

PHYTOCHEMICAL INVESTIGATIONS AND ANATOMICAL STUDY OF THREE SPECIES OF SIDA

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ABSTRACT

Plants and plant based products are bases of many modern pharmaceuticals that are currently in use today for various diseases. Several species of *Sida* commonly known as Bala, are known to have analgesic, anti-inflammatory, hypoglycemic and hepatoprotective activity. The root infusion is given in nervous and urinary diseases and also in disorders of blood. The present study was undertaken to analyse the various active phytochemical constituents and to study detailed anatomical features of three species of *Sida* (family:Malvaceae) i.e., *S. acuta*, *S. rhombifolia* and *S. cordifolia*. A qualitative analysis was performed for detection of alkaloids, anthraquinone derivatives, glycosides, terpenoids, steroids and flavanoids. HPLC (High Performance Liquid Chromatography) was used for quantitative analysis of vasicine and vasicinone. Anatomical features of various plant parts were found useful for correct easy identification of commercial drug for ensuring purity while quantitative analysis helped in identifying the elite genotypes of the three species of *Sida* present in the area of present study.

Keywords: *Sida* sps, Malvaceae, Phytochemical Investigation, Anatomical study.

INTRODUCTION

Phytochemical evaluation is one of the tools for quality assessment, which includes preliminary phytochemical screening. Use of chromatography for standardization of plant products was introduced by WHO and is accepted as a strategy for identification and evaluation of the quality of plant medicines (Anonymous, 1992; Farnsworth *et al.*, 1985). There are many species of *Sida* which are known by the name BALA, used in Ayurveda for treating the disorders of the nervous systems (Dhalwal *et al.*). According to Ayurveda "BALA" balances all the doshas-vata, pitta, kapha. The rejuvenating action of this herb extend to the nervous, circulatory and urinary systems (Chopra *et al.*, 1958). *Sida* sps.

(Family:Malvaceae) grows to a height of 3-5 feet and extensively used as a common herbal drug in India. The roots, leaves, stems and seeds of *sida* are used as traditional medicine against Chronic dysentery, asthma and gonorrhoea (Yusuf, 1999; Krishna Murari Kumar, 2013). *Sida* sps. (BALA), which grow as weed in Chandigarh, was screened for morphological, histological and anatomical characters. The present work reported the estimation of vasicine and vasicinone in three *sida* species i.e. *S. acuta*, *S. cordifolia*, *S. rhombifolia*.

MATERIALS AND METHOD

1. Plant Collection and Identification:

Fresh plant samples were collected from different localities (during flowering period for

easy identification). The various useful parts like roots, leaves and stem *etc.* separated and preserved for the study. Identification of various plants species done by comparing with authenticated herbarium specimens, later confirmed with the help of diagnostic keys and morphological description given in various floras.

2. Anatomical study of part used:

The roots and stem of all three *Sida* species were fixed in F.A.A. (*i.e.* Formalin acetic acid-alcohol, 1:1:18) after trimming them to correct dimensions. Hand sections of fresh stem and root (mature) were cut using a sharp blade. Thin transverse sections were stained in safranin and then fast green, passed through alcohol grades for dehydration, and then mounted in D.P.X. Observations were taken from these sections using light microscope. These sections were also photomicrographed. Special identifying features of the plant part(s) were studied and identified.

3. Phytochemical study:

The roots and stem were washed with a solution of 5% mercuric chloride for 5 minutes and then washed with distilled water, dried and then powdered. The powdered crude drug was macerated with 80% methanol. The solvent was then evaporated at a constant temperature of 72^o C until a very concentrated extract was obtained. Qualitative phytochemicals analysis was carried out from this plant extract.

3.1 Alkaloids:

Alkaloids are basic nitrogenous compounds with definite physiological and pharmacological activity. Alkaloid solution produces white yellowish precipitate when a few drops of Mayer's reagents are added (Siddiqui and Ali, 1997).

3.2 Anthraquinone derivatives:

Anthraquinone derivatives was detected by Borntrager's test in which root powder was treated with 3 ml of aqueous extract was shaken with 3 ml of benzene, filtered and 5 ml of 10% ammonia solution was added to the filtrate. The mixture was shaken and the presence of a pink, red or violet colour in the ammonical (lower)

phase indicates the presence of free anthraquinones (Njoku and Obi, 2009).

3.3 Glycosides :

To the solution of the extract in glacial acetic acid, few drops of ferric chloride and concentrated sulphuric acid are added, and observed for a reddish brown coloration at the junction of two layers and the bluish green color in the upper layer (Siddiqui and Ali, 1997).

3.4 Terpenoids and steroids:

Four milligrams of extract was treated with 0.5 ml of acetic anhydride and 0.5 ml of chloroform. Then concentrated solution of sulphuric acid was added slowly and red violet color was observed for terpenoid and green bluish color for steroids (Siddiqui and Ali, 1997).

3.5 Flavonoids:

Four milligrams of extract solution was treated with 1.5 ml of 50% methanol solution. The solution was warmed and metal magnesium was added. To this solution, 5-6 drops of concentrated hydrochloric acid was added and red color was observed for flavonoids (Siddiqui and Ali, 1997 and Vinata Naini, 2013).

4. High performance liquid chromatography Chromatographic Conditions and Procedure:

HPLC (Shimadzu, LC 2010A, Japan), Autosampler, UV-Detector was used for the analysis of vasicine and vasicinone. The data was acquired on the LC solution administrator data system (Japan). Phenomenex C18 column (250 mm X 4.6 mm, 5 µm) (California, USA) and a gradient mixture as mobile phase. The mobile phase was filtered through 0.45 µm Millipore filter and degassed by sonication for 30 min. The flow rate was adjusted to 1.0 ml/min. Injection volume was adjusted to 20 µl and detection was made at 280 nm.

RESULTS

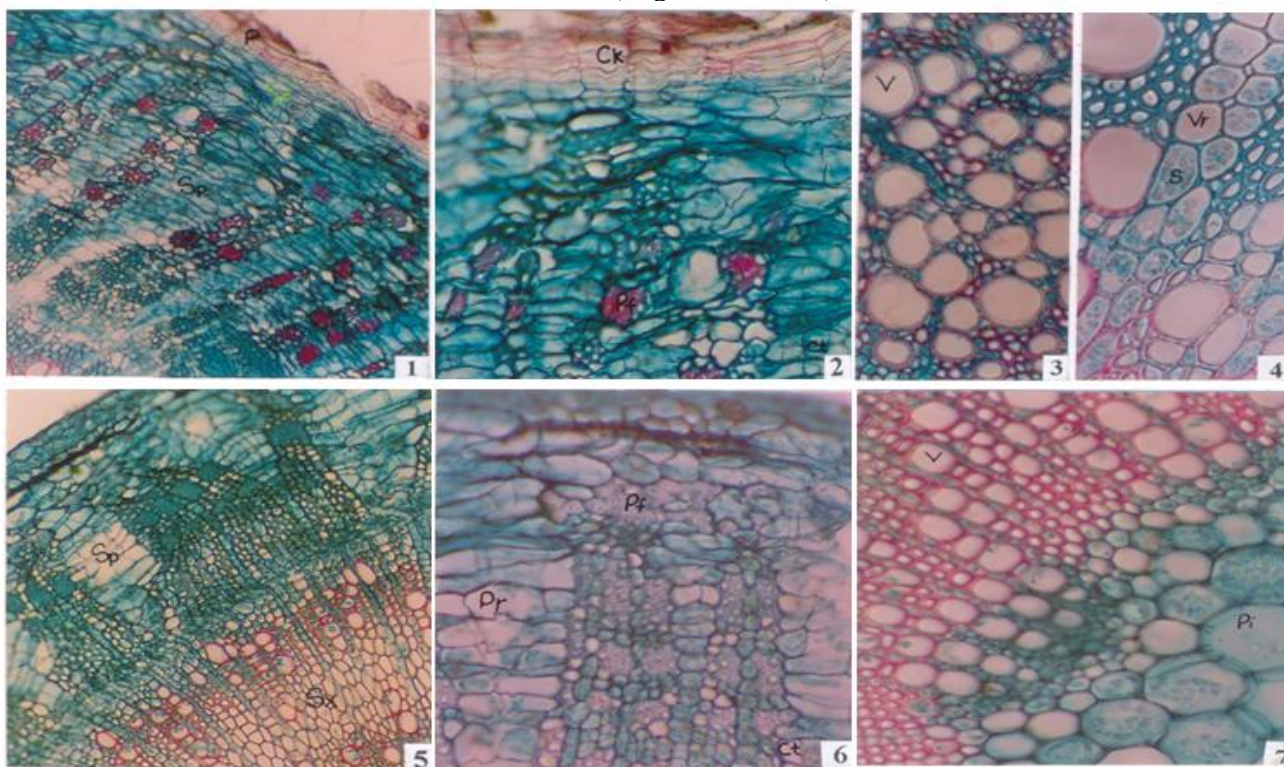
1. Anatomical study of plant:

The comparative account of anatomical features of plant parts *i.e.* root and stem of three *Sida* species are given in Table 1 and Table 2. and

Table- 1. The comparative account of anatomical features of root of three *Sida* species

characters	<i>S. acuta</i>	<i>S. rhombifolia</i>	<i>S. cordifolia</i>
ROOT	Cork, 6-11 layers.	Cork, 4-5 layers.	Cork, 4-5 layers
	Bark, Phloem fibres are present in tangential bands lying parallel to each other.	Bark, Phloem fibres are present in tangential bands.	Bark, phloem fibres broken in to tangential bands by presence of dilated phloem rays.
	Vessels, distributed mostly in short linear rows	Vessels, are arranged singly or in distinct oblique group's.	Vessels, solitary and in short radial multiples.
	Cortex, made up of thin walled parenchymatous cells. Which are not very distinct.	Cortex, in made up of a number of layers of parenchymatous cells.	Cortex, not very distinct.
	Cambium, 2-3 cell layers, not very distinct.	Cambium, 4-5 layer's	Cambium, not very distinct.
	Phloem, the phloem parenchyma cells shows starch deposits.	Phloem, dilated phloem ray parenchyma cells show starch presence.	Phloem, starch filled cells absent in the secondary phloem and cortex
	Calcium oxalate crystals found absent form the parenchyma cells of dilated phloem rays.	Calcium oxalate crystals found absent from the parenchyma cells of dilated phloem rays.	Abundant Calcium oxalate crystal occurs in the parenchyma cells of the dilated phloem ray.

Figure-1. The comparative account of anatomical features of plant parts i.e. root and stem of *Sida acuta* (Figures 1.1-1.7)

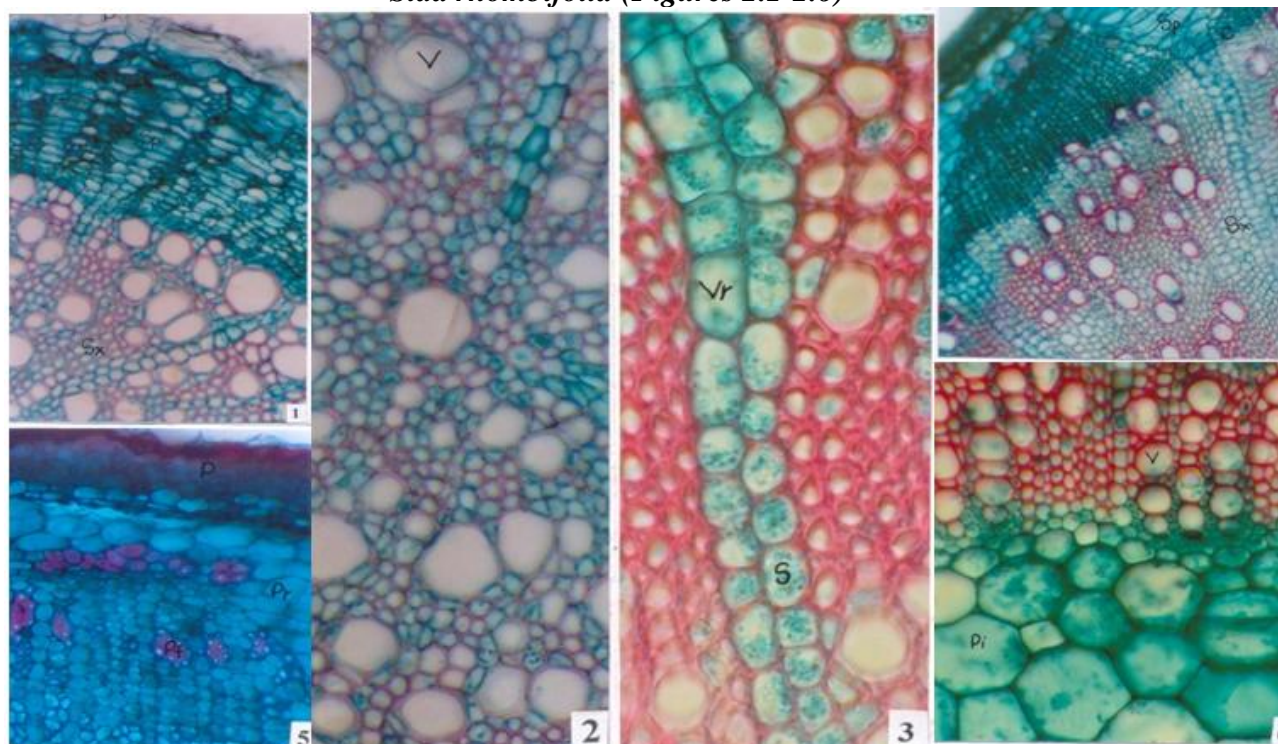


Root (Figures 1.1-1.4): Fig. 1.1-T.S of root, showing periderm, well developed secondary phloem x10. Fig. 1.2-T.S of root, through the secondary phloem region showing, scanty phloem fibres dilated phloem ray, phloem parenchyma and cork with 5-6 layers x 20. Fig.1.3- T.S of root, showing large round vessels and few xylem fibres x 20. Fig.1.4-T.S. of root, through vascular ray, multiseriate ray parenchyma showing starch deposits x 40. **Stem (Figures 1.5-1.7):** Fig. 1.5-T.S. of stem, showing well developed secondary phloem and secondary xylem x 10. Fig.1.6-T.S. of stem, through secondary phloem, few calcium oxalate crystals present in dilated phloem rays, stratification of phloem fibre. x 20. Fig.1.7-T.S. of stem, through the pith showing starch deposits; and secondary xylem with vessels in short radial multiples. x 20.

Table- 2. The comparative account of anatomical features of stem of three *Sida* species

STEM	Single layered epidermis produced into fine hair.	Single layered epidermis	Single layered epidermis with 3-4 layers of cork were present.
	Cortex, epidermis is followed by hypodermis and inner parenchymatous cells.	Cortex: epidermis followed by cortex, differentiated into outer hypodermis tissue richly supplied with chloroplast and inner parenchymatous cells.	Cortex: the stem cortex is reduced consisting of 2-3 layer of cells.
	Endodermis contain calcium oxalate crystals.	Endodermis not very distinct	Endodermis not distinct
	Vascular bundles, Separated by the presence of thin walled cells, continuous with the medullary rays and phloem parenchyma cells contain calcium oxalate crystals.	Vascular bundles, Phloem in present alternating with the dilated phloem ray cells and calcium oxalate crystals absent.	Phloem occur in tangential bands, the phloem rays contain calcium oxalate crystals.
	Vessels, Occur in radial rows	Vessels, arranged in radial rows	Vessels, are solitary in arrangement
	Vascular rays are predominantly uniseriate	Vascular rays vary from uni-multiseriate	Vascular rays vary from uni-multiseriate
	Pith, constituted by large sized rounded parenchymatous cells	Pith is composed of thin walled cells	Pith consists of thin-walled parenchymatous cells.

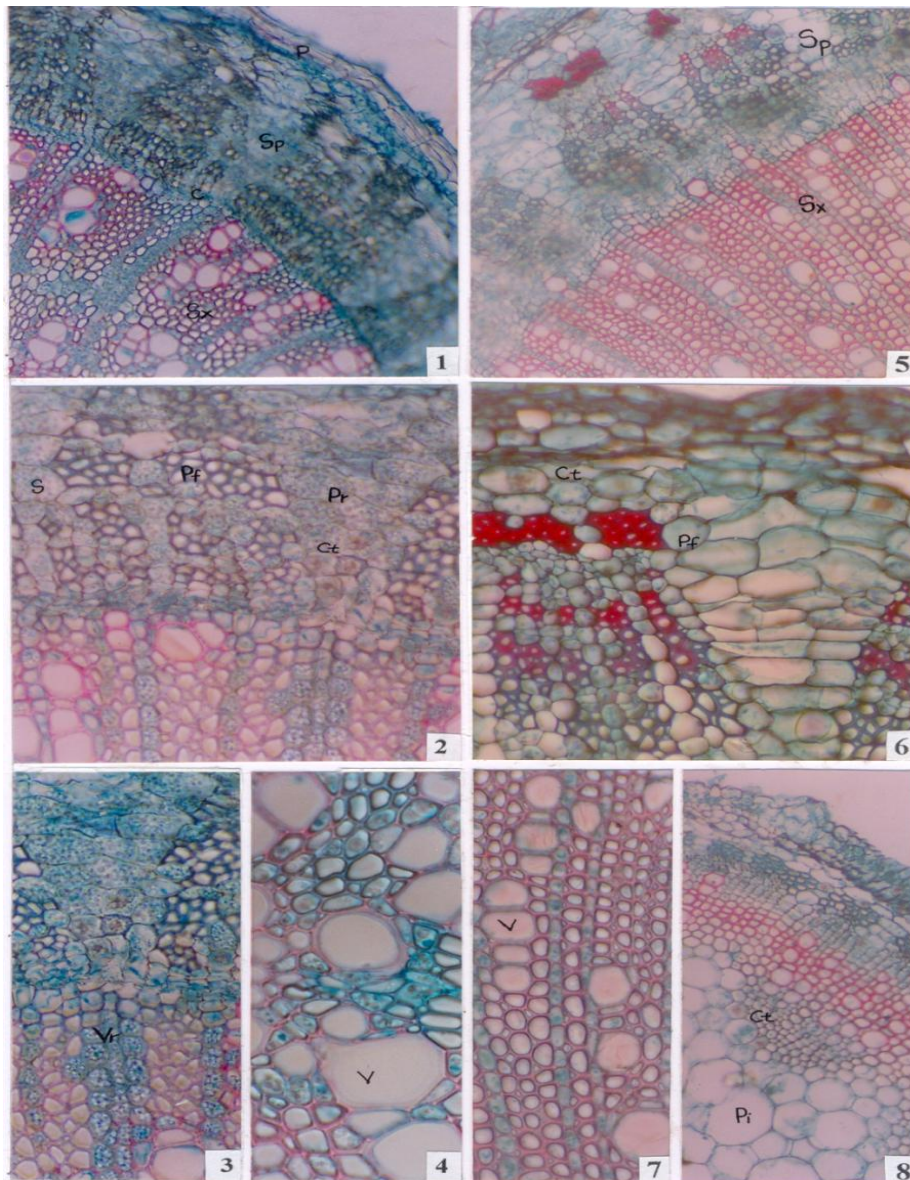
Figure-2. The comparative account of anatomical features of plant parts i.e. root and stem of *Sida rhombifolia* (Figures 2.1-2.6)



Root (Figures 2.1-2.3): Fig-2.1. T.S. of root, showing Periderm, 3-4 layers of cork, well developed secondary phloem with phloem fibres; few xylem fibres x 20. Fig-2.2. T.S. of root, showing solitary arrangement of vessels, which are oval to round in shape x 20. Fig-2.3. T.S. of root through vascular ray showing multiseriate rays with cell inclusions x 40.

Stem (Figures-2.4-2.6): Fig-2.4. T.S. of stem, showing well developed secondary phloem and secondary xylem x 10. Fig-2. 5. T.S. of stem through secondary phloem, showing sclerenchyma fibres of pericycle and dilated phloem rays and few phloem fibres x 20. Fig-2.6. T.S. of stem, showing solitary and short radial rows of vessels and large parenchymatous cells of pith x 20.

Figure-3. The comparative account of anatomical features of plant parts i.e. root and stem of *Sida cordifolia* (Figures 3.1-3.8)



Root (Figure 3.1-3.4)

Fig. 3.1 T.S. of root, showing periderm, secondary phloem and secondary xylem x 10.

Fig. 3.2 T.S. of root, through secondary phloem showing dilated phloem ray with abundant starch deposits. Phloem fibres arranged in tangential bands and cambium x 20.

Fig. 3.3 T.S. of root, through vascular ray, which is multi to uniseriate showing abundant, starch deposits x 20.

Fig. 3.4 T.S. of root, showing oval of polygonal shaped vessels,, solitary or in short radial rows x 40.

Stem (figure 3.5-3.8)

Fig. 3.5 T.S. of stem, shows well developed secondary xylem and secondary phloem x 10.

Fig. 3.6 T.S. of stem through secondary phloem showing dilated phloem ray with few calcium oxalate crystals. Phloem fibres in tangential bands and sclerenchyma fibres marking boundary of secondary phloem x 20.

Fig. 3.7 T.S. of stem showing vascular rays and solitary and short radial arrangement of vessels x 20. Fig. 3.8 T.S. of stem through the pith x 10.

hand sections are shown in Figure-1 to Figure-3.

2. Phytochemical studies:

Different phytochemical constituents analysed from three species of *SIDA* are shown in the table given below:

**3. High performance liquid chromatography
Extraction of plant sample:**

The air-dried, powdered plant samples of *Sida* *sps.* were passed through 20 mesh size sieve. The sieved material (100 g) was refluxed with 400 ml methanol (99%) at the temperature of 80-85°C for 2-3 hrs on a water bath.

The material was filtered and concentrated under vacuum using rota-vac (Heidolph, Schwalbach, Germany). Finally the material was air-dried after removal of methanol. Assaying of Vasicine and vasicinone is carried out from this extract.

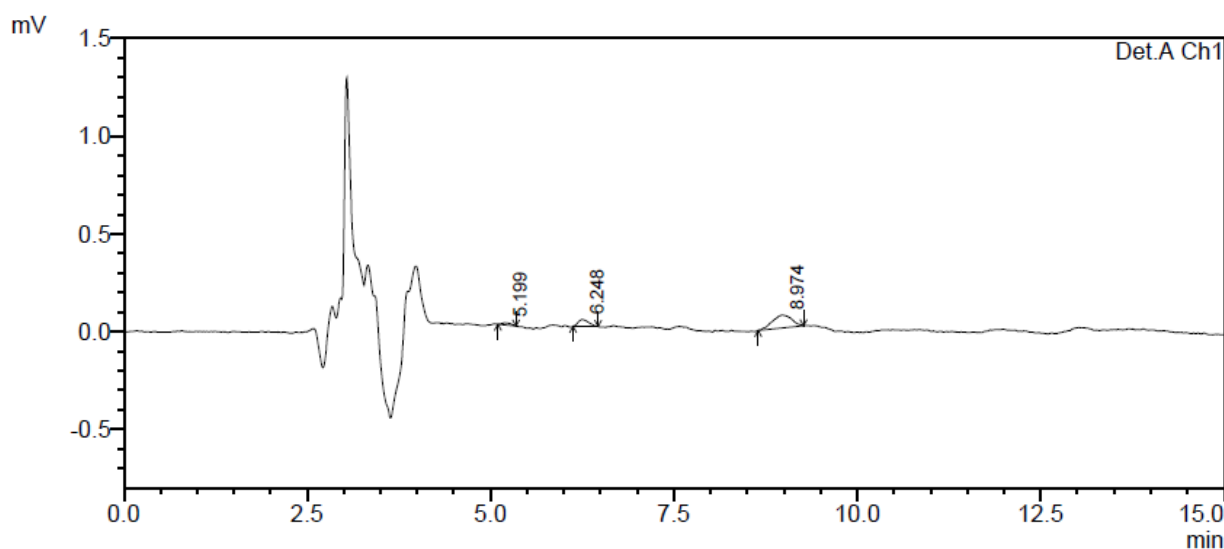
HPLC for assay Vasicine and vasicinone :

Under optimized conditions HPLC with C18 column and UV detector at 280 nm using gradient of acetonitrile and water as mobile phase gave well resolved symmetric peaks for Vasicine and vasicinone. The total run time of Vasicine and vasicinone was found to be 30 minutes and the Vasicine and vasicinone appeared on chromatogram at 5 and 8.7

Table-3. Phytochemical analysis of three species of SIDA

SIDA sp	Alkaloids	Anthraquinone derivatives	Flavonoids	Glycosides	Terpenoids	Steroids
<i>S. acuta</i>	+	-	+	+	+	+
<i>S. rhombifolia</i>	+	-	+	+	+	+
<i>S. cordifolia</i>	+	-	+	+	+	+

Figure-4. HPLC Chromatogram of *Sida acuta*

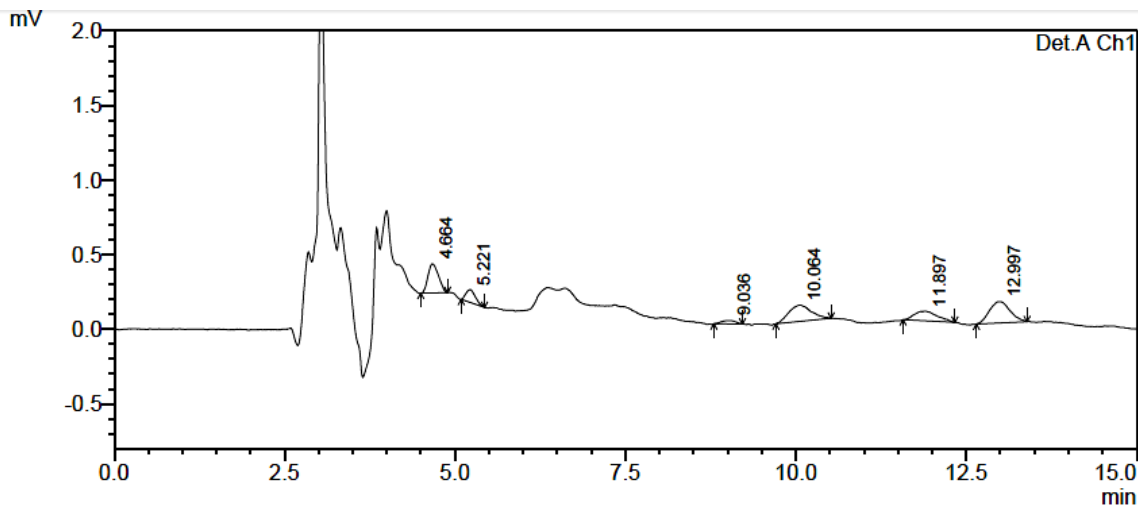


1 Det.A Ch1/300nm

PeakTable

Peak#	Ret. Time	Area	Height	Area %	Height %
1	5.199	83	11	5.082	9.451
2	6.248	354	36	21.522	31.836
3	8.974	1206	66	73.396	58.713

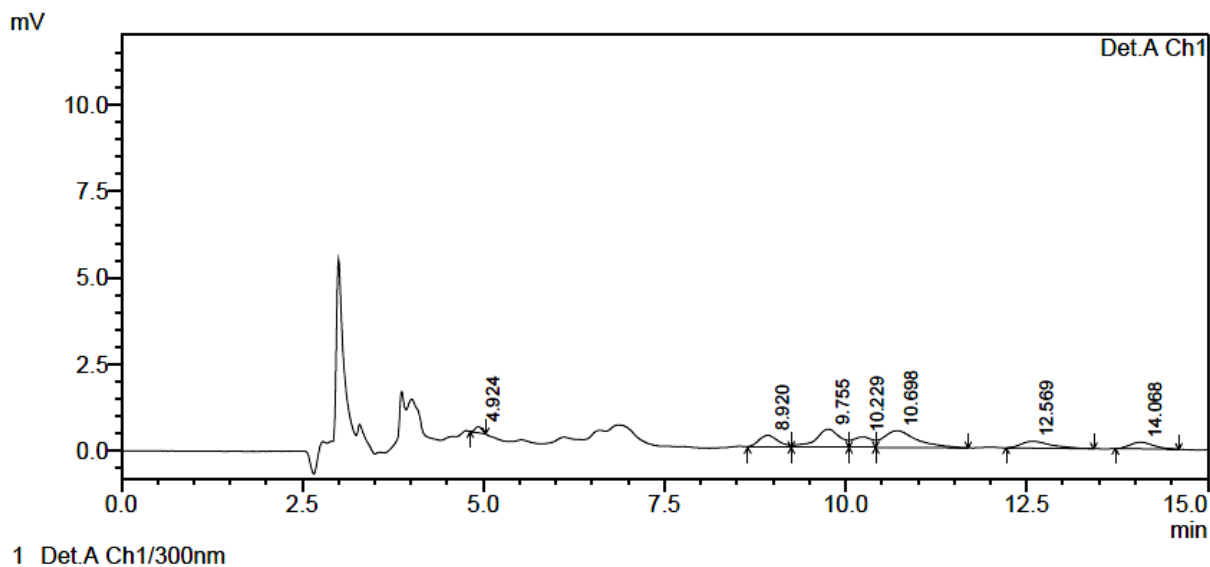
Figure-5. HPLC Chromatogram of *Sida rhombifolia*



PeakTable

Detector A Ch1 300nm						
Peak#	Ret. Time	Area	Height	Area %	Height %	
1	4.664	2082	195	20.372	31.431	
2	5.221	811	85	7.938	13.648	
3	9.036	323	24	3.157	3.807	

Figure-6. HPLC Chromatogram of *Sida rhombifolia*



PeakTable

Detector A Ch1 300nm					
Peak#	Ret. Time	Area	Height	Area %	Height %
1	4.924	1175	178	2.259	7.926
2	8.920	6745	350	12.970	15.600

respectively (Fig.4,5 and 6). The retention time of reference standard was observed to be same. (Dh (Dhalwa same.(Dhalwal *et al.* 2010).

When the same drug solution was injected 6 times, the retention time of the peak was found to be same.

DISCUSSION

Qualitative screening of phytochemical constituents of *Sida* species reveals the presence of alkaloid, flavanoid, terpenoids and steroids. Pharmacological studies have demonstrated that BALA possesses vasicine and vasicinone, which exhibits a wide range of properties of anti-inflammatory and analgesic activity. In present study, the observed alkaloids content is found maximum in *S. cordifolia* by observing their peak areas. The anatomical features of roots and shoots of the species of *Sida* which are of diagnostic value, have been studied. Since the drug in these species are primarily obtained from roots these have been studied in detail to avoid and prevent adulteration of commercial drug by users. Certain diagnostic features of morphology and anatomy have been found to be useful in the correct identification of the species of *Sida*, which are of medicinal value.

CONCLUSION

Approaches like screening, phytochemical profiling of these plants helped to get elite species among three *Sida* species i.e. *Sida cordifolia*. The correct botanical identification of the herbal drugs of commerce shall help to check piracy of these drugs and hence make available true botanicals to the consumer.

ACKNOWLEDGMENTS

This research was supported by UGC, New Delhi, India is greatly acknowledged. I am also very thankful to Department of Botany, Panjab University, Chandigarh for providing the necessary facilities.

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DOI:

<https://dx.doi.org/10.5281/zenodo.7214403>

Received: 10 April 2014;

Accepted; 23 May 2014;

Available online : 16 June 2014

