Total polyphenolic contents and *in vitro* antioxidant properties of eight *Sida* species from Western Ghats, India

M. D. Subramanya, Sandeep R. Pai[1], Vinayak Upadhya[2], Gireesh M. Ankad[2], Shalini S. Bhagwat, Harsha V. Hegde[1,2]

Department of Dravya Guna Vidnyana, Karnatak Lingayat Education University, BMK Ayurveda Mahavidyalaya, Belgaum, [1]Plant Biotechnology and Tissue Culture Division, [2]Herbal Medicine Division, Regional Medical Research Centre, Indian Council of Medical Research, Belgaum, Karnataka, India

ABSTRACT

Background: *Sida* L., is a medicinally important genus, the species of which are widely used in traditional systems of medicine in India. Pharmacologically, roots are known for anti-tumor, anti-HIV, hepatoprotective, and many other properties. Phenolic antioxidants help in reducing oxidative stress occurring during treatment of such diseases. **Objective:** The study aimed to evaluate and compare polyphenol contents and antioxidant properties of eight selected species of *Sida* from Western Ghats, India. **Materials and Methods:** Methanolic root extracts (10% w/v) of *Sida* species, viz., *S. acuta*, *S. cordata*, *S. cordifolia*, *S. indica*, *S. mysorensis, S. retusa, S. rhombifolia,* and *S. spinosa* were analyzed. **Results:** *Sida cordifolia* possessed highest total phenolic content (TPC: 1.92 ± 0.10 mg Caffeic Acid Equivalent/g and 2.13 ± 0.11 mg Tannic Acid Equivalant/g), total flavonoid content (TF: 2.60 ± 0.13 mg Quercetin Equivalent/g) and also possessed highest antioxidant activities in 2,2-diphenylpicrylhydrazyl (DPPH) radical scavenging (51.31 ± 2.57% Radical Scavenging Activity, (RSA); Trolox Equivalent Antioxidant Capacity: 566.25 ± 28.31μM; Ascorbic acid Equivalent Antioxidant Capacity: 477.80 ± 23.89 μM) and Ferric Reducing Antioxidant Power assays (TEAC: 590.67 ± 29.53 μM; AEAC: 600.67 ± 30.03 μM). Unlike DPPH and Ferric Reducing Antioxidant Power (FRAP) activity, 2, 2′-Azinobis (3-ethyl Benzo Thiazoline-6-Sulfonic acid) $ABTS^{+}$ antioxidant activity was highest in *S. indica* (TEAC: 878.44 ± 43.92 μM; AEAC 968.44 ± 48.42 μM). It was significant to note that values of AEAC (μM) for all the antioxidant activities analyzed were higher than that of TEAC. **Conclusion:** The high contents of phenolic compounds in the root extracts of selected *Sida* species have direct correlation with their antioxidant properties. Conclusively, roots of *S. cordifolia* can be considered as the potential source of polyphenols and antioxidants.

Key words: Antioxidant activity, *Bala*, *Sida,* total phenolic content, total flavonoids

INTRODUCTION

Genus *Sida* L., belonging to family Malvaceae, comprises about 200 species distributed throughout the world and 17 species are reported to occur in India.[1] Roots of many of the speceis are valued for their medicinal properites. The plant is also well documented in *Ayurveda*, ancient Indian system of medicine. *Bala* is an important plant in *Ayurvedic* system of medicine belonging to *Karpasa kula* (family of cotton plant),[2] used as *Rasayana*[3] to treat various ailments such as *Vatavyadhis* (degenerative and musculoskeletal diseases)[4] and *Pradara* (gynecological diseases).[5] It is known for various pharmacological activities such as hepatoprotective, anti-arthritic, treatment for gonorrhea, and also reported as immuno-enhancer.[6]

Even though the plants belonging to genus *Sida* are well known for medicinal properties, especially in the Indian classical systems, the crisis in their correct botanical identity still persists. As per classical references, *Bhavaprakasha nighantu*[7] mentions existence of four varieties of *Bala* (*Balachatushtayam*) while *Dhanvanthari nighantu*[8] mentions five varieties (*Panchabala*). Generally, as per the classics, *Bala* is referred to *Sida cordifolia* L., *Mahabala* is correlated to two plants viz. *Kshetrabala* (*Sida rhombifolia* L.)

Address for correspondence:
Dr. Gireesh M. Ankad, Scientist 1 (Herbal Medicine), Regional Medical Research Centre (ICMR), Nehru Nagar, Belgaum - 590 010, Karnataka, India.
E-mail: drgirishankad@gmail.com

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and *Sahadevi* (*Vernonia cinerea* L.). Similarly, the name *Atibala* is attributed to *Abutilon indicum* L. and *Nagabala* indicate three plants, i.e. *Bhumibala* (*Sida veronicaefolia* L.), *Kantakinibala* (*Sida spinosa* L.), and *Gudasharkara* (*Grewia hirsuta* Vanb.).[9] Other classical references mentioned about *Rajabala* or *Bruhad naga bala* as *Sida acuta* Burm.f.[10] There are differences of opinions regarding botanical identities of *Bala*, which are still controversial.[11] In addition, few other species of *Sida* such as *S. mysorensis, S. cordata, S. spinosa,* and *S. retusa* are also found to be used as substitutes for one or the other species of *Sida*.[6]

Phenolic compounds present in plants are considered to have a great deal of biologically active constituents and therefore have been studied extensively. One of the prominent properties of the phenolics is their excellent radical scavenging activity.[12] Flavonoids, a group of polyphenolic compounds, are well known for their biological properties, such as free radical scavenging activity, inhibition of hydrolytic and oxidative enzyme, and anti inflammatory action.[13]

In view of the existing crisis among different species of *Sida* as *Bala*, the present work was carried out to study and compare the total polyphenol contents with antioxidant activity in selected *Sida* species. Methanolic root extracts of eight *Sida* species, collected from Western Ghats were used to evaluate their total phenolic contents, flavonoid contents, and antioxidative potencies (DPPH, FRAP and ABTS assays).

MATERIALS AND METHODS

Collection of plant material and extraction

The plants of eight *Sida* species viz. *S. acuta*, *S. cordata*, *S. cordifolia*, *S. indica*, *S. mysorensis, S. retusa, S. rhombifolia,* and *S. spinosa* were collected in the month of April–May from Western Ghats regions of Belgaum district. Authenticated voucher specimens have been deposited at Regional Medical Research Centre (ICMR), Belgaum, Karnataka, India for future reference. [Voucher specimen Nos.: *Sida cordata* Boiss. (RMRC 475), *Sida spinosa* L. (RMRC 477), *Sida rhombifolia* L. (RMRC 479), *Sida acuta* Burm.f.(RMRC 484), *Sida cordifolia* L. (RMRC 938), *Sida indica* L. (RMRC 939), *Sida mysorensis* Wt. and Arn. (RMRC 970), *Sida retusa* L. (RMRC 971)].

The roots were cleaned properly, shade dried, and coarsely powdered. The powdered materials (10 g) were extracted with methanol (100 mL) by cold maceration and the extracts obtained were concentrated under reduced pressure at 40°C using rotary evaporator (Heidolf, Germany). These residues were stored at 4°C until further use.

Total phenolic content

Total phenolic content was quantified using modified Folin-Ciocalteu method.[14] The assay mixture was prepared using 0.5 mL of distilled water, 0.125 mL different concentrations of standard Tannic acid, and/or Caffeic acid with 0.125 mL of Folin-Ciocalteu reagent, incubated for 10 min in dark. After 10 min 1.25 mL 7% aq. sodium carbonate and 1 mL of distilled water was added and the reaction mixture was incubated in dark for 90 min at 37°C. The absorbance of blue color was read at 760 nm using distilled water instead of standards in the reaction mixture as blank on double beam spectrophotometer. Similarly, extracts prepared (10% w/v in methanol) were also quantified and the results were compared to the standard curves and expressed as mg tannic or caffeic acid equivalent per gram dry powder for the samples.

Total flavonoids

Total flavonoid contents were quantified using method explained by Luximon *et al.*[15] One milliliter of 2% methanolic $AlCl_3$ was reacted with 1 mL of different concentrations of standard quercetin for 10 min in dark. Absorbance was measured at 367 nm on double beam spectrophotometer using 2% methanolic $AlCl_3$ as blank. Standard was replaced with extracts prepared (10% w/v in methanol) and results were compared to the standard curves obtained. The results were expressed as mg quercetin equivalent per gram dry powder for the samples.

Antioxidant activities

DPPH radical scavenging assay

The antioxidant activities were determined as the measure of radical scavenging using DPPH assay.[16] Two milliliter of methanolic solution of DPPH (25 ppm) was mixed with 50 µl of 10% sample extract and the mixture was incubated for 30 min in dark. The absorbance at 515 nm was measured using methanol as blank. Similarly, different concentration of ascorbic acid and/or Trolox was used instead of plant extract as reference standard during the experiment. The inhibition percentage of DPPH (% DPPH) was calculated and the results were expressed as % RSA (Radical Scavenging Activity).

Ferric reducing antioxidant power (FRAP) assay

The Ferric Reducing Antioxidant Power (FRAP) assay[17] was used to measure the total antioxidant power of extracts. In FRAP assay, reductants (antioxidants) in the sample reduce Fe3+/tripyridyltriazine complex, present in stoichiometric excess, to the blue colored ferrous form, with an increase in absorbance at 593 nm. The Δ A is proportional to the combined (total) ferric reducing antioxidant power (FRA*P* value) of the antioxidants in

10% sample extracts. The FRAP assay results were expressed as μM ascorbic acid and/or Trolox equivalent antioxidant capacity (AEAC/TEAC).

2, 2′-Azinobis (3-ethyl Benzo Thiazoline-6-Sulfonic acid) (ABTS) method

ABTS method described by Re *et al.*[18]was used during the study. ABTS was dissolved in water to a concentration 7 mM. ABTS radical cation (ABTS•+) was produced by reacting ABTS stock solution with 2.45 mM potassium persulfate (final concentration) and allowing the mixture to stand in the dark at room temperature for 16 hours before use. The resulted ABTS•+ solution was diluted with methanol to an absorbance of 0.7 (±0.02) at 734 nm using spectrophotometer. Sample extracts (100 μL, 10% w/v) were allowed to react with 2 mL of the ABTS•+ solution for 30 min in dark condition. Then the absorbance was measured at 734 nm using a spectrophotometer. Different concentration of ascorbic acid and Trolox were used as reference standards. Results were expressed in μM ascorbic acid and/or Trolox equivalent antioxidant capacity (AEAC/TEAC).

RESULTS AND DISCUSSION

Total phenolic content

The total phenolic content (TPC) of selected *Sida* species were expressed in terms of caffeic acid/tannic acid equivalents using the standard curve equation as shown in Table 1 and the TPC are presented in Table 2. TPC were ranging between 0.72 ± 0.04 to 1.92 ± 0.10 mg CAE/g and 0.93 ± 0.05 to 2.13 ± 0.11 mg TAE/g. *S. retusa* possessed lowest TPC while *S. cordifolia* the highest. The selected *Sida* species may be arranged on basis of TPC from lowest as in *S. retusa*<*S. cordata*<*S. rhombifolia*<*S. acuta*<*S. indica* <*S. spinosa*<*S. mysorensis*<*S. cordifolia* to highest. Tannic acid equivalent TPC were higher than the caffeic acid equivalent TPC in all species.

Total flavonoids

The total flavonoids (TF) of selected *Sida* species were expressed in terms of quercetin equivalent using the standard curve equation as shown in Table 1 and the TF values are presented in Table 2. TF were ranging from 0.70 ± 0.03 to 1.26 ± 0.06 mg QE/g. *S. retusa* possessed lowest TF and *S. cordifolia* the highest. The selected species may be arranged on basis of TF from lowest as in *S. retusa*<*S. acuta*<*S. rhombifolia*<*S. cordata*<*S. indica*<*S. spinosa*<*S. mysorensis*<*S. cordifolia* to the highest.

Antioxidant activities

The result of antioxidant activities expressed in terms of μM AEAC/TEAC using the standard curve equations which are showed in Table 1. Similarly, the results of the different antioxidant activities (DPPH, FRAP, and ABTS) are summarized in Table 3.

DPPH radical scavenging assay

Table 3 summarizes % DPPH radical scavenging activity (% RSA) in the *Sida* species. The RSA ranged between 17.42 ± 0.87 to 51.31 ± 2.57% and observed a difference of 30% between lowest and highest % RSA. *S. cordifolia* showed highest radical scavenging activity with 51.31 ± 2.57% and *S. retusa* had lowest activity (17.42 ± 0.87%). The selected species may be arranged on basis of % RSA from lowest as in *S. retusa*<*S. cordata*<*S. acuta*<*S. rhombifolia* <*S. indica*<*S. spinosa*<*S. mysorensis*<*S. cordifolia* to highest. TEAC (μM) values for DPPH activity of the *Sida* species were higher than AEAC in all species.

Table 1: Standard curve equation

Activity	Standard	Concentration range μg/mL	Regression equation	Coefficient of determination (R^2)
TPC	Caffeic acid	10-800	y=0.0025x+ 0.0158	0.9995
	Tannic acid	10-800	y=0.0029x+ 0.0426	0.9968
TF	Quercetin	10-400	y=0.0059x- 0.0367	0.9992
DPPH	Trolox	10-800	y=0.0004x+ 0.0059	0.9964
	Ascorbic acid	10-800	y=0.0005x- 0.0074	0.9986
FRAP	Trolox	10-900	y=0.0003x- 0.0024	0.9801
	Ascorbic acid	10-900	y=0.0003x- 0.0054	0.9914
ABTS	Trolox	10-1000	y=0.0010x+ 0.0619	0.9632
	Ascorbic acid	10-1000	y=0.0009x- 0.0005	0.9950

TPC=Total phenolic content, TF=Total flavonoids, DPPH=2,2-diphenyl-1-picrylhydrazyl, FRAP=Ferric reducing antioxidant potential, ABTS=2,2′-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid)

Table 2: Total phenolic and flavonoid contents of *Sida* species

Species	TPC		TF
	mg CAE/g	mg TAE/g	mg QE/g
S. acuta	1.24±0.06	1.45±0.07	0.84±0.04
S. cordata	0.95±0.05	1.16±0.06	0.97±0.04
S. cordifolia	1.92±0.10	2.13±0.11	1.26±0.06
S. indica	1.24±0.06	1.45±0.07	1.03±0.05
S. mysorensis	1.66±0.08	1.87±0.09	1.18±0.05
S. retusa	0.72±0.04	0.93±0.05	0.70±0.03
S. rhombifolia	1.06±0.05	1.27±0.06	0.90±0.04
S. spinosa	1.35±0.07	1.56±0.08	1.09±0.05

Figures in tables are represented as mean of three readings±SD. TPC=Total phenolic content, TF=Total flavonoids, TAE=Tannic acid equivalent, CAE=Caffeic acid equivalent, QE=Quercetin equivalent

Table 3: Comparative antioxidant activities of *Sida* species

Species	DPPH			FRAP		ABTS	
	µM TEAC	µM AEAC	% RSA	µM TEAC	µM AEAC	µM TEAC	µM AEAC
S. acuta	324.00±16.20	284.00±14.20	29.83±1.49	363.33±18.17	373.33±18.67	876.56±43.83	966.56±48.33
S. cordata	204.25±10.21	188.20±09.41	19.22±0.96	359.33±17.97	369.33±18.47	613.78±30.69	703.78±35.19
S. cordifolia	566.25±28.31	477.80±23.89	51.31±2.57	590.67±29.53	600.67±30.03	877.22±43.86	967.22±48.36
S. indica	399.75±19.99	344.60±17.23	36.55±1.83	411.67±20.58	421.67±21.08	878.44±43.92	968.44±48.42
S. mysorensis	418.75±20.94	359.80±17.99	38.23±1.91	458.33±14.60	468.33±23.42	876.22±43.81	966.22±48.31
S. retusa	184.00±09.20	172.00±08.60	17.42±0.87	292.00±20.58	302.00±15.10	724.78±36.24	814.78±40.74
S. rhombifolia	370.50±18.53	321.20±16.06	33.95±1.70	369.33±18.47	379.33±18.97	877.89±43.89	967.89±48.39
S. spinosa	408.50±20.43	351.60±17.58	37.32±1.87	396.33±19.82	406.33±20.32	877.67±43.88	967.67±48.38

Figures in tables are represented as mean of three readings±SD. Abbreviation: DPPH=2,2-diphenyl-1-picrylhydrazyl, FRAP=Ferric reducing antioxidant potential, ABTS=2,2′-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid), AEAC=Ascorbic acid equivalent antioxidant capacity, TEAC=Trolox equivalent antioxidant capacity, RSA=Radical scavenging activity

FRAP assay

The values for FRAP activity assay ranged from 292.00 ± 20.58 to 590.67 ± 29.53 µM TEAC and 302.00 ± 15.10 to 600.67 ± 30.03 µM AEAC. *S. retusa* possess lowest activity and *S. cordifolia* the highest [Table 3]. The selected *Sida* species may be arranged on basis of activity (both TEAC and AEAC) from lowest as in *S. retusa*<*S. cordata*<*S. acuta*<*S. rhombifolia*<*S. pinosa* <*S. indica*<*S. mysorensis*<*S. cordifolia* to highest. $FRAP_{AEAC}$ was better than $FRAP_{TEAC}$.

ABTS method

The results for ABTS antioxidant activity is presented in Table. 3, which ranged from 613.78 ± 30.69 to 878.44 ± 43.92 µM TEAC and 703.78 ± 35.19 and 968.44 ± 48.42 µM AEAC. Here unlike DPPH and FRAP, *S. indica* possessed highest antioxidant activity and *S. cordata* showed lowest. The selected *Sida* species may be arranged on basis of activity (both TEAC and AEAC) from lowest as in *S. cordata*<*S. retusa*<*S. mysorensis* <*S. acuta* <*S. cordifolia*<*S. spinosa*<*S. rhombifolia*<*S. indica* to highest. $ABTS_{AEAC}$ was better than $ABTS_{TEAC}$.

The results suggest that the studied *Sida* species contained varied range of antioxidant activity in relation to polyphenolic contents. It is also observed that extracts with higher concentrations of polyphenolic contents have strong antioxidant effect. From our study, we note that extract of *S. cordifolia* is high in polyphenolic content and possess good antioxidant activity as compared to other selected species. The results were in accordance with observations made by Konate *et al.*[19] in *S. cordifolia* and in some herbs by Zheng *et al.*[20] Koh *et al.* also attributed higher antioxidant activity of *Cymbopogon citratus* to higher content of polyphenols.[21] Similar findings were reported by Zainol *et al.* indicated strong association between antioxidative activities and phenolic compounds, suggesting that phenolic compounds are probably responsible for the antioxidative activities of *Centella asiatica*.[22] Reports suggested that phenolic compounds were responsible for the antioxidant activity in some selected fruits, vegetables, grains, and medicinal plants.[23,24]

Furthermore, on the basis of antioxidant activity, the plants under studies can be classified in to 4 groups as Group I: *Sida cordifolia*: with high activity; Group II: *Sida spinosa*, *S. indica*, and *S. mysorensis*: having moderate activity; Group III: *Sida acuta*, and *S. rhombifolia*: low activity; and Group IV: *Sida cordata* and *S. retusa*: with poor activity [Table 3]. These variations in the activities can be attributed to the varied levels of TPC and TF, as the correlation between polyphenols and antioxidant activities has been well established.[25] It is interesting to note that the higher content of TPC and flavonoids in *Sida cordifolia, S. mysorensis, S. spinosa,* and *S. indica* (arranged high to low) are also associated with higher antioxidant activity.

Sida is one of the important medicinal plant species used to treat various diseases in *Ayurveda* and other traditional systems of medicine. The study provides a comprehensive comparison on polyphenolic contents and antioxidant activities of root extracts of eight *Sida* species. Based on the results of the present study, it can be concluded that methanolic root extract of *S. cordifolia* can be a good source of polyphenolics, which also exhibited highest antioxidant activity among the eight selected species.

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