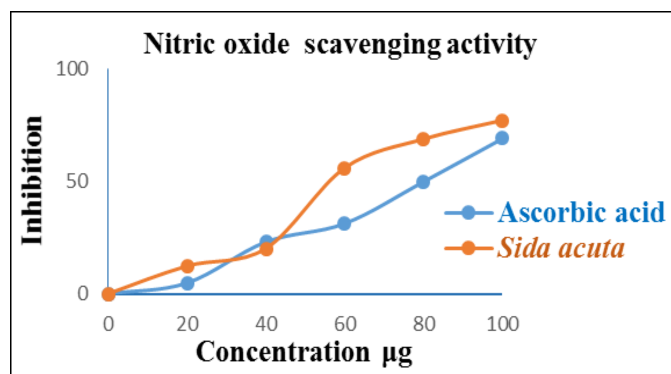

FIG. 3: NO RADICAL SCAVENGING ACTIVITY OF ETHANOLIC EXTRACT OF *SIDA ACUTA* LEAVES. Values are expressed as mean ± SD for triplicate

Hydroxyl Radical Scavenging Activity: As shown in **Fig. 4** *Sida acuta* leaf extract also demonstrated hydrogen peroxide scavenging activity in a concentration-dependent manner with an IC_{50} of 72 ± 0.52 µg. The IC_{50} of standard ascorbic acid was slightly higher with 74 ± 0.53 µg. This indicates an increased scavenging potential of the leaf extract when compared to the standard ascorbic acid.

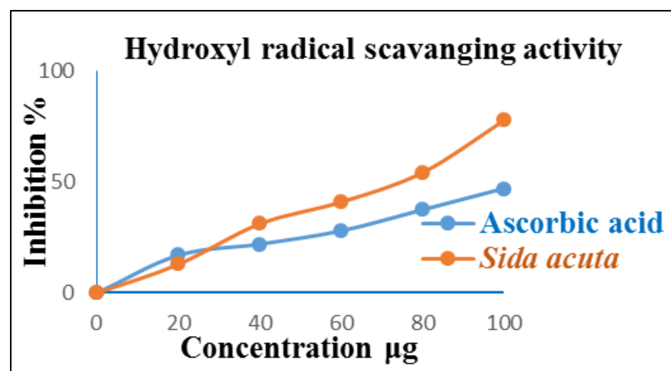

FIG. 4: HYDROXYL RADICAL SCAVENGING ACTIVITY OF ETHANOLIC EXTRACT OF *SIDA ACUTA* LEAVES. Values are expressed as mean ± SD for triplicate

Nevertheless, the graph displayed an intersection of the radical scavenging activity of the sample and standard at a concentration of near to 30 µg wherein the antioxidant potential of the plant extract and standard was likely to be similar.

$ABTS^+$ Radical Scavenging Activity: The ethanolic leaf extract of *Sida acuta* was an effective scavenger of the ABTS radical. It is shown in **Fig. 5** and this activity was comparable to that of ascorbic acid. It exhibited potent scavenging effects against ABTS radical with an IC_{50} value of 150 ± 0.12 µg and standard ascorbic acid (IC_{50} value 100 ± 0.5 µg). The activity of the leaf extract of *Sida acuta* and ascorbic acid increased proportionately.

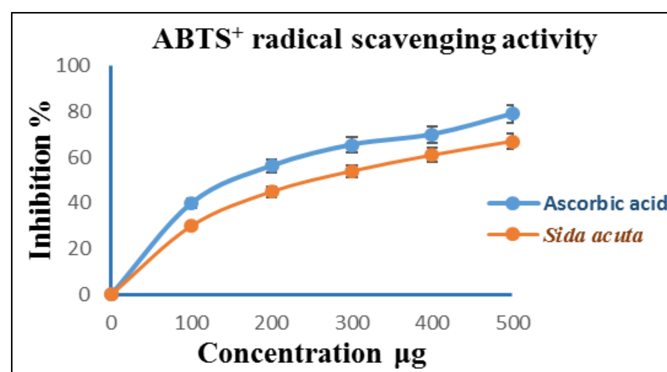

FIG. 5: $ABTS^+$ RADICAL SCAVENGING ACTIVITY OF ETHANOLIC EXTRACT OF *SIDA ACUTA* LEAVES. Values are expressed as mean ± SD for triplicate

HPTLC Profiling of Alkaloids Present in Ethanolic Extract of *Sida acuta* Leaves: The ethanolic extract of *Sida acuta* leaves yielded 12 prominent bands, among which three bands were identified to alkaloids as they produced yellow/orange-yellow zones **Fig. 6**.

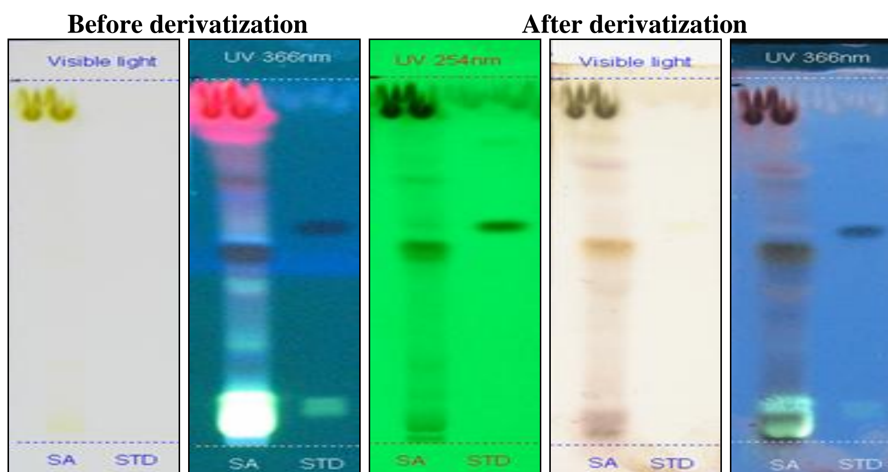

FIG. 6: HPTLC FINGERPRINTING PROFILE FOR ALKALOIDS PRESENT IN ETHANOLIC EXTRACT OF *SIDA ACUTA* LEAVES

TABLE 4: PEAK TABLE-ALKALOIDS PROFILE

Track	Peak	R_f	Height	Area	Assigned substance
Sample SA	1	0.02	259.8	9317.2	Unknown
Sample SA	2	0.11	49.8	823.8	Alkaloid 1
Sample SA	3	0.19	56.7	1967.1	Unknown
Sample SA	4	0.31	15.4	289.3	Unknown
Sample SA	5	0.34	25.2	413.7	Unknown
Sample SA	6	0.39	50.3	1847.6	Unknown
Sample SA	7	0.53	438.6	23992.4	Alkaloid 2
Sample SA	8	0.59	102.5	3791.2	Unknown
Sample SA	9	0.71	149.4	4096.9	Alkaloid 3
Sample SA	10	0.77	50.0	1518.4	Unknown

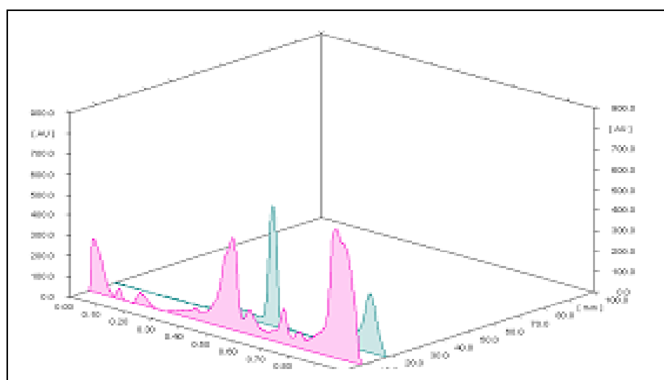

FIG. 7: 3D-DISPLAY OF HPTLC DENSITOGRAM OF ALKALOID PRESENT IN ETHANOLIC EXTRACT OF *SIDA ACUTA* LEAVES. Note: The pink and green peaks represent the sample and standard peaks, respectively.

The alkaloids were observed in the following R_f values such as 0.11, 0.53 & 0.71 **Table 4**. The 3D-peak densitogram profile for alkaloids present in *Sida acuta* leaf extract is indicated in **Fig. 7**. The standard colchicine was spotted in a separate track. The standard band was very prominent indicating the absence of the contaminants. The densitogram analysis of the obtained peak suggested that among the identified alkaloids, the alkaloids numbered as 2 possessed higher area percentage which was a more likely indication of higher concentration.

HPTLC Profiling of Flavonoids Present in Ethanolic Extract of *Sida acuta* Leaves: The ethanolic extract of *Sida acuta* leaves yielded only 7 distinct bands, among which three were identified as flavonoids as they produced yellow/bluish-yellow color zones at UV region **Fig. 8**. The flavonoids were observed in the following R_f values of 0.46, 0.56 & 0.77 **Table 5**. The 3D-peak densitogram profile for flavonoids present in *Sida acuta* leaf extract is indicated in **Fig. 9**. The standard rutin was spotted in a separate track. The densitogram analysis of the obtained peak suggested that flavonoids#2 and flavonoids#3 possessed higher area percentage. Interestingly, the R_f value of flavonoids#1 detected from the sample and the R_f value of standard rutin were similar at 0.46.

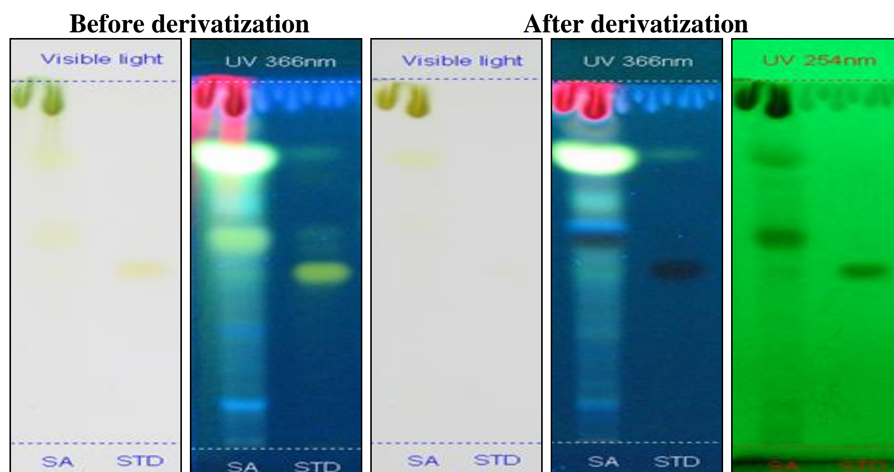

FIG. 8: HPTLC FINGERPRINTING PROFILE FOR FLAVONOIDS PRESENT IN ETHANOLIC EXTRACT OF *SIDA ACUTA* LEAVES

TABLE 5: PEAK TABLE- FLAVONOIDS PROFILE

Track	Peak	R_f	Height	Area	Assigned substance
Sample SA	1	0.09	31.3	1177.8	Unknown
Sample SA	2	0.20	25.6	775.1	Unknown
Sample SA	3	0.32	14.8	296.5	Unknown
Sample SA	4	0.46	106.6	3201.4	Flavonoid 1
Sample SA	5	0.56	550.0	32307.7	Flavonoid 2
Sample SA	6	0.77	477.8	23462.6	Flavonoid 3
Sample SA	7	0.86	10.1	149.1	Unknown
Sample SA	8	0.92	296.8	15307.0	Unknown
STD	1	0.46	556.5	22477.4	Rutin

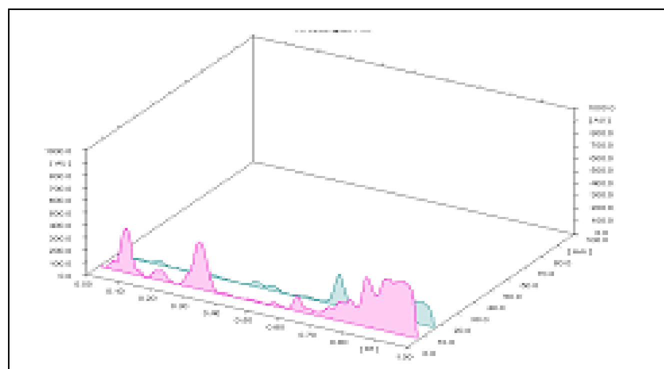

FIG. 9: 3D-DISPLAY OF HPTLC DENSITOGRAM OF FLAVONOIDS PRESENT IN ETHANOLIC EXTRACT OF *SIDA ACUTA* LEAVES

HPTLC Profiling of Tannin Present in Ethanolic Extract of *Sida acuta* Leaves: The ethanolic extract of *Sida acuta* leaves yielded only 15 different peaks, wherein five of them were designated as tannins as they produced Brown, Yellowish brown or Green color zones at visible light **Fig. 10**. The tannins were observed in the following R_f values of 0.08, 0.31, 0.78, 0.84 & 0.91 **Table 6**. The 3D-peak densitogram profile for tannins present in *Sida actua* leaf extract is indicated in **Fig. 11**. The standard gallic acid was used in a separate track.

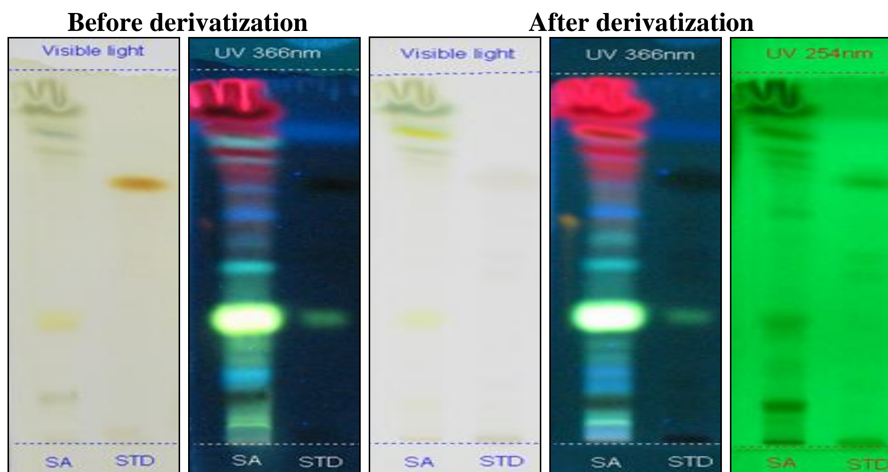

FIG. 10: HPTLC FINGERPRINTING PROFILE FOR TANNINS PRESENT IN ETHANOLIC EXTRACT OF *SIDA ACUTA*

TABLE 6: PEAK TABLE-TANNINS PROFILE

Track	Peak	R_f	Height	Area	Assigned substance
Sample SA	1	0.05	74.4	1345.7	Unknown
Sample SA	2	0.08	349.3	11017.8	Tannin 1
Sample SA	3	0.19	76.3	2748.8	Unknown
Sample SA	4	0.31	374.3	15992.5	Tannin 2
Sample SA	5	0.40	14.5	307.9	Unknown
Sample SA	6	0.47	11.0	277.4	Unknown
Sample SA	7	0.55	24.4	668.4	Unknown
Sample SA	8	0.62	122.1	3020.3	Unknown
Sample SA	9	0.65	39.1	760.7	Unknown
Sample SA	10	0.72	85.9	2212.6	Unknown
Sample SA	11	0.76	156.7	4430.5	Unknown
Sample SA	12	0.78	188.8	4615.9	Tannin 3
Sample SA	13	0.84	395.7	14241.9	Tannin 4
Sample SA	14	0.91	423.3	16545.1	Tannin 5
Sample SA	15	0.95	423.9	20973.2	Unknown
STD	1	0.70	245.8	7991.8	Gallic acid standard

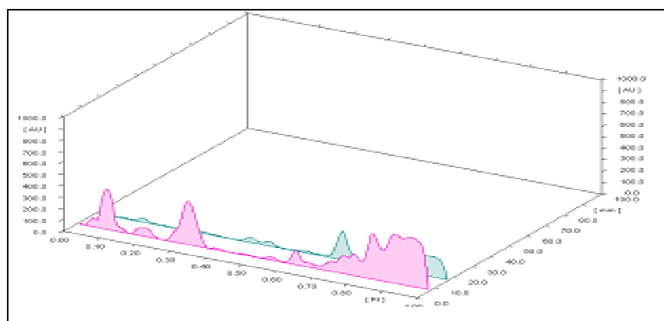

FIG. 11: 3D-DISPLAY OF HPTLC DENSITOGRAM OF TANNINS PRESENT IN ETHANOLIC EXTRACT OF *SIDA ACUTA* LEAVES

HPTLC Profiling of Glycoside Present in Ethanolic Extract of *Sida actua* Leaves: The ethanolic extract of *Sida acuta* leaves exposed the existence of 11 different peaks, wherein six of them were named glycoside as they produced Brownish-Yellow bands in the visible light after spraying with Liberman-Burchard reagent **Fig. 12**. The glycosides were observed in the following R_f values of 0.11, 0.28, 0.42, 0.65, 0.74 & 0.84 **Table 7**. The standard glycoside swertiamarin was spotted in a separate track and was prominent at R_f value 0.64. The 3D-peak densitogram profile for glycoside present in *Sida acuta* leaf extract is indicated in **Fig. 13**.

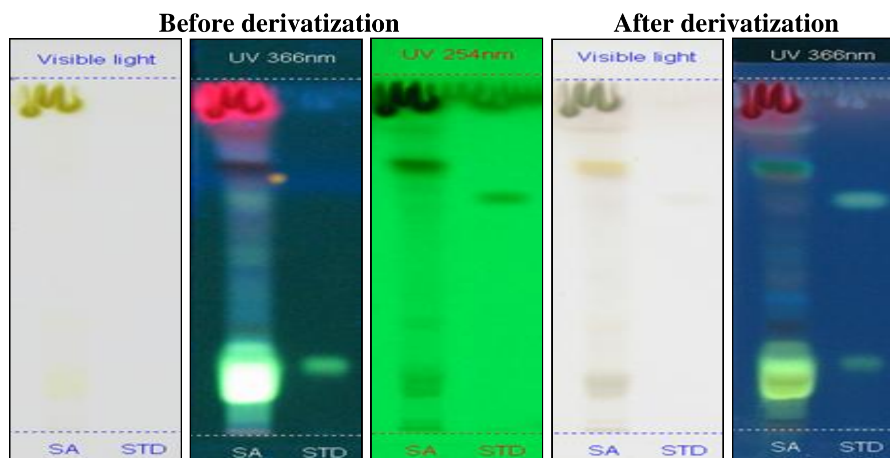

FIG. 12: HPTLC FINGERPRINTING PROFILE FOR GLYCOSIDES PRESENT IN ETHANOLIC EXTRACT OF *SIDA ACUTA* LEAVES

TABLE 7: PEAK TABLE- GLYCOSIDES PROFILE

Track	Peak	R_f	Height	Area	Assigned substance
Sample SA	1	0.11	375.4	27454.9	Glycoside 1
Sample SA	2	0.22	51.0	1284.0	Unknown
Sample SA	3	0.28	78.3	2537.8	Glycoside 2
Sample SA	4	0.36	19.1	382.1	Unknown
Sample SA	5	0.41	22.0	426.4	Unknown
Sample SA	6	0.42	22.6	598.3	Glycoside 3
Sample SA	7	0.55	25.2	430.4	Unknown
Sample SA	8	0.65	69.5	3804.3	Glycoside 4
Sample SA	9	0.74	422.1	20177.8	Glycoside 5
Sample SA	10	0.84	279.6	11873.2	Glycoside 6
Sample SA	11	0.92	617.9	42976.2	Unknown
STD	1	0.64	184.4	5438.3	Swertiamarin standard

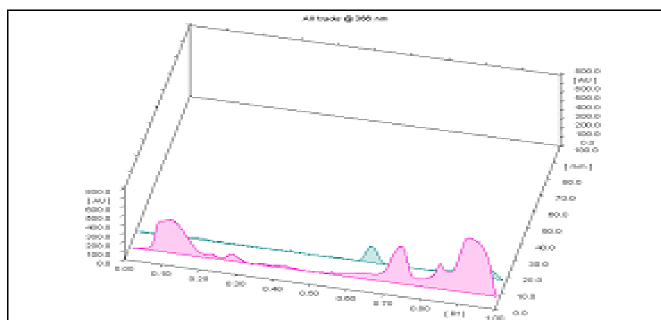

FIG. 13: 3D-DISPLAY OF HPTLC DENSITOGRAM OF GLYCOSIDE PRESENT IN ETHANOLIC EXTRACT OF *SIDA ACUTA* LEAVES

HPTLC Profiling of Steroids Present in Ethanolic Extract of *Sida acuta* Leaves: The ethanolic extract of *Sida acuta* leaves exposed the existence of 15 different peaks. The presence of bluish/bluish-violet colored zones of the standard stigmasterol and in the sample track affirmed the presence of steroids **Fig. 14**. The steroids were observed in the following R_f values of 0.07, 0.10, 0.16, 0.24, 0.40, 0.50, 0.63, 0.82 & 0.94 **Table 8**. The standard stigmasterol was spotted in a separate track and was prominent at R_f value 0.47. The 3D-

peak densitogram profile for steroid present in *Sida acuta* leaf extract is indicated in **Fig. 15**.

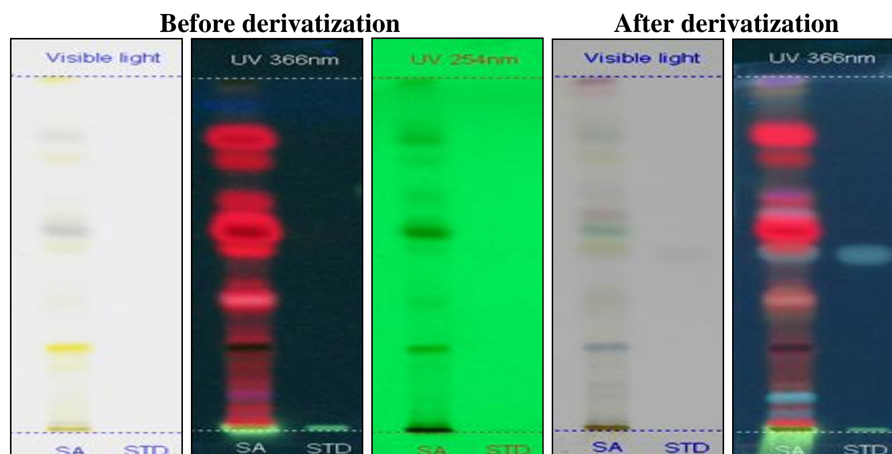

FIG. 14: HPTLC FINGERPRINTING PROFILE FOR STEROIDS PRESENT IN ETHANOLIC EXTRACT OF *SIDA ACUTA* LEAVES

TABLE 8: PEAK TABLE- STEROIDS PROFILE

Track	Peak	R_f	Height	Area	Assigned substance
Sample SA	1	0.07	91.3	2023.8	Steroid 1
Sample SA	2	0.10	136.6	2402.5	Steroid 2
Sample SA	3	0.13	32.1	352.6	Unknown
Sample SA	4	0.16	34.6	644.5	Steroid 3
Sample SA	5	0.21	214.9	4485.8	Unknown
Sample SA	6	0.24	22.8	299.8	Steroid 4
Sample SA	7	0.34	124.9	5823.5	Unknown
Sample SA	8	0.40	44.7	557.1	Steroid 5
Sample SA	9	0.50	142.3	4484.7	Steroid 6
Sample SA	10	0.55	428.4	20311.2	Unknown
Sample SA	11	0.63	122.0	5376.0	Steroid 7
Sample SA	12	0.75	59.4	1749.6	Unknown
Sample SA	13	0.82	245.7	10122.6	Steroid 8
Sample SA	14	0.94	32.5	511.5	Steroid 9
Sample SA	15	0.98	156.3	2551.1	Unknown
STD	1	0.47	84.1	3021.9	Stigmasterol

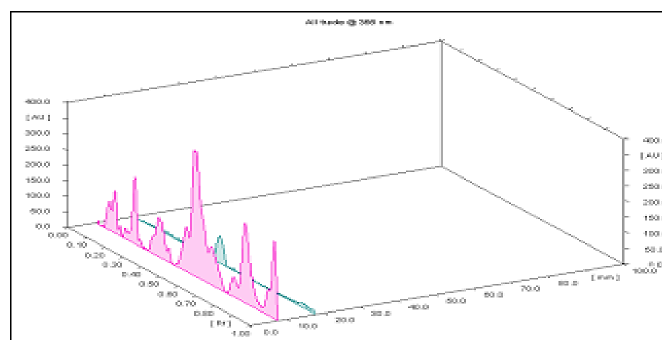

FIG. 15: 3D-DISPLAY OF HPTLC DENSITOGRAM OF STEROID PRESENT IN ETHANOLIC EXTRACT OF *SIDA ACUTA* LEAVES

GC-MS Analysis Results of Ethanolic Extract of *Sida acuta* Leaves: GC-MS is one of the latest techniques used for the identification of bioactive components present in the plant extract. The GC-MS analysis results of ethanolic extract of *Sida acuta* leaves revealed the presence of 35 different compounds each belonging to the different class of phytochemicals such as sterols, flavonoids, terpenes, heterocyclic aromatic compounds, phenols, polyunsaturated & monounsaturated fatty acids, vitamins, alkaloids & sesquiterpenoids **Table 9**. These compounds were identified based on their peak area, retention time, molecular formula and molecular mass. The peak chromatogram obtained is displayed in **Fig. 16**.

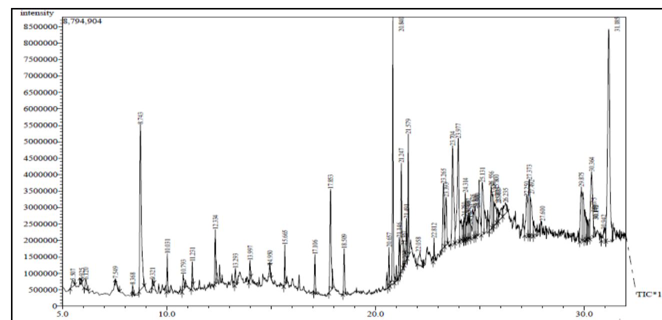

FIG. 16: GC-MS CHROMATOGRAM OBTAINED FROM THE ANALYSIS OF ETHANOLIC EXTRACT OF *SIDA ACUTA*

TABLE 9: GC-MS RESULTS OF THE ETHANOLIC EXTRACT OF *SIDA ACUTA* LEAVES

S. no.	RT	Name of the compound	Molecular formula	Molecular weight	Peak area %
1	8.743	2-Methoxy-4-Vinylphenol	$C_9H_{10}O_2$	150	7.81
2	10.79	O-Isopropylphenetole	$C_{11}H_{16}O$	164	0.52
3	12.33	3',5'-Dimethoxyacetophenone	$C_{10}H_{12}O_3$	180	1.43
4	13.29	Quinoline, 4-Methyl-, 1-Oxide	$C_{10}H_9NO$	159	0.49
5	14	Mehyl ester (4-Isopropylidene-7-methyl-6-Methylene-2-Octenoic acid	$C_{14}H_{22}O_2$	222	0.49
6	15.67	Neophytadiene	$C_{20}H_{38}$	278	0.8
7	17.11	Palmitic acid methyl ester	$C_{17}H_{34}O_2$	270	0.89
8	17.85	L-(+)-Ascorbic Acid 2,6-Dihexadecanoate	$C_{38}H_{68}O_8$	652	3.71
9	18.51	Palmitic acid ethyl ester	$C_{16}H_{32}O_2$	256	1.27
10	20.66	Linolenic acid, methyl ester	$C_{19}H_{32}O_2$	292	0.88
11	20.84	3,7,11,15-Tetramethylhexadec-2-en-1-ol	$C_{20}H_{40}O$	296	6.04
12	21.01	Stearic acid methyl ester	$C_{19}H_{38}O_2$	298	0.44
13	21.15	cis,cis-Linoleic acid	$C_{18}H_{30}O_2$	280	1.18
14	21.25	alpha-Linolenic acid	$C_{20}H_{34}O_2$	278	3.66
15	21.37	3,4,5-Trimethoxyphenylacetic Acid	$C_{11}H_{14}O_5$	226	0.73
16	21.49	N-Propyl 9,12-Octadecadienoate	$C_{21}H_{38}O_2$	322	1.25
17	21.58	Ethyl 9,12,15-Octadecatrienoate	$C_{20}H_{34}O_2$	306	2.54
18	22.06	6,9,10-Trimethoxy-12h-Benz(6,7)Oxepino(2,3,4-I,J)Isoquinoline	$C_{19}H_{17}NO_4$	323	0.9
19	22.81	Carbonic acid, 2-dimethylaminoethyl neopentyl ester	$C_{10}H_{21}NO_3$	203	0.43
20	23.27	1-Demethylcolchicine	$C_{18}H_{14}FNO_3$	311	2.5
21	23.7	3-Fluoro-5-methyl-11-Oxo-5,6,6A,11-Tetrahydroisoindolo[2,1-A]quinoline-10-carboxylic acid	$C_{18}H_{16}O_4$	296	4.77
22	24.14	Trans-4,4'-Dimethoxy-Beta-Methylchalcone	$C_{18}H_{32}O_2$	280	1.25
23	24.25	Benzo[C]thiophene-1-carboxylic acid, 4,5,6,7-tetrahydro-, (2-Dimethylaminoethyl)amide	$C_{13}H_{20}N_2O$	252	0.52
24	24.31	Carbamic Acid, 2-(Dimethylamino)Ethyl ester	$C_5H_{12}N_2O2$	372	0.92
25	24.68	Palmitic acid beta.-monoglyceride	$C_{27}H_{54}O_5Si_2$	514	1.39
26	24.8	7-(2-Hydroxy-ethyl)-1,3-dimethyl-8-morpholin-4-yl-3,7-dihydro-purine-2,6-dione	$C_{13}H_{19}N_5O_4$	309	1.13
27	24.9	1.Alpha.-18o-1,25-Dihydroxycholecalciferol	$C_{27}H_{44}O_3$	416	0.8
28	25.13	2-Methyl-11h-Indeno[1,2-B]Quinoxaline	$C_{16}H_{12}N_2$	232	2.74
29	25.65	1-Cyano-3-Phenylindolizine	$C_{15}H_{10}N_2$	218	1.31
30	25.8	1,3-Dimethyl-1,3-bis(4-methylphenyl)-1,3-disilacyclobutane	$C_{18}H_{24}Si_2$	296	1.37
31	25.88	Spiro[2-cyano-cyclopropane,-1,9',-4'-Azafluorene	$C_{16}H_{13}N$	219	0.44
32	26.24	1-(5-Phenyl-5H-1-thia-3,5,8-triazaacenaphthylen- 2-yl)ethanone	$C_{16}H_{11}N_{3O}S$	293	0.91
33	27.25	5,10-Dihydro-2-morpholino-5,10-ethanophenazine	$C_{18}H_{19}N_{3O}$	293	2.41
34	27.37	13-Tetradecenyl acetate	$C_{16}H_{30}O_2$	254	2.25
35	30.36	Campesterol	$C_{28}H_{48}O$	400	4

DISCUSSION: The therapeutic potential of the medicinal plants appears to be due to the presence of various secondary metabolites such as polyphenols, alkaloids, flavonoids, glycosides, tannins, saponins, and steroidal compounds. The ethanolic extraction of phytochemicals from the leaves of *Sida acuta* followed by their quantitative estimation revealed the presence of flavonoids, tannins, and phenols in copious quantity and alkaloids in negligible quantity. These compounds are known to possess a broad range of biological activities including antioxidant, anti-inflammatory, antibacterial, and anticancer activities [26]. These findings would not only convince the traditional use of *Sida acuta* in the treatment of free radical-induced diseases but also serve as a source of bioactive compounds against a plethora of diseases. Thus, the quantitative estimation is useful to relate the concentration of bioactive compounds, which consequently leverage the discovery and development of new classes of drug. The results are in harmony with the previous findings, wherein, the

authors screened the presence of phytochemicals qualitatively and identified that the leaf extract of *Sida acuta* contained alkaloids, steroids, saponins, tannins, flavonoids, glycosides and polyphenols [27].

Reactive oxygen species (ROS) are synthesized in the plants as a consequence of metabolic process happening inside them such as photosynthesis, respiration and defending against plant-pathogens. While encountering a major difference in the balance between the antioxidant defense enzymes and ROS production, the plants are susceptible to abiotic stress [28]. It is interesting to note that the plant-based antioxidant enzymes are also beneficial for humans to keep free radical-induced damages away. In general, the ability to counter the stress-induced diseases is considerably reduced by developing the intake of antioxidant enzyme rich foods or antioxidant enzyme supplements which could potentially lead to physiological well-being and optimal health [29]. As a consequence, antioxidant enzyme rich plant parts are effective against various diseases. The results of the present study suggest that the fresh leaves of *Sida acuta* may be considered as a significant source of antioxidant enzyme and non-enzymatic antioxidants.

Thus, the results reinstate the caliber of the plant to be used against free radical-induced ailments. In a previous study, the authors determined the activity of the following enzymes such as SOD, POD, PPO, and CAT from the plant *Rumex obtusifolius* and the results have revealed that the plant contains appreciable quantities of the antioxidant enzymes and hence recommended for the treatment of diseases induced by oxidative damage [30].

The consumption of allopathic medicines involves greater risk of undesirable side effect causing damage to the internal organs. An alternative way to reduce the risk is to promote the use of naturally available antioxidants from the medicinal plants owing to their lessened side effects. To determine the antioxidants property of ethanolic extract of Sida acuta five methods were used involving different oxidizing agents to decipher the best possible results. This is because a recent study reported that evaluation of the antioxidant property of the plant extracts requires unique methods that address not only the radical scavenging potential but also the reaction kinetics. Accordingly, inhibited autoxidation is suitable for chain-breaking antioxidants, and other methods are essential for preventive antioxidants [31]. The results of the present study revealed that the ethanolic extract of *Sida acuta* leaf showed considerable radical scavenging activity against the tested compounds such as DPPH, ABTS, ferric, hydroxyl and nitric oxide. This could be attributed to the number of phytochemicals present in the extract. Ideally, the chemical structure of polyphenols made them good antioxidant towards physiological and exogenous free radicals. The higher number of the hydroxyl groups linked to the aromatic backbone of flavonoids enabled them to undergo a redox reaction that helped them to scavenge the free radicals [32]. The phytochemical investigation of the current study depicted the increased presence of polyphenols (flavonoids and tannins). Hence, it was plausible that the reducing potential of the leaf extract of *Sida acuta* might be associated with the presence of reductions that could have quenched the propagation of free radical chain by donating hydrogen atoms. Prior report has shown that nitric oxide (NO) radical is synthesized by phagocytes and endothelial cells, which further decompose to form OH-radical.

The NO radical is otherwise known for its crucial role in inflammation [33]. The radical scavenging effect of the present study reveals a significant reduction in the level of nitric oxide radical, which is suggestive of the tendency of the plant extract to be used as an anti-inflammatory agent. The results are in concordance with our previous results which evaluated the radical scavenging ability of the chloroform extract of *Sida acuta* against the same radicals used in the present study except ABTS [34]. It is observed that radical scavenging activity of the chloroform extract and ethanolic extract of *Sida acuta* leaves increased in a dose-dependent fashion.

In the present study, the HPTLC analysis of an ethanolic extract of *Sida acuta* leaves showed the presence of alkaloids, flavonoids, glycosides, tannins, and saponins. HPTLC fingerprint studies affirmed the results of quantitative phytochemical analysis by portraying various colored bands at UV and visible light with specific solvent systems, representing the presence of particular phytoconstituents.

The chromatographic fingerprint was suitable for monitoring the identity and purity of a plant extract. Also, HPTLC technique also provided semi-quantitative information about the major phytoconstituents present in a plant extract, thus enabling an assessment of plant extract quality [35]. Plant-based bioactive compounds were striking molecules for drug development. The HPTLC results suggested that the leaf extract of *Sida acuta* was a rich source of polyphenols, glycosides, and steroids. Interestingly, some considerable amounts of alkaloids were also detected in HPTLC technique, whereas, quantitative alkaloid results were observed only in negligible amounts. This proved the improved sensitivity of the technique over the existing ones. The recovery of the target compounds could conclude the choice of the optimal solvent system for a particular plant extract. The difference in the R_f value of the phytochemical provided an important hint about the selection of the ideal solvent system for separation of isolated bands in HPTLC [36].

In the present HPTLC study flavonoids and tannins were detected in higher amounts with different R_f values. Earlier reports suggested that flavonoids were one of the most efficient molecules to exhibit antioxidant activity. This happened because of the ability of flavonoids to quench the free radicals, enhancing the production of antioxidant defense enzymes and chelating radical intermediate compounds [37]. It was also reported that tannins were 15-30 times capable of quenching peroxy radicals than simple phenolic compounds. Previous, epidemiological research reported that dietary intake of flavonoids might reduce the risk of tumors of the breast, colon, lung, prostate, and pancreas [38]. Glycosides comprised the next category of phytochemical present ubiquitously in almost all plants. Plants store folklore significant molecules in the form of inactive glycosides.

The aglycone moiety of glycosides comprises the functional property and elicits the chemical effects on the body. Glycosides possess various roles such as cardioprotection, laxatives, counterirritants, analgesics, renal disinfectants, antirheumatics, anti-inflammatory, antituberculosis, expectorant and antispasmodic action [39]. Plant-origin steroid compounds possess a large group of substances that mediate a varied set of biological responses. Naturally occurring steroids are also used for the treatment of various diseases such as hypersensitive reactions, arthritis, cancer and diseases resulting from hormone deficiencies or disorders [40].

The capability of phytosterols to reduce serum cholesterol levels as well as the abrogation of myocardial infarction risk has made them a class of preferred food supplements. In the current scenario, plant extracts enriched with phytosterols have become a healthy supplementary tool to lower LDL-cholesterol levels traditionally. Furthermore, groups of naturally occurring chemical compounds that contain majority of basic nitrogen atoms are known as alkaloids. Similar to the other phytochemical alkaloids do possess therapeutic properties such as muscle relaxant, anticancer, antioxidant, antimicrobial, and many more [41].

The GC-MS displayed the presence of a wide range of phytochemical compounds in the leaf extract of *Sida acuta*. In this present study, the higher antioxidant power of the ethanolic extract of the *Sida acuta* was presumably due to the detection of phytochemical compounds such as ascorbic acid, neophytadiene, campesterol, and dihydroxychole-calciferol. These compounds were reported to contain outstanding antioxidant tendency [42]. Moreover, the GC-MS results also revealed the presence of beneficial unsaturated fatty acids, specifically, the linoleic acid and linolenic acid.

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 [43]. The plant-based sterols have been reported to contain various roles in the prevention of human pathologies [44].

Accordingly, Stigmasterol was reported to have anti-arthritic, anti-venom, and glucose regulatory activities [45]. Nonetheless, the antiangiogenic activity of campesterol has already reported [46]. The presence of these compounds in the *S. acuta* leaf extract reestablishes the folklore importance of the plant leaves. It is to be emphasized that the esters of quinoline molecule are reported to possess phospholipase A_2 inhibitory potential, which may

allegedly support the anti-inflammatory property of the same or nearly similar compounds [47]. Since ancient times, colchicine and its derivatives have been used for the treatment of a broad range of clinical cases such as gout, mediterranean fever, liver cirrhosis, and cancer [48]. Despite these promising results, more research efforts are warranted to isolate, characterize, and assess the functionality of these compounds from the leaves of *Sida acuta* to validate their traditional significance.

CONCLUSION: The quantitative results of this study observed the presence of various bioactive compounds such as flavonoids, phenols, tannins and alkaloids in the ethanolic extract of *Sida acuta* leaves. This may be partly responsible for its different physiological functions or antioxidant activities. The free radical scavenging assay results suggest the promising antioxidant tendency of the extract. The HPTLC results document the presence of many important phytochemicals. GC-MS analysis identifies reasonable levels of medicinally active compounds from a different category of phytochemicals. Overall results indicate the amplified scope of *Sida acuta* leaf extract in the development of novel therapeutic agents capable of countering existing diseases stimulated by oxidative damage. Future studies are envisaged to isolate the individual bioactive compounds from the extract and also to extrapolate the safe concentration that can be used to improve existing drugs.

ACKNOWLEDGEMENT: The authors are thankful to our Chancellor, Chief Executive Officer, Vice-Chancellor and Registrar of Karpagam Academy of Higher Education for providing facilities and encouragement to complete this research work in fine fulfillment.

CONFLICT OF INTEREST: The authors declare no conflict of interest.

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How to cite this article:
Muneeswari P, Bhaskaran SK and Poornima K: Identification of active pharmaceuticals of *Sida acuta* Burm. F leaves using GC-MS and HPTLC fingerprinting. Int J Pharm Sci & Res 2019; 10(3): 1194-07. doi: 10.13040/IJPSR.0975-8232.10(3).1194-07.

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