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Phytochemical comparison of selected *Sida* species using spectrophotometric and multiple chromatographic analyses

Cheruthazhakkat Sulaiman^{1*} , Ankitha Saji¹, Erayur Mana Anandan² and Indira Balachandran¹

Abstract

Background Bala is an important Ayurvedic drug used for the treatment of many diseases. *Sida cordifolia* (L.), Malvaceae family, is the genuine source drug of Bala as per Ayurvedic Pharmacopeia of India. The other species of *Sida* like *Sida acuta*, *Sida rhombifolia*, *Sida alnifolia* and *Sida cordata* are also used as adulterants or substitutes of Bala. The objective of the present study is to identify a scientifically validated substitute for Bala (*Sida cordifolia*) by detailed phytochemical evaluation of its allied species.

Results Preliminary analysis showed that all the selected species contain similar class of compounds like alkaloids, phenolics, flavonoids, etc. Quantitative estimation of major class of compounds such as total alkaloids, total phenolics and total flavonoids was done by spectrophotometric methods. Polyphenolic contents of selected species are almost comparable. In the case of alkaloids, variations have been observed among the species. Chemical profiles of selected species were compared by HPTLC and HPLC analysis. Major chemical constituents are found to be common for the selected species. HPLC profiles also showed similarity in their peak pattern.

Conclusion Based on the phytochemical studies, the species like *S. alnifolia*, *S. acuta* and *S. rhombifolia* contain almost similar types of phytochemicals. However, the chemical constituents of *Sida cordata* are found to be different from other allied species. Further studies including pharmacological evaluation are required to ensure the therapeutic properties of allied species to confirm the substitute.

Keywords *Sida cordifolia*, Bala, Ayurveda, HPTLC, HPLC

Background

Bala is an important Ayurvedic drug used for the treatment of many diseases. Bala is described as Rasayana, Vishaghna, Balya and Pramehaghna in the classical Ayurveda texts. *Sida cordifolia* (L.) is the genuine source drug of Bala as per Ayurvedic Pharmacopeia of India, and it is widely used as source of Bala in northern parts

of India. But the traditional physicians of Kerala have adopted *Sida rhombifolia* as source drug of Bala. The other species of *Sida* like *Sida acuta*, *Sida alnifolia* and *Sida cordata* are also adulterated or substituted with this drug [1]. In Ayurveda, Bala is used for the treatment of rheumatism and it forms a chief ingredient of several important Ayurvedic preparations like *Kshirabala*, *Balaguluchyadi Kashayam*, *Dhanvantaram kashayam*, *Balarishtam*, *Rasnadhi kashayam* etc. [2]. Root is the official part of the drug and is reported to be cool, sweet, demulcent, aphrodisiac and tonic. The drug is also useful in neurological disorders like hemiplegia, facial paralysis, sciatica, general debility, headache, ophthalmia, dysuria, leucorrhoea, tuberculosis, diabetes, fever and uterine disorders [3].

*Correspondence:

Cheruthazhakkat Sulaiman
sulaimanct@aryavaidyasala.com; slmnc@gmail.com

¹ Phytochemistry Division, Centre for Medicinal Plants Research, Arya Vaidya Sala, Kottakkal, Malappuram, Kerala 676503, India

² Product Development Department, Arya Vaidya Sala, Kottakkal, Kerala, India

Table 1 Details of plant collections

Sl. No	Species name	Collection locality	Voucher specimen No.
1	<i>Sida cordifolia</i>	Anamalai Tiger Reserve, Tamil Nadu, India	CMPR 11269
2	<i>Sida alnifolia</i>	Chinnar, Munnar, Kerala, India	CMPR 11270
3	<i>Sida acuta</i>	Arangattukara, Palakkad, Kerala, India	CMPR 11271
4	<i>Sida rhombifolia</i>	Nellimattom, Kothamangalam, Kerala, India	CMPR 11273
5	<i>Sida Cordata</i>	Vadakkenchery, Thrissur, Kerala, India	CMPR 11274

Table 2 Sample details of selected *Sida* species

Sl. No	Sample code	Sample name
1	SCR	<i>Sida cordifolia</i> root
2	SALR	<i>Sida alnifolia</i> root
3	SAR	<i>Sida acuta</i> root
4	SRR	<i>Sida rhombifolia</i> root
5	SCOR	<i>Sida Cordata</i> root
6	SC AP	<i>Sida cordifolia</i> aerial part
7	SAL AP	<i>Sida alnifolia</i> aerial part
8	SA AP	<i>Sida acuta</i> aerial part
9	SR AP	<i>Sida rhombifolia</i> aerial part

The genus *Sida* is one of the most diverse in the Malvaceae family, with about 200 species distributed around the world. Alkaloids, flavonoids and phytosteroids are the major phytochemicals of *Sida* [4]. *Sida rhombifolia* is known as arrow leaf *Sida* by natives. It is a short-lived perennial shrub which can grow up to 1.5 m in height. The leaves of the plants are simple, narrowly ovate to lanceolate with entire leaf blade and without foliar nectarines. Flowers are solitary, axillary with cup-shaped calyx and yellow mericarps with awns, and glabrous with free petals [5]. *Sida alnifolia* is distributed at higher and lower altitudes in South India. It is an annual herb seen in Decan and Karnatic Districts. The leaves are obtuse, pedicels jointed near the flower [6]. *Sida acuta* Burm. f. is commonly known as stubborn weed. It is an erect, branched, small perennial shrub with a woody tap root, and hairy branches up to 1 m high. It is predominant on roadsides, waste areas, grazing land, disturbed land and abandoned farmlands [7]. *Sida cordata* is native to India that grows wild in wastelands and along roadsides. They are found throughout the tropical and sub-tropical plains all over India and Sri Lanka. The herb grows to 30–80 cm. The plant though seasonal, is available throughout the year, and is especially luxuriant during the months of October to December. The leaves, 1–5.5 cm long, are heart shaped one at each node. The flowers, 7–8 mm across, are produced at the leaf axils, and are yellow in colour.

Table 3 Physiochemical properties of various *Sida* species

Physicochemical properties (%)	Samples				
	SCR	SALR	SAR	SRR	SC AP
Total Ash	2.1361	3.052	4.6675	4.777	7.0148
Acid insoluble ash	0.4252	0.5012	0.629	0.9455	1.5499
Water insoluble ash	1.9864	2.6500	2.9448	4.0233	6.0846
Sulphated ash	2.5494	4.3232	6.4665	3.4566	11.5975
Water soluble extractive	6.224	5.424	6.384	5.1	18.976
Alcohol soluble extractive	4.744	7.784	5.696	2.664	8.712

The availability of genuine Bala in the raw drug market is a concern of Ayurveda industry. Various species are admixed and being traded as Bala. The genuine source drug of Bala is facing acute shortage with regard to the huge requirement of the industry. The objective of the present study is to identify a scientifically validated substitute for Bala (*Sida cordifolia*) by detailed phytochemical evaluation that may lead to addressing the scarcity of this drug.

Methods

Collection of plant materials

Various *Sida* species such as *Sida cordifolia*, *Sida acuta*, *Sida cordata*, *Sida rhombifolia* and *Sida alnifolia* were collected from different agroclimatic conditions of south India and from secondary sources also. All the samples were authenticated and voucher specimens were deposited in CMPR Herbarium.

Physicochemical evaluation and preliminary screening

All the collected materials were subjected to physicochemical evaluation to ensure the quality. Preliminary phytochemical analysis was carried out using standard methods to identify the major group of phytochemicals [8].

Extraction of materials

The plant materials were shade dried and pulverized. Five gram each of the sample was successively extracted

Table 4 Preliminary phytochemical screening of various *Sida* species

Phytochemicals	SCR	SALR	SAR	SRR	SCAP
Phenolics	++	++	++	++	++
Flavonoids	+	+	+	++	+
Carbohydrate	+++	+++	+++	+++	+++
Amino acid	++	++	+++	+	+++
Protein	++	++	+++	+	+++
Sterols	+	++	+	+	–
Saponins	–	–	–	–	–
Alkaloids	+++	+++	+++	+++	+++

with various solvents like n-hexane, chloroform and ethanol by refluxing for 6 h. Crude extracts were also prepared separately with water and hydroalcohol (ethanol:water 50:50) using reflux extraction method. The process was repeated in triplicates. The final extracts were pooled and concentrated at 40 °C using Rotary evaporator (Heidolph, Germany), and it was made up to 100 ml with respective solvents in standard flasks. The extracts were kept under refrigerator until various phytochemical analyses.

Estimation of total alkaloids

Total alkaloid content (TAC) was estimated using bromocresol green spectrophotometric method. Atropine was used as standard and the total alkaloid content

was expressed as milligram equivalents of atropine. The method consisted of extraction of alkaloid by acid treatment of the extracts followed by basification and extraction with chloroform. Different concentrations of atropine were used to plot calibration curve. The absorbance of the complex in chloroform was measured at 470 nm against blank [9].

Estimation of total phenolics

The total phenolic content (TPC) was determined using Folin-Ciocalteu method with gallic acid as standard. The absorbance was measured at 550 nm [10, 11]. TPC was expressed as gallic acid equivalents (GaE) in mg/g of sample.

Estimation of total flavonoid content

Total Flavonoid content (TFC) of aqueous, ethanol and hydroalcohol extracts of selected *Sida* species was estimated. The TFC of various extracts of *Sida* species was estimated spectrophotometrically at 510 nm by using aluminium chloride colorimetric assay and was expressed as milligram equivalent of Quercetin (mg QE/g) [11].

High performance thin layer chromatographic (HPTLC) analysis

HPTLC analysis was performed using CAMAG HPTLC system (Switzerland). Samples were applied using automatic sample applicator (CAMAG ATS-IV) on aluminium backed pre-coated silica gel 60F₂₅₄ TLC plate (Merck India). Mobile phase was standardized as toluene, ethyl acetate in the ratio of 8:2 for n-hexane and chloroform extracts, Toluene, Ethyl acetate, Methanol, Formic acid in the ratio of 6:4:1:0.5 for alcoholic extracts, 5:5:1:0.5 for hydroalcohol and 5:5:1:0.5 for aqueous extracts. The chromatogram was developed in a saturated Twin Trough chromatographic chamber (Camag, Switzerland). The developed plate was visualized under UV at 254 nm and 366 nm and in visible light after derivatizing with anisaldehyde sulphuric acid reagent followed by heating at 105 °C for 5 min.

Table 5 Total phenolics, Total flavonoids and flavonoid to phenolics ratio of various extracts of selected *Sida* species

Sl. No	Sample code	TPC	TFC	F/P
1	SCR A	2.352 ± 0.000	1.485 ± 0.049	0.631505
2	SCR E	1.94 ± 0.024	1.604 ± 0.203	0.827216
3	SCR HA	3.547 ± 0.019	3.28 ± 0.117	0.926219
4	SALR A	2.091 ± 0.022	0.824 ± 0.013	0.394213
5	SALR E	1.611 ± 0.008	0.545 ± 0.047	0.338672
6	SALR HA	2.109 ± 0.022	2.03 ± 0.235	0.964865
7	SAR A	2.242 ± 0.023	1.126 ± 0.027	0.502632
8	SAR E	1.927 ± 0.013	1.222 ± 0.076	0.634406
9	SAR HA	2.408 ± 0.012	1.979 ± 0.165	0.821885
10	SRR A	2.277 ± 0.041	1.302 ± 0.112	0.571893
11	SRR E	1.969 ± 0.07	0.888 ± 0.036	0.45099
12	SRR HA	2.607 ± 0.024	2.066 ± 0.036	0.79275
13	SC AP A	4.621 ± 0.039	2.775 ± 0.153	0.600649
14	SC AP E	4.426 ± 0.026	3.73 ± 0.198	0.844826
15	SC AP HA	5.812 ± 0.028	4.945 ± 0.145	0.850981

Table 6 Total Alkaloid content of various *Sida* samples

Sl. No	Sample code	Total alkaloid content (mg Eq At/g)
1	SCR	3.3439 ± 0.4428
2	SALR	1.4402 ± 0.1539
3	SAR	9.1846 ± 0.1299
4	SRR	0.7640 ± 0.0180
5	SC AP	0.7952 ± 0.0360

High performance liquid chromatography (HPLC) analysis

Comparative HPLC profiling was done using Agilent 1200 High Performance Liquid Chromatographic system equipped with Luna[®] 5 µm HILIC 200A[°] column (150×4.6 mm) and quaternary pump with Diode Array Detector (DAD) in combination with Agilent Chemstation software. The mobile phase used for the separation was HPLC grade 0.1% acetic acid in water (A) and HPLC grade Acetonitrile (B) in a gradient elution method; % A—5–85, 10–75, 15–65, 20–55. The samples were injected using Agilent auto-sampler with an injection volume of 20 µl. The column temperature was maintained at 30 °C, and the flow rate was maintained to 0.8 ml/min. The DAD signal was recorded @ 260 nm with a total run time of 20 min.

Results

Collection of materials

Various species such as *Sida cordifolia*, *S. alnifolia*, *S. acuta*, *S. rhombifolia* and *S. cordata* were collected from wild and secondary sources (Fig. 1). The plant collection details and sample details are given in Tables 1 and 2.

Phytochemical studies

Physicochemical properties and preliminary screening

The Pharmacopoeial standards such as physicochemical properties are the important quality parameters of herbal drugs. Physicochemical and phytochemical characterizations are required to be carried out for establishing their identity, purity, and quality standards. Physicochemical standards were generally used for deciding the identity, purity and strength of the drug source. These parameters were also used to detect the adulterants if present in the plant material. Physicochemical properties such as total ash, acid insoluble ash, water insoluble ash, sulphated ash, water soluble extractive and alcohol soluble extractive were evaluated in all the samples of *Sida* species. The result is presented in Table 3. In the case of water soluble extractives, the highest extractive value was observed for aerial parts of *Sida cordifolia* (18.976%) and the least value was showed by root of *Sida rhombifolia* (5.1%). Highest alcohol soluble extractive value was noted for aerial parts of *Sida cordifolia* (8.712%). The Pharmacopoeial monograph of *Sida cordifolia* is not available in Ayurvedic Pharmacopoeia of India [1].

Qualitative test for the detection of major class of compounds is a common preliminary screening of plant-based crude drugs as it ensures the minimal phytochemical contents present in the respective extracts.

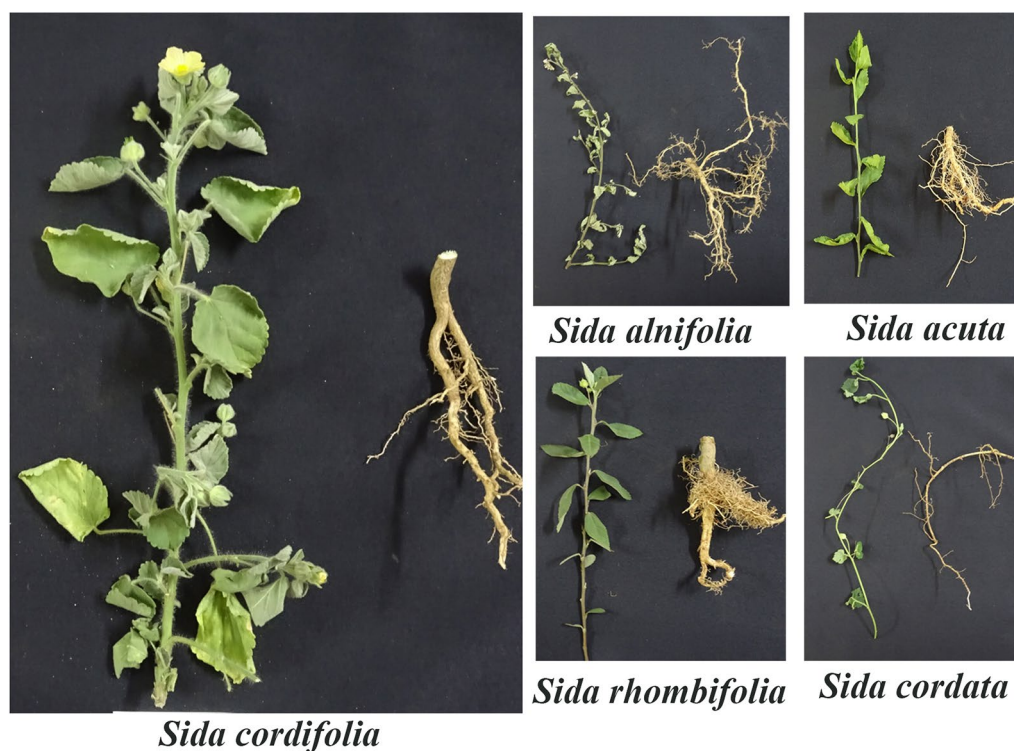


Fig. 1 Photos of selected *Sida* species

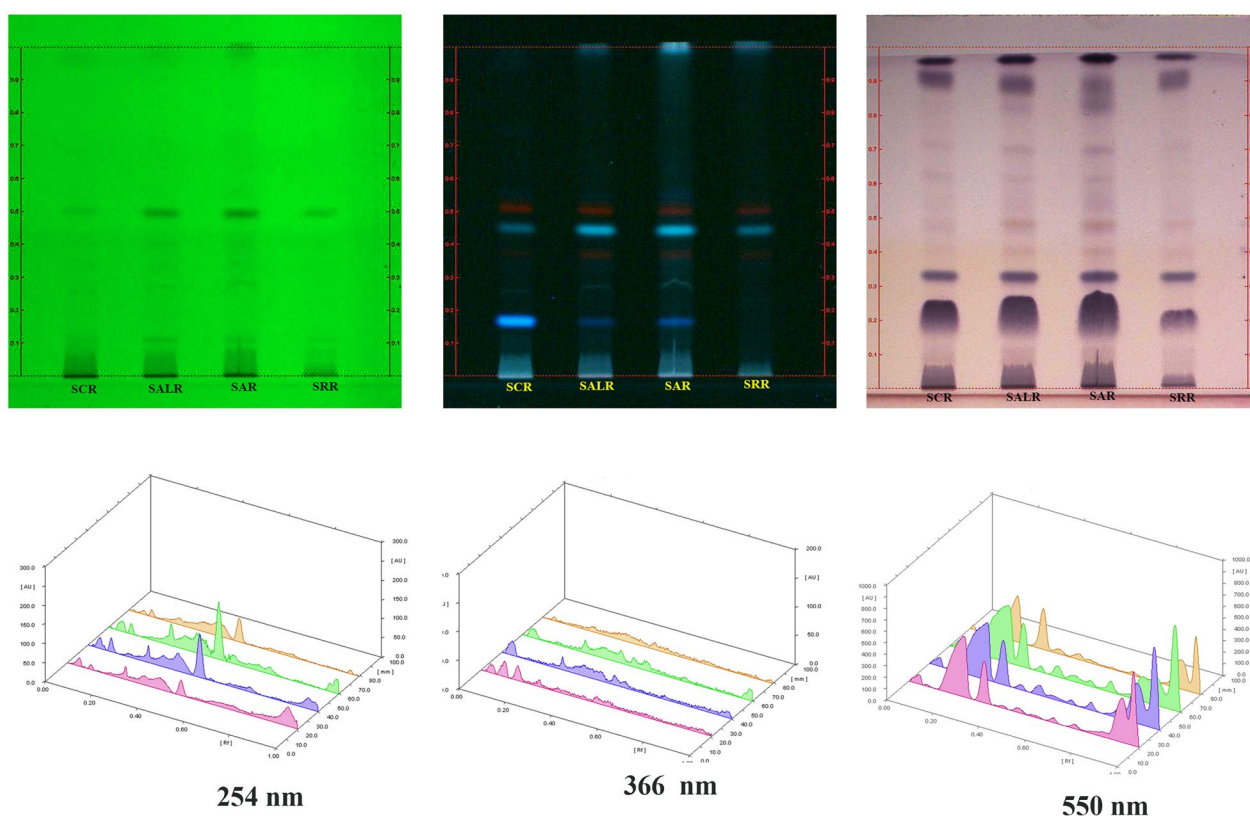


Fig. 2 HPTLC profiling of n-Hexane extracts of selected *Sida* species. Tracks: SCR; SALR; SAR; SRR

Result of preliminary phytochemical screening of selected *Sida* species is presented in Table 4. The preliminary phytochemical analysis showed presence of phenols, flavones, alkaloids, carbohydrates and proteins as major compounds in all the selected species.

Estimation of total phenolic content (TPC) and total flavonoid content (TFC)

Total polyphenolics such as Total Phenolic Content (TPC) and Total Flavonoid Content (TFC) of selected *Sida* species were estimated specifically for aqueous (A), ethanol (E) and hydro alcohol (HA) extracts (Table 5). TPC was calculated from calibration curve of gallic acid ($R^2=0.9991$). The highest phenolic content (TPC) was observed in the hydro alcohol extract of aerial parts of *Sida cordifolia*. Total poly phenolics are almost comparable for all the selected species. TPC of aqueous extracts of various samples of *Sida* species is as follows SCR (2.352), SALR (2.0913), SAR (2.2426), SRR (2.2772), SCA (4.6216). The highest phenolic content (TPC) was observed in the hydro alcohol extract of aerial parts of *Sida cordifolia* (5.8121) followed by SCR (3.5479), SRR (2.6072), SAR (2.4085) and SAL (2.1095), respectively. Total polyphenolics are almost comparable for all the

selected species. TPC of water extracts is lower than that of hydroalcoholic extracts. It might be due to the higher extraction capacity of water alcohol combination.

Total Flavonoid Content (TFC) of selected species was estimated for different extracts such as water, ethanol and hydroalcohol. TFC was calculated from calibration curve of Quercetin ($R^2=0.994$). Highest TFC was showed by aerial parts of *Sida cordifolia* (4.9459) followed by roots of *Sida cordifolia* (3.285) in hydroalcohol extract. Aqueous extract of *Sida cordifolia* contains 1.485 mg equivalents of flavonoids. *Sida alnifolia* showed less flavonoid compared to other species.

Flavonoid/phenolics ratio was also calculated for each sample to specify the abundance of flavonoids among the total polyphenolics. Hydroalcoholic extracts of root of *S. alnifolia* and *S. cordifolia* showed highest f/p ratio (0.96 and 0.92) followed by hydroalcoholic extract of areal part of *S. cordifolia* (0.85), ethanolic extract of aerial part of *S. cordifolia* (0.84) and hydroalcoholic extract of root of *S. acuta* (0.82).

Total alkaloid content

The occurrence of alkaloids in *Sida* species has chemotaxonomic relevance to Malvaceae family, since it occurs

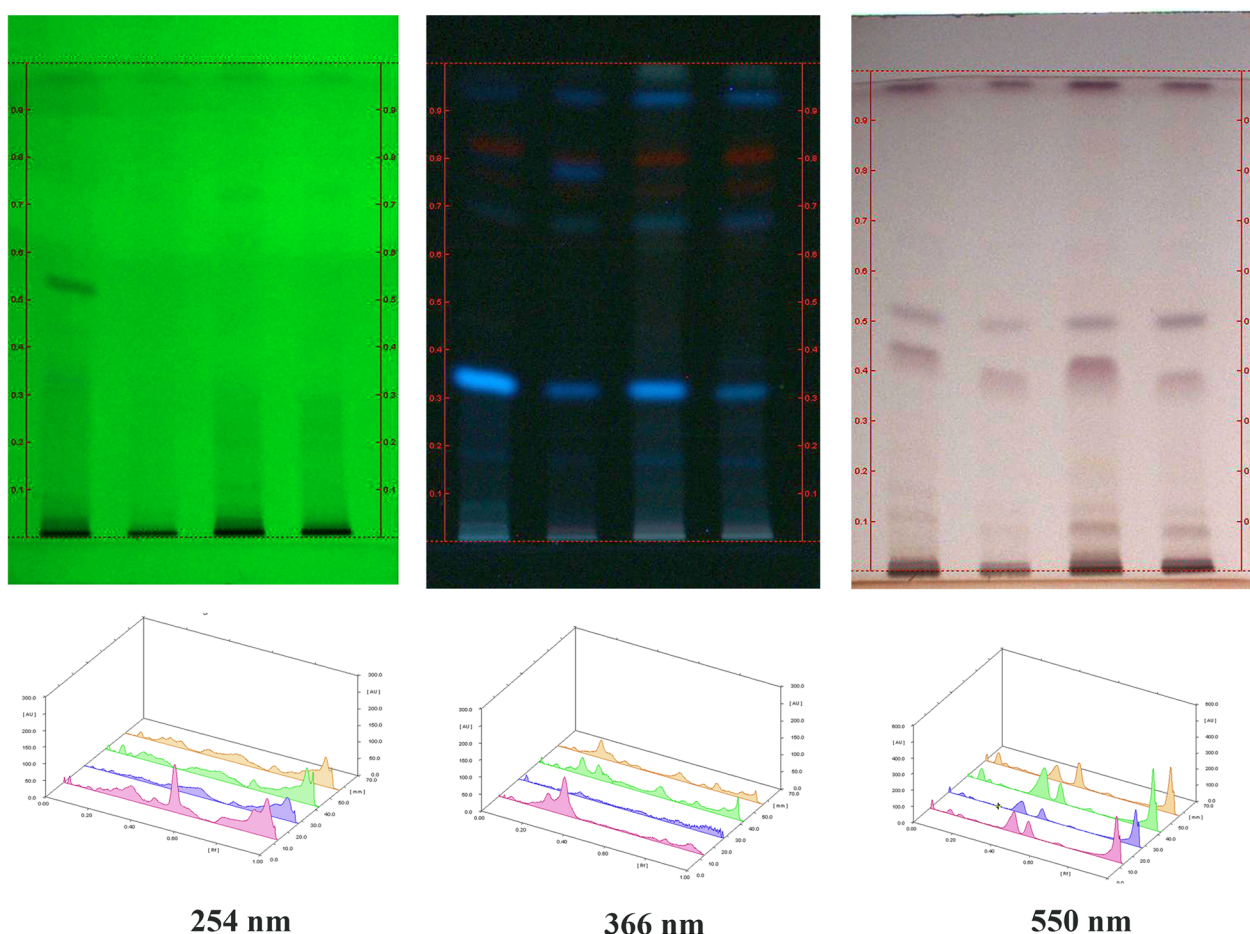


Fig. 3 HPTLC profiling of chloroform extracts of selected *Sida* species. Tracks: SCR; SALR; SAR; SRR

almost exclusively in *Sida* genus [12–14]. Total alkaloid content (TAC) of various *Sida* species has been estimated using Bromocresol green spectrophotometric method with atropine standard. TAC was calculated from calibration curve of Atropine ($R^2=0.991$). Root of *S. acuta* showed highest quantity of Alkaloids (9.1846) followed by roots of *S. cordifolia* (3.3439), *S. alnifolia* (1.4402), aerial parts of *S. cordifolia* (0.7952) and root of *S. rhombifolia* (0.7640). The TAC of selected species is given in Table 6.

HPTLC profiling

Comparative HPTLC profiles were developed for root of various *Sida* species with sequential extracts such as n-hexane, chloroform and ethanol. At 254 nm, n-hexane extract (Fig. 2) showed a common band with Rf 0.50 for all the selected species. At 366 nm, compound at Rf 0.38 (red), 0.45 (blue), 0.51 (red) were found to be common for all the selected species. A fluorescent band at 0.17 was detected in all other species except for *S. rhombifolia*. On evaluating under 550 nm, common bands are observed

at Rf 0.22, 0.33, 0.48, 0.63, 0.70, and 0.90. HPTLC profile of chloroform extract (Fig. 3) showed a major band at Rf 0.54 which is specific for *S. cordifolia*. At 366 nm, common bands are observed at Rf 0.32, 0.50, 0.67 and 0.81 for all the selected species. Under 550 nm, bands with Rf 0.40 and 0.50 are common for all the species.

At 254 nm, sequential ethanol extract showed common bands at Rf 0.17 and 0.26. Two bands at 0.08, 0.81 were observed for *S. alnifolia*, *S. acuta* and *S. rhombifolia*. Major bands were observed at 0.05, 0.17, 0.36, 0.62, 0.89 as common bands in all the selected species. On visualizing under 550 nm, common bands with Rf 0.07, 0.20, 0.26, 0.64, and 0.76 are observed for all the selected species.

HPTLC profile of crude ethanol extracts (Fig. 4) is almost similar in their banding pattern, and it indicates the presence of similar phytoconstituents in all the selected species. At 366 nm, major common bands were observed at Rf 0.08, 0.22, 0.40, 0.64 and 0.90. Band at Rf 0.11 and 0.83 are two additional common compounds for all other species except *Sida cordifolia*. A band at

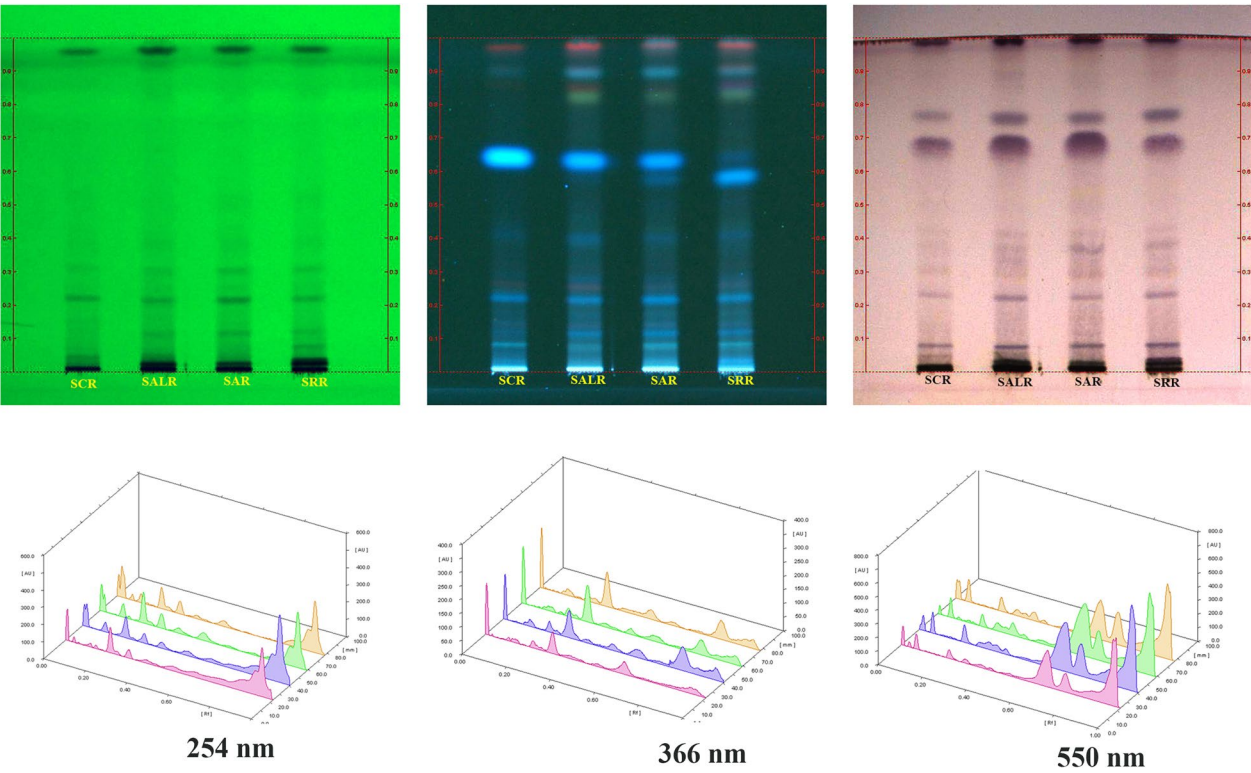


Fig. 4 HPTLC profiling of ethanol extracts of selected *Sida* species

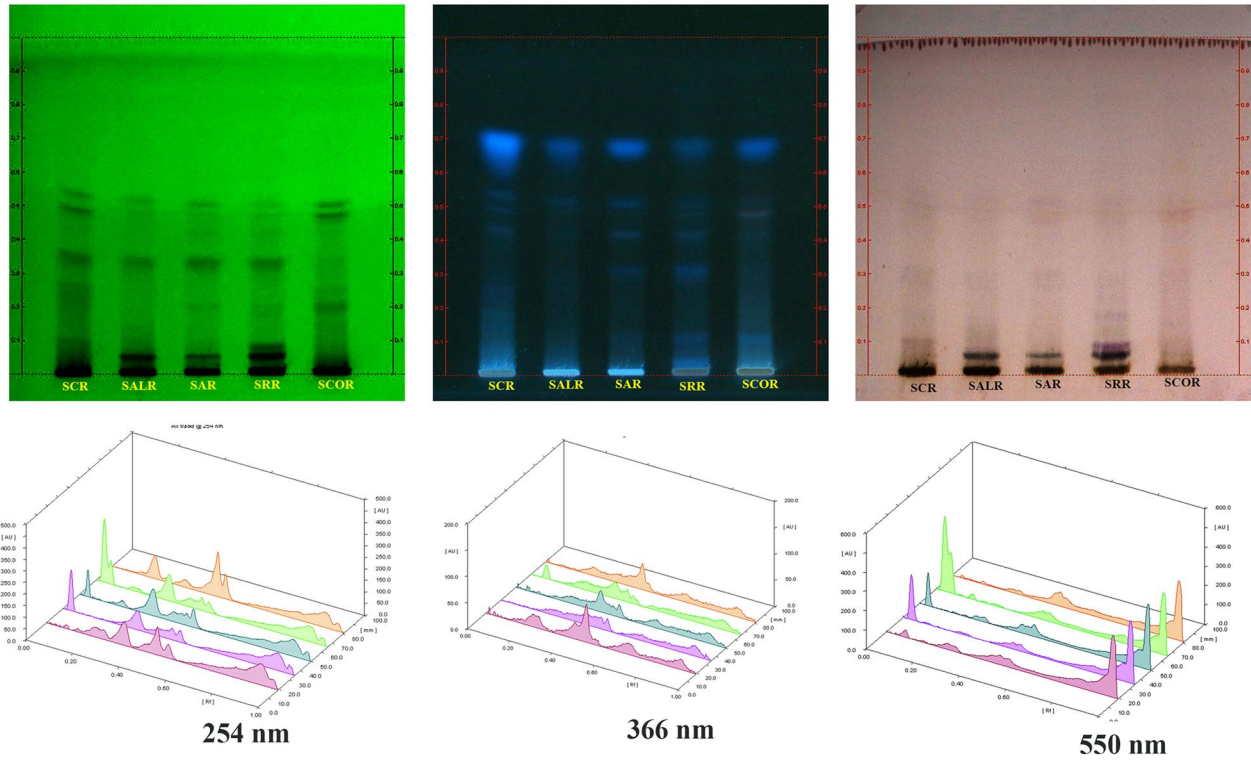


Fig. 5 HPTLC profiling of hydroalcohol extracts of selected *Sida* species. Tracks: SCR; SALR; SAR; SRR; SCOR

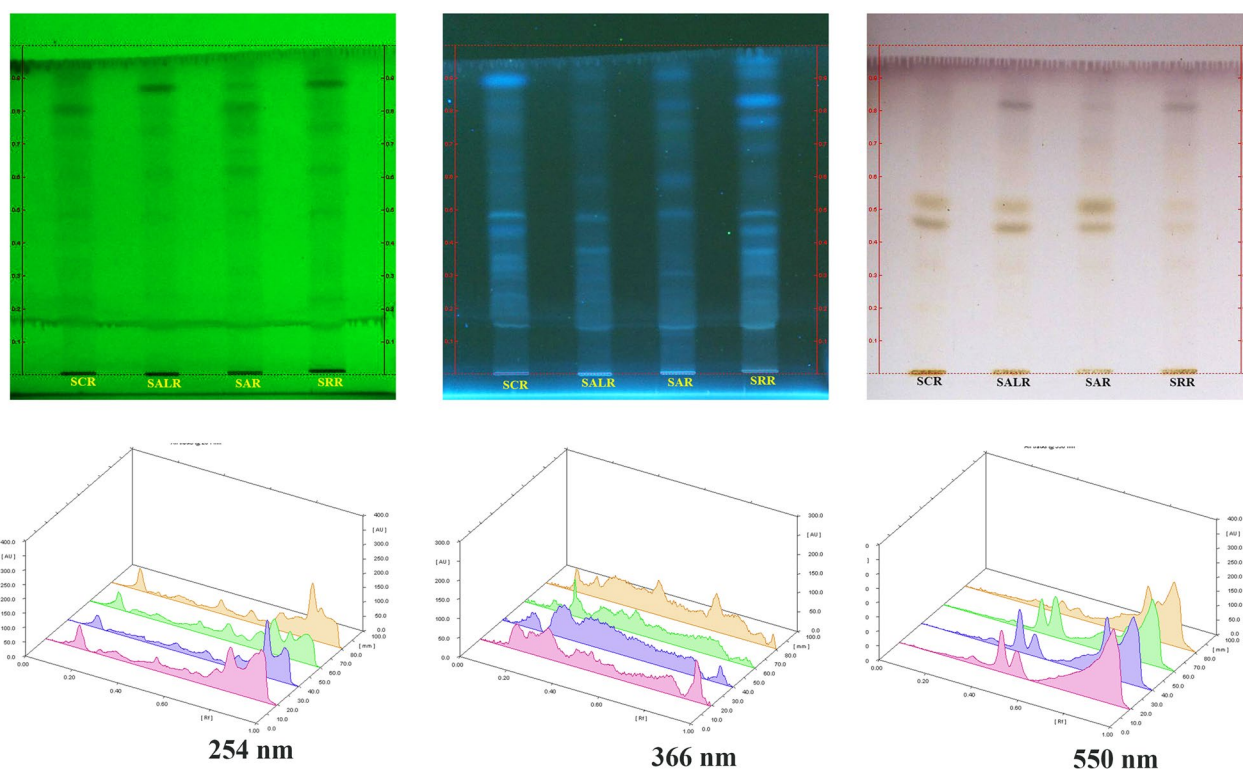


Fig. 6 HPTLC profiling of aqueous extracts of selected *Sida* species. Tracks: SCR; SALR; SAR; SRR

0.59 was present only for *S. acuta* and *S. rhombifolia*. On evaluating under 550 nm, common bands at R_f 0.08, 0.22, 0.69, 0.77 were observed for all the selected species.

Comparative HPTLC profile of hydroalcohol extracts of selected *Sida* species is presented in Fig. 5, At 254 nm, common band 0.34, 0.51 was observed. A band at R_f 0.20 was observed for *S. acuta* and *S. rhombifolia*. On visualizing under 366 nm, a major band at 0.52, 0.67 (blue) is found to be common for all the species. A band at 0.42 was present only for *S. cordifolia*, *S. acuta* and *S. rhombifolia*. At 550 nm. A band at 0.06 is absent for *S. cordifolia* and *S. cordata*. A specific band at 0.09 was observed for *S. rhombifolia* only.

Chromatogram for crude water extracts were documented by HPTLC visualizer at 254 nm and 366 nm (Fig. 6). At 254 nm, major common bands were observed at 0.45, 0.6. Common bands at 0.32, 0.45 and 0.60 were observed at 366 nm for all the selected species. At 366 nm, specific bands at 0.13, 0.17 were observed for *S. cordifolia*. A common band at 0.32 was observed for *S. acuta* and *S. rhombifolia*. A band at 0.11 was found to be present only for *S. rhombifolia*. At 550 nm common bands were observed at 0.34, 0.46 and 0.52 for all the species.

The extracts such as ethanol, water and hydroalcohol showed maximum similarity in their banding pattern.

HPTLC profiling of various extracts of selected *Sida* species showed that most of the species except *S. cordata* contain almost similar type of phytochemicals. Number of matching bands for aqueous and hydro alcohol extracts shows the close similarities in their phytochemical characteristics. There are minor variations in the band intensities of separated compounds which might be due to the variation of their concentration. Variations of abundance of some compounds have been observed for the selected species. However, most of the major bands are found to be common with almost similar band intensity.

HPLC analysis

Comparative HPLC profiles were developed for the aqueous extracts of selected species. DAD chromatograms were documented at 260 nm (Fig. 7). Major compounds for *S. cordifolia* were observed at R_t 1.1, 1.2, 1.3, 1.8, 3.6, 4.6, 5.1, 6.6, 7.3, 9.4 and 10.5. Compounds with R_t 1.1, 1.2, 3.6, 6.6 and 9.4 are common for all the species with variation in peak area percentage. Some specific peaks were observed at 10.5, 11.1, 12.4, 13.5 and 14.9 for *S. cordifolia* only. Seven compounds are found to be common for *S. cordifolia* and *S. alnifolia*. The peak at 9.4 showed highest area percentage for *S. alnifolia* (28.76%) followed by *S. rhombifolia* (6.65%), *S. acuta* (3.7%) and *S. cordifolia* (1.87%).

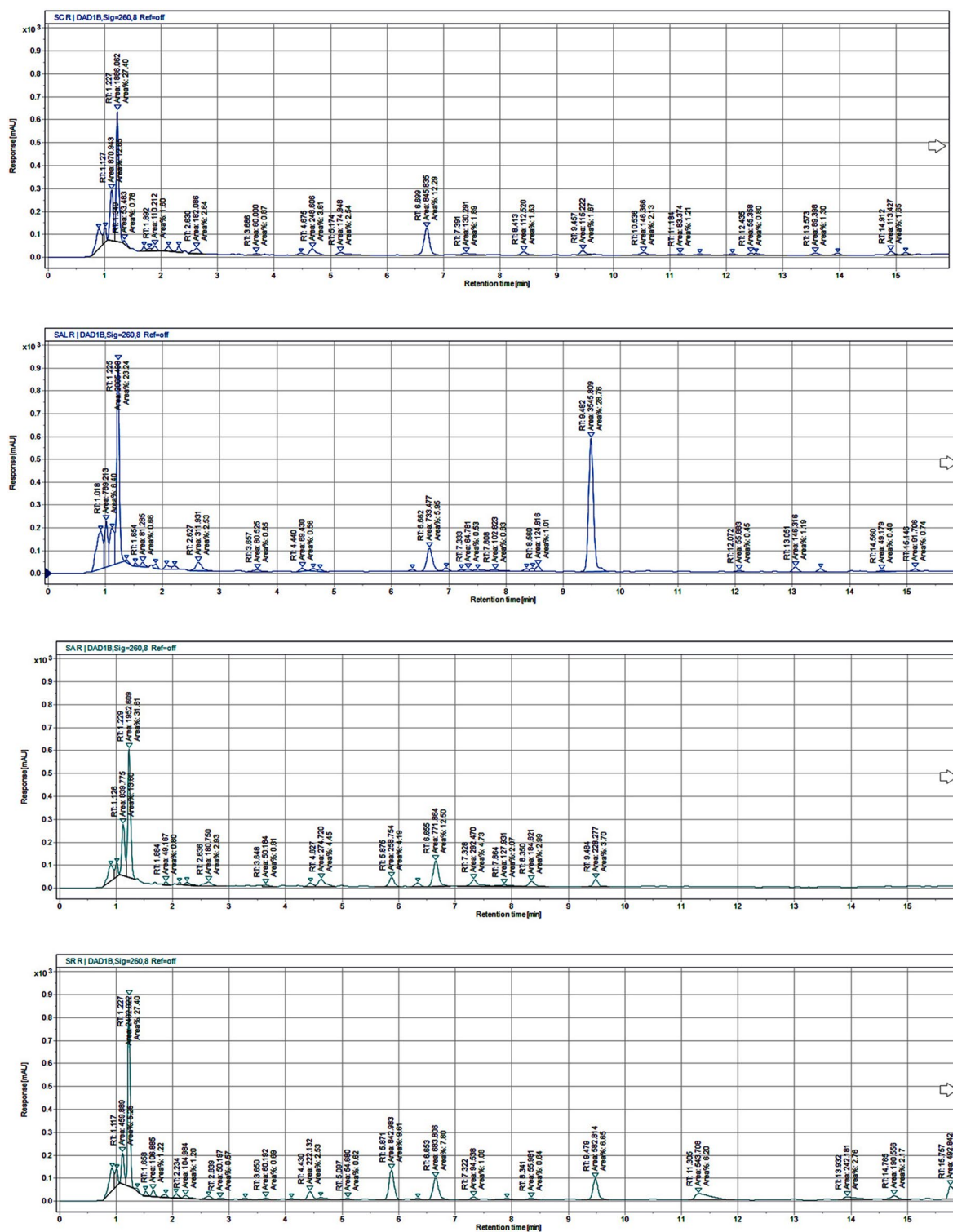


Fig. 7 HPLC profiling of aqueous extracts of selected *Sida* species. **A:** SCR; **B:** SALR; **C:** SAR; **D:** SRR

Discussion

Bala is one of the important drugs used in Ayurveda for the treatment of many diseases. In Ayurveda classics, it is attributed *Balya*, *Kantikarka*, *Grahi*, *Vrishya*, *Ojovardhaka*, *Stambhana*, *Brihmana*, *Sothahara*, *Rasayana* and *Hridya* properties. *Sida cordifolia* is the genuine source drug for Bala. However, in the raw drug market various other species like *S. acuta*, *S. alnifolia*, *S. rhombifolia* etc. are being traded as Bala. The present study is aimed at identification of scientifically validated substitute for Bala by detailed phytochemical studies. Phytochemical comparison of *S. cordifolia* has been done with its allied species such as *S. alnifolia*, *S. acuta* and *S. rhombifolia*. The literature survey revealed that no comparative phytochemical studies of selected species are available. However, anatomical comparison of *Sida rhombifolia*, *S. alnifolia*, *S. scabrida* and *S. rhomboidea* has been reported previously [15]. Antioxidant activities of species such as *Sida acuta*, *S. cordata*, *S. cordifolia*, *S. indica*, *S. mysorensis*, *S. retusa*, *S. rhombifolia* and *S. spinosa* were also reported earlier [16].

Preliminary analysis showed that all the selected species contain similar class of compounds like alkaloids, phenolics, flavonoids, etc. Quantitative estimation of major class of compounds such as total alkaloids, total phenolics and total flavonoids was done by spectrophotometric methods. Polyphenolic contents of selected species are almost comparable. In the case of alkaloids, variations have been observed among the species. Chemical profiles of selected species were compared by HPTLC and HPLC analysis. HPTLC finger printing of various extracts of selected species has been done and the chromatograms were documented under UV and visible light. Various matching bands were observed in the HPTLC profiles of ethanol, water and hydroalcohol extracts. Major chemical constituents are found to be common for the selected species. HPLC profiles also showed similarity in their peak pattern. However, variation in area percentages of some separated peaks might be due to differences in its abundance. The pharmacological studies of selected species are under progress and it will be reported later.

Conclusion

Phytochemical studies provided valuable information regarding the chemical constituents of selected *Sida* species. Major compounds are found to be common for the selected species such as *S. cordifolia*, *S. alnifolia*, *S. acuta* and *S. rhombifolia*. Chemical profile of *S. cordata* showed major variations when compared to other species. Based on the phytochemical studies, the species like *S. alnifolia*, *S. acuta* and *S. rhombifolia* contain

almost similar types of phytochemicals. Further studies including pharmacological evaluation are required to confirm the possible substitute for Bala.

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Plant authentication

Plant authentication was done by Plant Systematics and Genetic Resources Division of the institute.

Author contributions

All authors have read and approved the manuscript. SCT designed and executed the work, carried out data analysis and drafted the manuscript. AS carried out the sample preparation, formal analysis and data compilation. AEM and IB participated in planning and edited the manuscript.

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Availability of data and materials

All data and material are available upon request.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interest

The authors declare that they have no competing interests.

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