



Research Article

COMPARATIVE EVALUATION OF PRELIMINARY PHARMACOGNOSY AND PHYTOCHEMISTRY OF TWO SOURCE PLANTS OF *BALA* (*SIDA CORDIFOLIA* LINN. AND *SIDA RETUSA* LINN.)

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ABSTRACT

"Bala"- a predominant herbal drug, has wide selection of uses as a single drug and also is an ingredient in most of the popular and extensively used Ayurveda formulations. Ayurvedic Formulary of India accepts *Sida cordifolia* Linn as *Bala*. *Sida cordifolia* being not so common in Southern parts of India has paved way for use of *Sida retusa* Linn., presently known as *Sida alnifolia* a common plant to be used as source plant of *Bala* in Kerala. In the present study, preliminary pharmacognostical, physical and phytochemical evaluation including chromatography of *Sida cordifolia*, Linn. and *Sida retusa* Linn. was done to evaluate their use as source plants of *Bala*. The pharmacognostical study by virtue of organoleptic evaluation and microscopy of Transverse section of the two species were performed and documented based on the standard procedure. The findings are valuable source of information as it may assist identification of the 2 drugs. Preliminary physical and phytochemical analysis of genuine sample of the study drug *Sida cordifolia*, Linn and *Sida retusa* Linn were conducted as a part of the study. These tests are simple and easy to carry out and give valuable information about the identity, genuineness and purity of the drug. The preliminary pharmacognostical and phytochemical screening of *Sida cordifolia* and *Sida retusa* suggests that either of the plants could be the source plants of *Bala* due to their immense similarity. It makes it highly probable that the pharmacological activities and therapeutic efficacy could possibly be same.

KEYWORDS: *Bala*, *Sida cordifolia*, Linn., *Sida retusa* Linn., Pharmacognosy, Phytochemistry.

INTRODUCTION

"Bala"- a predominant herbal drug, has wide selection of uses as a single drug and also is an ingredient in most of the popular and extensively used Ayurveda formulations. The drug has immense therapeutic value in Ayurveda treatment. It is *Tridosahara*, *Ojovardhaka* and has *Rasayana guna*¹. Acharya Charaka includes the drug in *Brimhaniya*², *Balya*, *Prajasthapana-mahakasaya* and in *Madhuraskanda*. Charakacharya has described the drug *Bala* as one of the best medicine or *Agryaousadha* stating that "*Bala sangrahitakabalya vataharanaam*"³.

The drug is ingredient in most of the popular formulations in Ayurveda like *Rasnaadikashaya*, *Astavaragamkashaya*, *Balaguloochyadikashaya*, *Balajeerakadi kashaya*, *Maharasnadikashaya* etc. *Taila* preparations like *Bala taila*, *Balaaswagadhaitaila*, *Balahataditaila*, *Balaguloochyaditaila*, *Karpaasaasthiyadi Taila*, *Ketakee mooladitaila*, *Ksheerabala taila*, etc. have *Bala* as an important ingredient. Many *Lehya* preparations to encompass this drug. It is among the most common and popular drugs used in Ayurveda treatment.

According to National Medicinal Plants Board (NMPB), *Bala* is the 3rd most consumed drug in Ayurveda pharmaceutical industry and is mostly collected from the wild⁴. Presently many *Sida* species are employed as "*Bala*" throughout the country. *Sida cordifolia*, Linn. is proposed as source plant of *Bala* in Ayurveda Formulary of India⁵ whereas by literature review it was found that *Sida retusa* Linn.^{6,7} is being used in Kerala. In the present study,

preliminary pharmacognostical, physical and phytochemical evaluation including chromatography of *Sida cordifolia*, Linn. and *Sida retusa* Linn. was done to evaluate their use as source plants of *Bala*. The pharmacognostical study by virtue of organoleptic evaluation and microscopy of Transverse section of the two species were performed and documented based on the standard procedure. Preliminary physical and phytochemical analysis of genuine sample of the study drug *Sida cordifolia*, Linn. and *Sida retusa* Linn. were conducted as a part of the study.

MATERIALS AND METHODS

Collection of source plants of *Bala*

The two source plants of *Bala* taken *Sida cordifolia*, Linn. and *Sida retusa* Linn. are found as weed and in wilderness. *Sida cordifolia* Linn. was collected from Neyyatinkkara, Thiruvananthapuram district Kerala in the months of May to June 2016. *Sida retusa* Linn. was collected from natural surroundings from Nedumangadu Thiruvananthapuram district Kerala and Aluva, Ernakulam district Kerala in the months of March to June 2016.

Authentication and preparation of study drug

The 2 species of *Sida* were identified by taxonomist of Pharmacognosy Unit, Govt. Ayurveda College Thiruvananthapuram and a herbarium of the same has been submitted to the department of Dravyaguna Vijnana. The roots of both source plants were thoroughly cleaned, shade dried and powdered for stored in air tight

containers for phytochemical analysis. For the pharmacognostical evaluation, it was decided to find out the macroscopic (organoleptic) and microscopic features of the roots of *Sida cordifolia* and *Sida retusa*.

STUDY SETTINGS

Drug Standardisation Unit, Government Ayurveda College, Thiruvananthapuram, Kerala.

1. Macroscopic evaluation

Macroscopic evaluation is the method of qualitative evaluation established on the study of morphological and sensory profiles of whole plant. Fresh, full-grown and healthy plant of both species was collected and washed in pure water to remove all the impurities. The root was separated by cutting with a sharp blade. The samples were subjected to macroscopic evaluation by observation with naked eyes and by tactile and other sensory inspection. A magnifying lens with a dissecting microscope was used for a better evaluation of surface characters.

2. Microscopic evaluation

The microscopic evaluation is used for studying the histological features of transverse section of root of *Sida cordifolia*, Linn. and *Sida retusa* Linn. A cylindrical portion of the root near the stem part was selected and the root hairs were removed. Enough number of sections was taken. The sections were carefully transferred to a petri dish containing water and few thin sections that floated in water were selected. A stained section was carefully transferred on a clean glass micro slide using thin brush. With the help of a forceps and a needle a clean cover slip was placed gently over the section. With the help of a blotting paper excess water was removed and the slide was placed on a digital microscope (Olympus digital- CS41, Japan, with CCD camera with analysis software digital image- Pro) for histological examination and direct images were taken.

3. Preliminary physical and phytochemical analysis

Preliminary physical and phytochemical analysis of genuine sample of the study drug *Sida cordifolia*, Linn. and *Sida retusa* Linn. were conducted as a part of the study. These tests are simple and easy to carry out and give valuable information about the identity, genuineness and purity of the drug. The procedures were done as per standardised procedures. Physical standards⁸ for the drug were determined (results shown in Table 3) and phytochemical analysis⁹ was done (results shown in Table 4) as per standard protocol.

4. HPTLC (High Performance Thin Layer Chromatography)

High performance thin layer chromatography (HPTLC) is an invaluable quality assessment tool for the evaluation of herbal chemical constituents. 2 µl of Ethanolic extract of each sample of *Sida* was applied as 8mm band length in the 10x 200 silica gel 60F254 TLC plate using Hamilton syringe and CAMAG LINOMAT 5 instrument. The plate with the samples applied were kept in TLC twin trough developing chamber (after saturation with solvent paper) with respective mobile phase [Toluene: ethyl acetate: formic acid in 9:1: 1 drop ratio] up to 70mm. The plate was dried and kept in photo documentation chamber (CAMAG REPROSTAR 3). Before derivatisation, the plate was fixed in scanner stage (CAMAG TLC SCANNER) and scanning was done at UV 254nm and UV 366nm. The peak table and peak densitogram for each profile was noted. The software used was Win CATS 1.3.4 version. HPLC grade reagents were used.

Observations and Results

The organoleptic evaluation and the microscopy of the root of *Sida cordifolia* and *Sida retusa* were conducted and the observations were noted. The data was analysed and results were interpreted from it.

Table no. 1 Organoleptic evaluation of root of *Sida cordifolia* and *Sida retusa*

Features	<i>Sida cordifolia</i> root	<i>Sida retusa</i> root
Size	Length varying from 7 cm to 12 cm	Length varying from 7.5 to 15 cm
Shape	Cylindrical, branched, tortuous	Cylindrical, profusely branched, tortuous
Lateral roots	Thin, wiry	Thin, wiry, numerous in number especially arising from upper region of root
Rootlets	Almost absent	Present
Colour	Pale yellow	Pale yellow
Odour	No characteristic smell	No characteristic smell
Taste	Slightly bitter	Slightly bitter

Microscopic evaluation

The microscopic evaluation of transverse section of roots of *Sida cordifolia* and *Sida retusa* were carried out. The transverse section of the 2 roots are described below

Table 2: Results of microscopic evaluation of transverse section of roots of *Sida cordifolia* and *Sida retusa*

Transverse section of root of <i>Sida cordifolia</i>	Transverse section of root of <i>Sida retusa</i>
<ul style="list-style-type: none"> 4-6 rows of thin walled tangentially elongated cells formed the cork. The outermost 2 layers were slightly ruptured and light brown in colour. Inner to the cork was the Phellogen consisting of a single layer of narrow, thin walled tangentially elongated cells. <p>Cortex:</p> <ul style="list-style-type: none"> The cortex is made up of 3-4 group of parenchymatous 	<ul style="list-style-type: none"> The outermost cork consists of 4 to 7 rows of thin walled, rectangular tangentially elongated cells. The Phellogen is composed of a single row of narrow, thin walled tangentially elongated cells The cambium is composed of 1 or 2 rows of narrow thin walled cells which contains stellate crystals. The phloem appeared in cortical strands and are much narrower and linear than in <i>Sida cordifolia</i>. The strands were separated by the medullary rays.

<p>cells.</p> <ul style="list-style-type: none"> • Calcium oxalate crystals were present and few sparsely distributed starch grains were seen. • Secondary phloem occurs in conical strands. Each strand consists of 6-8 tangential bands of thick walled phloem fibre groups alternating with thin walled phloem elements. • Medullary rays were many in number, long, uniseriate, extending and reaching up to the cortex in a straight course. • Some of the phloem parenchyma cells at the outer region contain cluster crystals. • Vascular cambium was distinct. • Vessels were many, occurring solitary or in scattered groups of 2 to 4. • Xylem parenchyma cells were thick walled, surround the vessels and contained starch grains. 	<p>Some phloem parenchyma contained calcium oxalate crystals.</p> <ul style="list-style-type: none"> • The wood was composed of vessels, parenchyma, fibres and medullary rays. • The vessels were many and vary in size. • Only few number of parenchyma cells contained starch grains. • Medullary rays were numerous, long and straight. • The medullary rays gradually become bigger towards the outer region. • Towards the distal end the medullary rays the cells are tangentially elongated and contain small cluster crystals of calcium oxalate.
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Results of Physical and Phytochemical evaluation of *Bala*

The screening of the physical and phytochemical parameters of *Sida cordifolia* Linn. and *Sida retusa* Linn. were done as per the standard procedures. Physical characters like moisture content, total ash, acid insoluble ash, water extractive and alcohol soluble extractives were analysed.

Table no 3. Results of evaluation of physical parameters of *Sida cordifolia* Linn. and *Sida retusa* Linn.

Parameter	<i>Sida cordifolia</i>	<i>Sida retusa</i>
1. Foreign matter	Nil	Nil
2. Moisture content	8%	10%
3. Volatile oil	Nil	Nil
4. Cold water extractive	8.12%	10.2%
5. Alcohol extractive	3.18%	2.78%
6. Total ash	2.64%	3.88%
7. Acid insoluble ash	1.78%	1.31%
8. Total sugar	Traces	.49049%
9. Reducing sugar	Traces	0.33225%
10. Fibre content	42.24%	46.04%

Results of phytochemical screening of ethanol extracts

Table no 4. Results of phytochemical screening of ethanol extracts

Sl no.	Test done	<i>Sida cordifolia</i>	<i>Sida retusa</i>
1.	Alkaloid	+	+
2.	Steroid	+	+
3.	Phenols	+	+
4.	Flavanoids	+	+
5.	Saponin	-	-
6.	Tannin	-	-

+ for Present, - for absent

HPTLC Profile

HPTLC analysis of ethanolic extract of *Sida cordifolia* and *Sida retusa* was performed with the solvent system Toluene: ethyl acetate: formic acid in the ratio 9:1:1 drop. 4 peaks were obtained for *Sida cordifolia* at Rf values 0.26, 0.34, 0.38 and 0.94. 3 peaks were obtained for *Sida retusa* at 0.27, 0.34 and 0.95Rf values. The HPTLC profile is shown in figure 4. In figure 4, Track I represents the spots obtained for *Sida cordifolia* and Track II represents the spots of *Sida retusa*.

DISCUSSION

The pharmacognostical study of the whole root of *Sida cordifolia* Linn. and *Sida retusa* Linn. was done. The 2 species displayed significant difference in macroscopic evaluation of root. In *Sida retusa* the lateral roots were

mainly concentrated in the upper region and there was the presence of a greater number of lateral roots and rootlets which clearly stood out in comparison to *Sida cordifolia* Linn. which had few lateral roots and negligible number of rootlets. The histology of roots was almost similar except for the presence of large number of cluster crystals in the expanded distal ends of the medullary rays in *Sida retusa*. There was also a noteworthy comparative scantiness of starch grains in the cortex region in *Sida cordifolia*.

The phytochemicals present in a plant determine its pharmacological and therapeutic actions. The physical and phytochemical parameters were similar in *Sida cordifolia* and *Sida retusa*. Alkaloids, Flavanoids, Steroids and Phenols were present in both species. The

phytochemical analysis is relevant here as it highlights that both could bear similarity in the pharmacological action as the quality of phyto-constituents is similar. The HPTLC profile too points that both the species could have similar phyto-constituents.

CONCLUSION

The results of the present study analysis can be used as a standard for future comparison since no data is available regarding the phytochemical parameters of the drug in the Ayurvedic pharmacopoeia of India (API). These tests are simple and easy to carry out and give valuable information about the identity, genuineness and purity of the drug.

The preliminary pharmacognostical and phytochemical screening of *Sida cordifolia* and *Sida retusa* suggests that either of the plants could be the source plants of *Bala* due to their immense similarity. It makes it highly probable that the pharmacological activities and therapeutic efficacy could possibly be same.

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Figures

Figure no. 1 T.S of root of *Sida cordifolia*

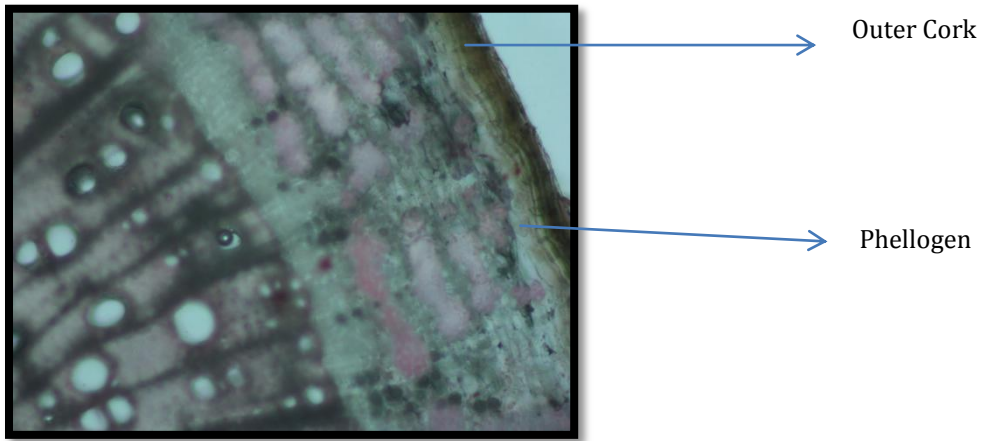


Figure 2. TS of root of *Sida cordifolia*

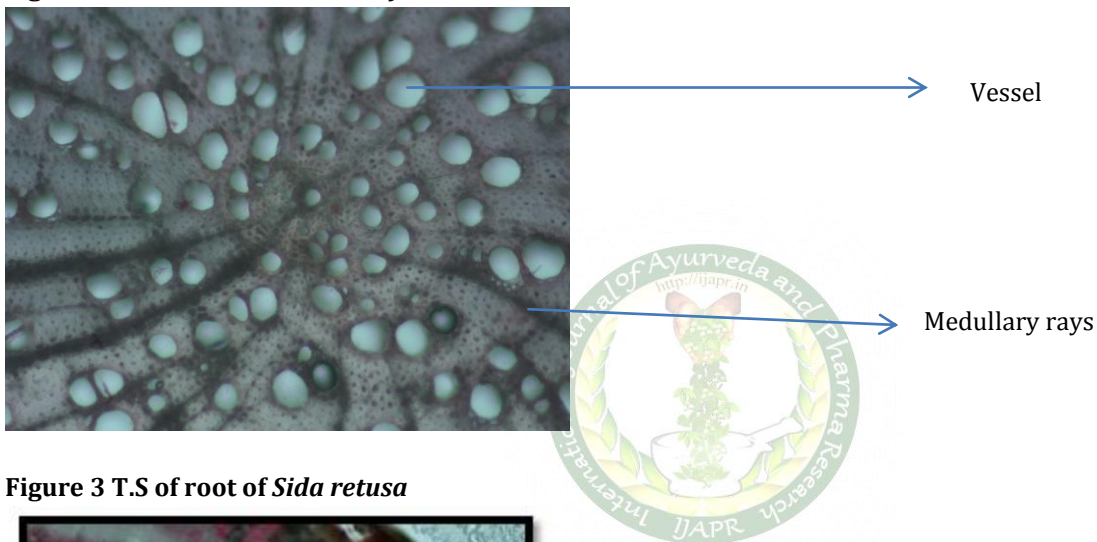


Figure 3 T.S of root of *Sida retusa*

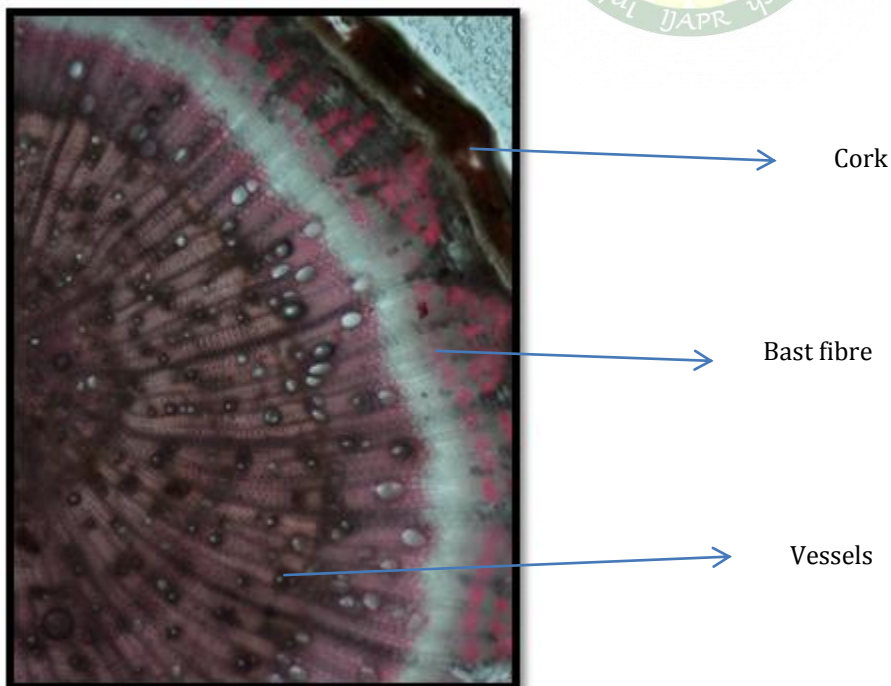


Figure 4. TS of root of *Sida retusa*

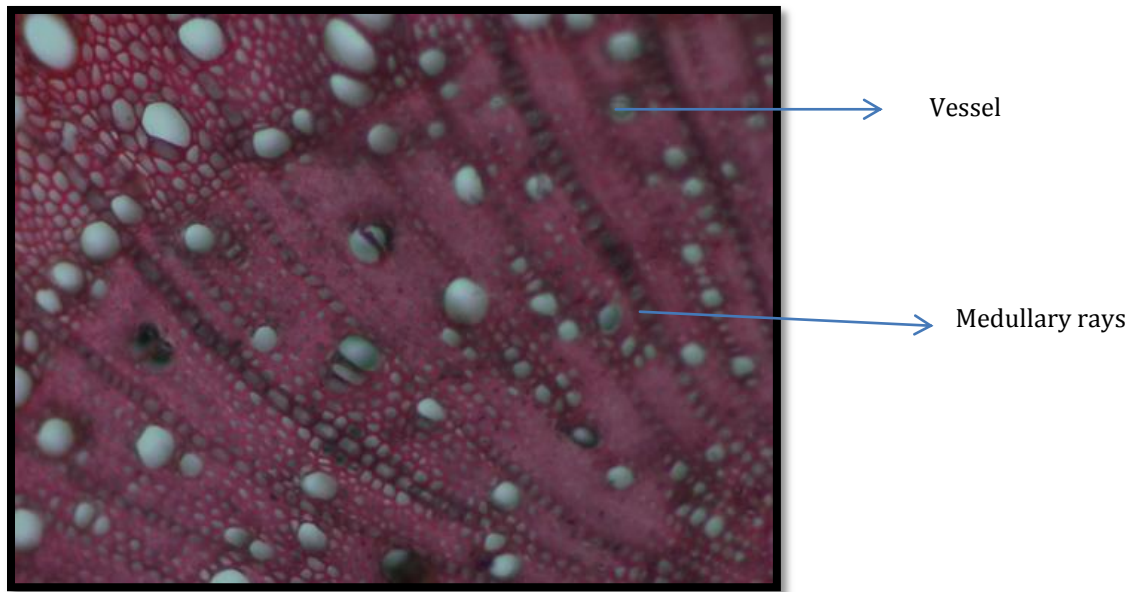


Figure 5: HPTLC profile of both *Sida* species

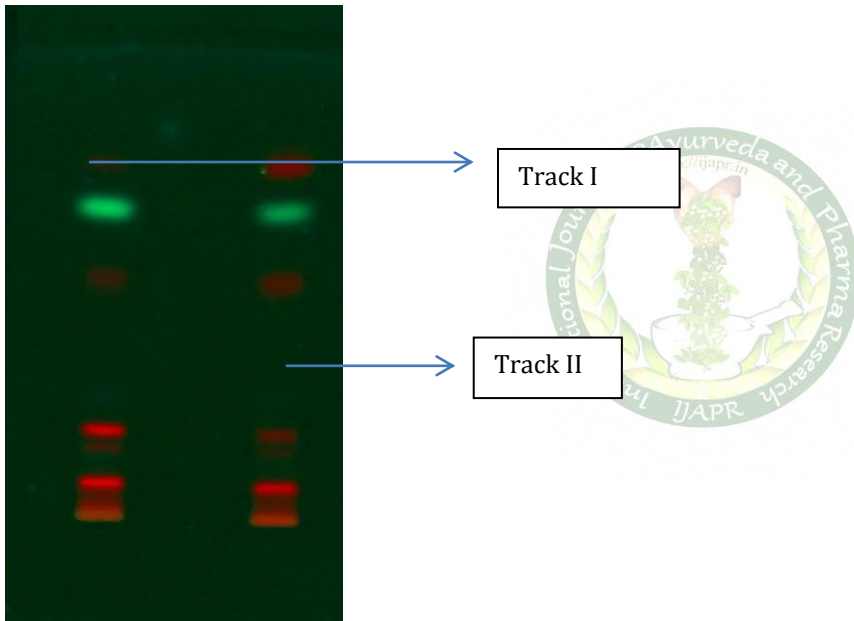


Figure 6.3D scan profile at 254 nm (densitometric)

