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Phytochemical profiling and antibacterial activity of selected *Sida* species against common human pathogenic bacteria: An in vitro study

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Abstract
In the present study the acetone, methanol and aqueous extracts of *Sida acuta* Burm. F, *Sida alnifolia* L., *Sida fryxelli* Sivarajan & Pradeep (I.C.) and *Sida rhombifolia* L., whole plant were screened for the presence of phytochemical components and tested for antibacterial activity against *Escherichia coli*, *Staphylococcus aureus* and *Klebsiella pneumonia.* Result revealed the presence of phenolics, saponins, flavonoids, alkaloids, tannins, steroids, carbohydrates and proteins. The study indicates that phytochemical constituents of the four species of *Sida* are different. Acetone extract of *Sida* didn't show significant activity compared to the standard amoxicillin. Aqueous and methanolic extracts showed significant antibacterial property at higher dose. The different species of *Sida* studied didn't show significant antibacterial competence against *E. coli*. Prominent anti-bacterial activity was shown by *Sida acuta* against *Staphylococcous aureus* (20.5 mm) at 200 µg concentration in methanolic extract. In UV-VIS studies of the extracts in the range of 190nm to 400nm showed large number of peaks indicated the presence of active components in the extract.

Keywords: plant extract, phytochemical screening, antibacterial activity

Introduction
Nature has provided a complete store house of remedies to treat all ailments of mankind (Kokate *et al*., 2007) [1].Within the wide range of living organisms available on the earth including higher plants, animals, fungi, and marine organisms, the databases of natural products have recorded more than 200,000 compounds from almost all part of the world (Fullbeck *et al*., 2006) [2]. Plants have been by far the most extensively studied source of medicinal compounds. Medicinal plants are the centre stone of traditional medicine. The W.H.O. has reported that 80% of the world populations primarily rely on traditional medicines and major part of traditional therapies involve the use of crude extracts of plants and herbs or their active constituents (WHO, 1993) [3]. In the present scenario, there is an urgent and continuous need of looking at and development of cheaper, effective new plant based drugs with enhanced bioactive potential and without any side effects.
The genus *Sida* L., includes a significant group of plants belonging to Malvaceae. The plants are large herbs or small shrubs profusely growing in the tropics and sometimes even assuming weed status. Many species of *Sida* are widely used as the ayurvedic raw drug '*Bala'* in different regions for treating rheumatism (Remashree *et al*., 2008) [4]. The present study deals with the comparative phytochemistry, antibacterial properties and UV-Vis studies of four species of *Sida*, which are *Sida acuta* Burm.f, *Sida alnifoia (*syn. *Sida rhombifolia ssp.retusa), Sida fryxelli* Sivarajan& Pradeep *(*I.C.*)* and *Sida rhombifolia* L. Phytochemical studies give an insight into the chemical constituent present in a plant.

Materials and Methods
Sample collection
Four species of Sida plants were collected from Maliankara, Ernakulam District, Kerala during the month of November 2016. Whole plants were uprooted and soil and other debris adhered on the plants were removed. The collected plants were packed separately in polythene bags and brought to lab for identification. Identification of the plants was confirmed with the help of Dr. C.N. Sunil, Associate Professor from Sree Narayana Mangalam College, Maliankara.

Preparation of the extract
The whole plant of four species were washed thoroughly in water and dried in an oven at 45ºC. Acetone, methanolic and aqueous extracts were prepared by mixing 20 g of each of the powder samples with 200 ml of each solvent.

After 24 hour the extract were filtered and refrigerated for further use.

Preliminary phytochemical analysis

The extract was subjected to the preliminary qualitative chemical tests by following standard methods described by Harborne (1998) [5].

Antibacterial study

Disc diffusion method was used for the antimicrobial assay (Bauer *et al.*, 1996) [6]. Using a sterile cotton swab lawn cultures of the test organisms were made on nutrient agar plates under aseptic conditions. Filter paper discs of 6mm diameter (Whatman filter paper No.1) were prepared using paper punch and sterilized. The obtained discs were put in tubes containing different concentration of plant extract and kept for 1-2 days at 40^0C. The discs will absorb the drug. For the present study the concentration used are 50 µg, 100µg and 200µg. Discs impregnated with Amoxillin served as positive control (standard) and the filter paper disc soaked in solvent were used as negative control. The discs were placed on the surface of nutrient agar with flamed forceps and gently pressed down to ensure complete contact of the disc with the agar plate. The prepared plates were incubated at 27^0C for 18-24 hours. Inhibition zones are calculated as the difference between disc diameter and the diameter of the inhibition. Antibacterial activity of each extract was expressed in terms of the mean diameter of zone of inhibition (in mm) produced by respective extract at the end of incubation period. The test organisms used were *Escherichia coli, Staphylococcus aureus* and *Klebsiella pneumonia.*

UV-VIS spectroscopic analysis

One gram of plant powder was added to 10 mL of solvent such as acetone, methanol and aqueous and then filtered. An aliquot of the filtered sample was scanned using UV- Visible Spectrophotometer (Shimadzu, Japan), at a range of 190 - 400 nm, to detect the characteristic wavelength of the plant extract.

Result and Discussion

The present study was carried out to evaluate the phytochemical and antibacterial activity of four species of *Sida* against pathogenic bacteria *Escherichia coli, Staphylococcus aureus and Klebsiella pneumoniae* in acetone, methanolic and aqueous extract. The qualitative screening of phytochemical constituents of *Sida* species reveals the presence of phenolics, saponins, flavonoids, alkaloids, tannins, steroids, cabohydrates and proteins (Table 1). The study indicates that there is a variation in phytochemical constituents among the four species of *Sida*. When we comparing the different extracts it was found that most number of phytochemicals were present in the methanolic extract of all the four species, followed by aqueous extract. The least number of phytochemicals were present in acetone extract. Extraction efficiency of different solvents is affected by the chemical nature of phytochemicals, the extraction method used, sample particle size, the solvent used, as well as the presence of interfering substances (Stalikas, 2007) [7]. Similar result was obtained in studies conducted by Quy *et al*. (2014) [8] on *Limnophylla aromatic*. Preliminary photochemical studies revealed that most number of phytochyemicals were present in *Sida acuta* methanolic extract. The presence of flavanoid was observed in all plant species in all the crude extracts. All the plants show presence of alkaloids, carbohydrates, proteins, steroids and phenolics. Cardiac glycoside was present only in *Sida acuta*, all the three species of *Sida* except *Sida rhombifolia* showed presence of saponins. Our result was similar to study conducted by Richa et al, (2014), but [9] it contradicted regarding cardiac glycoside which was present only in *Sida acuta* but in their study cardiac glycoside was present in all the 3 species of Sida studied. According to the studies conducted by Beena (2016) [10] the presence of saponin was not detected in methanolic root extract of different species of Sida, but in the present study saponins was absent only in *Sida rhombifolia*.

The antibacterial efficiency of the three different extract of *Sida* species was evaluated against both gram positive and negative bacteria. The extract showed a dose dependant variation in the antibacterial activity. The different concentration used for the present study was 50, 100 and 200µg. As the dose increase the zone of inhibition also improved. Acetone extract of *Sida* didn't show prominent activity compared to the standard used amoxicillin. Aqueous and methanolic extracts showed significant antibacterial property at higher dose. *Sida* species show antibacterial effect against both gram positive and gram negative species (Table 2). Prominent antibacterial activity was shown by *Sida acuta* against *Staphylococcous aureus* (20.5 mm) at 200 µg concentration in methanolic extract. This is in agreement with the work of Ekpo et al. (2009). In aqueous extract the *Sida acuta* showed maximum activity against *Klebsiella pneumoniae* (15.33 mm). *Sida fryxelli* showed significant activity against *Staphylococcous aureus* in methanolic extract. In the study by Ekpo *et al*., (2009) [11], it was observed that the ethanolic extracts had a significantly higher antimicrobial activity than the aqueous extract. This difference is attributed to the solubility of the active component in different solvents. Our study is in agreement with this result. The different species of *Sida* studied didn't show antibacterial competence against *E. coli*.

In studies conducted by Anani *et al*. (2000) [12], it was noted that methanolic extract of *Sida acuta* had a significant activity on *S. aureus, E. coli, B. subtilis* and *Mycobacterium phlei* but no inhibition effect recorded on *Streptococcus faecalis* and *Klebsiella pneumoniae*. But it contradicted our study regarding the activity of *E.coli* and *Klebsiella pneumonia*. It was observed that methanolic extract of *Sida acuta* had a significant activity on Klebsiella *pneumonia* but no significant inhibition effect was reported on *E.coli.*

UV- VIS profile of the plant extract was studied at a wavelength range of 190 to 400 nm. The UV–VIS profile of *S. acuta* methanol extracts showed 16 peaks high number of peaks was present between 300 and 380 nm. The UV–VIS profile *of S. acuta acetone* extracts showed the 12 peaks and peaks with maximum absorbance was seen at 432, 410 and 211nm The qualitative UV–VIS spectrum profile of *S. acuta aqueous* extracts showed peaks at 289, 273, 224, 218 and 193 nm with the absorption 2.329, 0.782, 4.958, 5.334 and 3.537.

The UV–VIS profile of *S. alnifolia* methanol extracts showed peaks at 306, 284, 266, 262, 258, 248,238, 227, and 214 nm with the absorption 4.518, 5.564, 7.036, 5.600, 5.661, 5.674, 5.503, 5.109 and 4.600 resp. The UV–VIS profile *of S. alnifolia acetone* extracts showed 14 different peaks and the highest peak was at 410 and 211 nm with absorbance of 2.583 and 1.863. The qualitative UV–VIS spectrum profile of *S. alnifolia aqueous* extracts showed peaks at 289, 196 and 192 nm with the absorption 0.872, 23.833 and 3.001.

The UV–VIS profile of S. *fryxellii* methanol extracts showed peaks at 399, 365, 262, 256, 250, 218 and 194 nm with the absorption 1.122, 1.159, 2.361, 2.646, 2.713, 4.397 and 1.305 resp. The UV–VIS profile *of S. fryxellii acetone* extracts showed the peaks at 409, 307, 300, 295, 282, 268, 250 and 212 nm with the absorption 2.593, 1.072, 0.743, 0.751, 0.676, 0.690, 0.633 and 1.842. The qualitative UV–VIS spectrum profile of *S. fryxellii aqueous* extracts showed peaks at 289, 196 and 192 nm with the absorption 0.526, 2.808 and 2.652.

The UV–VIS profile of *S. rhombifolia* methanol extracts showed peaks at 262, 256, 234 and 225 nm with the absorption 3.305, 3.853, 4.832 and 4.716 resp. The UV–VIS profile *of S. rhombifolia acetone* extracts showed 15 peaks highest peak was at 212 nm with the absorption 1.672. The qualitative UV–VIS spectrum profile of *S. rhombifolia aqueous* extracts showed peaks at 318, 289, 284, 266, 207and 192 nm with the absorption 1.146, 1.2228, -0.523, 1.337, 4.642 and 3.183.

In the UV-VIS spectra the appearance of one or more peaks in the region from 200 to 400 nm is a clear indication of the presence of unsaturated groups and heteroatoms such as S, N, O (Njokua *et al.*, 2013) [13]. Nevertheless, the use of UV-visible spectrophotometery in the analysis of complex media is limited by the inherent difficulties in assigning the absorption peaks to any particular constituents in the system. Thus, UV–VIS findings must be supplemented with some other analytical technique such as GC/MS etc, to enable proper extract characterization and constituent identification (Karpagasundari *et al*., 2014) [14].

The studies conducted by Pavithra *et al., (*2010)[15], Okoro *et al.,(*2010) [16], Selime *et al*., (2010) [17] and Faizyi *et al., (*2003) [18] reported that the relative wide range of antibacterial properties for the crude extract and fractions can be explained by the presence of various classes of potentially active secondary metabolites detected in them. Indeed saponins, phenols, tannins and alkaloids, identified in the tested crude plant extracts have been reported to possess antimicrobial activities. The relative wide range of antimicrobial properties may results from the individual or from the joint modes of action of compounds belonging to the identified groups of constituents. Neha *et al., (*2006) [19] reported that the spectra for phenolic compounds (tannins) and flavanoids lie in the range of 230-290 nm. In our study highest spectral peaks are observed at wavelength 262 and 289 nm in all the crude extract of Sida sp that showing antibacterial activity and methanolic extract shows maximam peak when compared to aqueous and acetone extracts. So from the phytochemical and UV-VIS spectral analysis we can confirm that the presence of tannins and flavanoids in the species of Sida that provide antibacterial activity to the extract.

Table 1: Phytochemical screening of four species of *Sida* in three different extracts

Phytochemicals	*S. acuta*			*S. alnifolia*			*S. fryxelli*			*S.rhombifolia*		
	AE	**ME**	**AQ**	**AE**	**ME**	**AQ**	**AE**	**ME**	**AQ**	**AE**	**ME**	**AQ**
Phenolics	-	+	+	-	+	+	-	+	+	-	+	+
Saponins	-	+	+	-	+	+	+	-	+	-	-	-
Flavanoids	+	+	+	+	+	+	+	+	+	+	+	+
Alkaloids	-	+	+	-	+	-	-	+	+	-	+	+
Tannins	-	+	+	-	-	+	-	-	+	-	-	-
Cardiacglycoside	-	+	-	-	-	-	-	-	-	-	-	-
Steroids	-	+	+	-	+	-	-	+	-	-	+	+
Coumarins	-	-	-	-	-	-	-	-	-	-	-	-
Carbohydrates	-	+	+	+	+	+	-	+	+	-	+	+
Proteins	-	+	+	+	+	+	-	+	+	-	+	+
Phlobatannins	-	-	-	-	-	-	-	-	-	-	-	-
+ : Presence of phytochemical -: Absence of phytochemical AE- Acetone extract ME- Methanol extract AQ- Aqueous extract												

Table 2: Antibacterial screening (Zone of inhibition) of four species of *Sida* in three different extracts

	Dose (µg/ml)	**E.coli**			***K. pneumoniae***			***S. aureus***		
		AE	***ME***	***AQ***	***AE***	***ME***	***AQ***	***AE***	***ME***	***AQ***
Ampicillin		14.93± .57	14.93±0.57	14.93±0.57	25.3±0.57	25.3 ± 0.57	25.3 ± 0.57	26.3±1.52	26.3± 1.52	26.3± 1.52
Sida acuta	50	6.5±0	6.5 ± 0	6.4±0	6.5±0	10.2 ± 0.29	10.33±0.58	6.47±0.06	7.03± 0.06	6.9±0.06
	100	6.5±0	6.5 ± 0.15	6.43±0.06	6.47±0.06	11.6 ± 0.51	11.66±0.58	6.5±0	19.7± 0.25	6.9±0.06
	200	6.5±0	6.6±0.15	6.5±0	6.5±0	15.3±0.57	15.33±0.58	6.5±0	20.5±0.01	7±0.057
Sida alnifolia	50	6.4±0	6.4 ± 0.06	6.45±0.06	6.43±0	7 ± 0	6.4±0.17	6.4±0	6.4 ± 0	6.3±0
	100	6.4±0.06	6.5±0	6.5±0	6.5±0	8.55 ± 0.5	6.9±0	6.5±0	7.9 ± 0.06	6.3±0
	200	6.5±0	6.6±0.11	6.5±0	6.5±0	9.33±0.28	7±0	6.5±0	8.9±0.11	6.4±0
Sida fryxelli	50	6.5±0	6.4 ± 0.10	6.86±0.06	6.5±0	6.5 ± 0	6.5±0	6.5±0	6.5 ± 0	6.5±0
	100	6.5±0	7 ± 0	7±0	6.53±0.06	6.86 ± 0.06	6.56±0.06	6.5±0	9.9 ± 0.10	6.5±0
	200	6.5±0	7.3±0.29	7±0	6.93±0.06	7.1±0.11	6.86±0.06	6.5±0	13.9±0.32	6.96±0.06
Sida rhombifolia	50	6.4±0	6.6 ± 0.12	6.43±0.06	6.41±0	7.53 ± 0.06	6.73 ±0.06	6.45±0	7 ± 0	6.83±0.06
	100	6.46±0.06	7 ± 0.06	6.53±0.06	6.5±0	8.4 ± 0.32	6.86±0.06	6.5±0	10.0± 0.45	6.86±0.06
	200	6.46±0.06	7.1±0.17	6.7±0.06	6.5±0	10.8±0.25	7.5±0.06	6.5±0	10.9±0.11	7±0

Values are Mean ± Standard Deviatio

Conclusion

The four species of Sida *i.e*., *Sida alnifolia* L., *Sida rhombifolia* L., *Sida acuta* Burm.f, *Sida fryxelli* Sivarajan & Pradeep (I.C.) differed in their phytochemical constituents. Of the 3 different solvents used methanolic extract showed maximum number of phytochemical constituents. Most promising antibacterial activity was showed by *Sida acuta* methanolic extract against *S. aureus* all species were not efficient against E. coli. In Uv-vis studies of the extracts in the range of 190nm to 400nm showed large number of peaks

which confirm the presence of large number of potentially active secondary metabolites.

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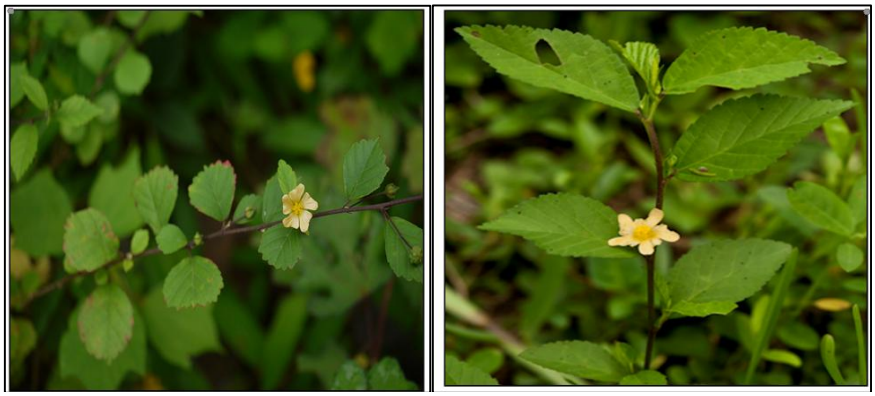
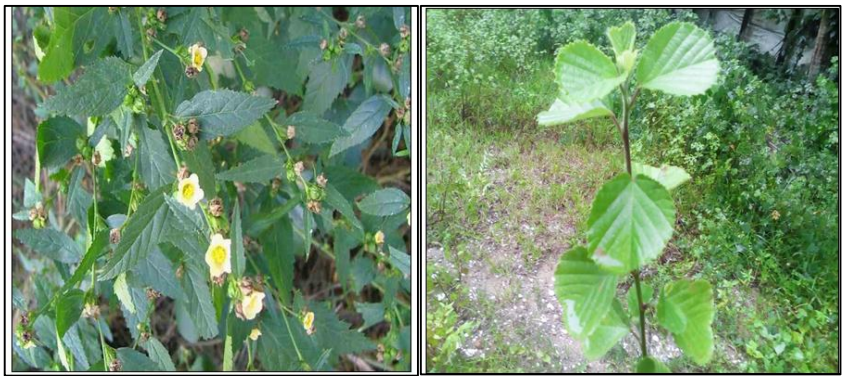


***Sida alnifolia* L.** ***Sida rhombifolia* L.**

Plate 1. Habit of selected species of *Sida* L.



Sida acuta Burm. f ***Sida fryxellii* Sivarajan & Pradeep**

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