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PHARMACOGNOSTICAL STUDIES AND QUALITY CONTROL PARAMETERS OF *SIDA RHOMBIFOLIA*

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ABSTRACT

Introduction: *Sida rhombifolia* is a perennial plant & grows on tropical or subtropical lands. *Sida rhombifolia* belongs to Malvaceae family. *Sida rhombifolia* has various pharmacological actions as they contain biologically active compounds. **Material & Methods:** Pharmacognostical and quality control parameter involves study of macroscopic, microscopic, microchemical, TLC & Qualitative chemical examination *of Sida rhombifolia* **Result:** All pharmacognostical and quality control parameters of *Sida rhombifolia* were carried out. The morphological evaluations were done to ascertain the standard reference values for standardization of the plant materials where as the microscopy, the section study of the leaves of *Sida rhombifolia* shows the presence of xylem, fibers, trichomes, crystal, phylum, and anisocytic stomata is present.

Keywords: *Sida rhombifolia,* Pharmacognostical parameters, quality control parameters

INTRODUCTION

Sida rhombifolia (Arrow leaf Sida) is a perennial or sometimes annual plant (family- Malvaceae) native to Tropical and subtropical countries **(Figure 1) [1]**. Common name are huang hua mu (china), countary mallow (english), bala, mahabala (hindi), atibala (sanskrit), chittamadi (srilanka), petoria bassie (africa). These plant contains chemical constituents like Alkaloids, ascorbic-acid, beta-carotene, beta-phenethylamine, calcium, carbohydrates, ephedrine, gums, ephedrine, indole alkaloids, mucilage, phenolic compounds, protein, pseudoephedrine, saponin, steroids, tannins, triterpenoids, vascin, vasicine, flavonoids **[2]**.

The traditional uses of *Sida rhombifolia* are reported like fever, ulcers, boils, urinary diseases, toothaches, relive constipation and treatment of gout etc. **[3]**. The pharmacological uses of *Sida rhombifolia* show hepatoproctive, cytotoxic, antibacterial, antifungal, anti-arthritic, anti-gout, anthelmintic, and hypoglycemic activities **[4, 5]**.

Figure 1: Flower of *sida rhombifolia*

MATERIALS AND METHODS

Collection and Authentication of plant material

The arial parts of *sida rhombifolia* **[7, 8]** were procured from F.R.I. Dehradun, Uttarakhand. The Plant material was authenticated at National Ayurveda Dietetics Research Institute (Ay), Bangalore by Dr. Shiddamallayya N (senior scientist). DrugAuthentication/SMPU/NADRI/ BAG/2011-12/540.

Extraction

Air dried powder materials of the *sida rhombifolia* were allowed to successive extraction with the help of soxhlet apparatus by using pet. ether, chloroform, acetone, ethanol & water as a solvent **[9]**.

Standardization of sida rhombifolia

Macroscopic Examination

Macroscopic parameters like size, colour, surface characteristics, texture, odour and taste was considers in above examination **[10]**.

Microchemical Tests

Microchemical tests parameters was performed by detections of following test - cellulose, chitin, suberin and cutin, starch, mucilage, protein, fixed oil and fats, tannins, calcium carbonate, inulin, cellulose cell walls, calcium oxalate **[11]**.

Microscopic Examination

Leaf microscopy, powdered microscopy, determination of total ash, acid insoluble

ash, water soluble ash, moisture content, swelling index, extractable matter (hot extraction & cold extraction), foaming index, was performed in microscopic examination **[12-14]**.

Thin layer chromatography (TLC)

Thin layer chromatography is particulary valuable for the qualitative determination of small amounts of impurities. The silica gel g slurry was made & prepare TLC plates then allowed all plates for dry in hot air oven **[14]**. The mobile phase was made by the mixture of toluene: chloroform: ethanol [28.5: 57: 14.5] and all plates was stand in a suitable jar for allowed to run of mobile phase. The spots of pet.ether, chloroform, acetone, ethanol & aqueous extracts of *sida rhombifolia* showed a particular chemical constituent at a particular rf value. The color of the spot visualized after apply visualizing agent like dragendorff's reagent **[15-17]**.

Qualitative chemical examination of extracts

The following tests was performed under chemical examination of extracts-

Detection of alkaloids (mayer's test, dragendorff 's test, hager's test & wagner's test) was performed. detection of carbohydrates (molich's test, barfoed's test). detection of glycoside (modified borntrager's test, legal's test, baljet's test), detection of saponins (foam test), detection of steroids (libermann- burchard's test), detection of fixed oils & fats (stain test, saponification test), detection of resins (acetone-water test), detection of phenol (ferric chloride test), detection of tannin (gelatin test), detection of flavonoid (lead acetate test, zinc hydrochloric acid reduction test), detection of proteins and amino acids (millon's test, ninhydrin test, biuret test) **[18, 19]**.

RESULT AND DISCUSSION

Plant authentication

Sida rhombifolia were authenticated at national ayurveda dietetics research institute (ay), Bangalore. (Drug authentication/smpu/nadri/bag/2011-12/540).

Extraction

The extraction was done by successive solvent extraction method using the various solvents like petroleum ether, chloroform, acetone, ethanol and aqueous solvents.

Standardization

Macroscopic examination

Macroscopic examination of leaves

The leaves are 2.5-5cm long, dark green in color. The taste and odor are characteristic with obovate, acute, stellatly hair beneath surface, leaves are arranged alternately along the stem **(Table 1)**.

Macroscopic Examination of Stem

The stems are soft and branched, growing 50 to 120 cm in height, with the lower sections being woody with 25-30 cm. The colour and odour of *Sda rhombifolia* are

greenish and characteristic respectively **(Table 2)**.

Microchemical Test

Sida rhombifolia show the presence of cellulose, lignin, fixed oils and fats, suberin and cutin, tannins, starch, mucilage, proteins, calcium oxalate crystal, cellulose cell wall, calcium carbonate, while chitin absent **(Table 3)**.

Microscopic Examination

Stomatal number and Stomatal index

The leaves of *sida rhombifolia* plant contain anisocytic type of stomata. The stomatal number and stomatal index were 8 and 40 respectively. The number of epidermal cells was 12 **(Table 4, Figure 1)**.

Powder Microscopy

Powder microscopy of *sida rhombifolia* was done under trinocular microscope and observed xylem, fibers, trichomes, crystal and medullary rays are present **(Figure 3)**.

Determination of total ash

The total ash, acid insoluble ash and water soluble ash of 2gm of dried arieal part of *sida rhombifolia* were found to be 54.50mg/g, 32.5mg/g and 12mg/g respectively. The total ash value are more than acid insoluble and water soluble ash. Total ash of arial part of *sida rhombifolia* was found to be 54.50 mg/g **(Table 5)**.

Acid-insoluble ash

The acid insoluble ash of *sida rhombifolia* was found out to be 32.5 mg/g **(Table 6)**.

Water soluble ash

The water soluble ash of *sida rhombifolia* was found out to be 12 mg/g **(Table 7)**.

Determination of water matter

Loss on drying- The water content or moisture content of aerial part of *sida rhombifolia* was found to be 175 mg/g **(Table 8)**.

Determination of swelling index

The swelling index aerial part of *sida rhombifolia* was observed 0.25 ml **(Table 9)**.

Determination of extractable matter

The extractive value of *sida rhombifolia* in petroleum ether extract was found out to be 45 mg/ml, chloroform extract 32.5 mg/ml, acetone extract 47.5 mg/ml, ethanol extract and in aqueous extract 65 mg/ml. with cold maceration, the extractive value of petroleum ether extract was found to be 12.5 mg/ml, chloroform extract 12.5 mg/ml, acetone extract 17.5 mg/ml, ethanol extract 15 mg/ml and in aqueous extract it was found to be 47.5 mg/ml **(Table 10)**.

Foaming index

The height of foam measured was less than 1 cm in every test tube. Therefore the foaming index was found to be less than 100 in plant **(Table 11)**.

Chromatographic studies

The TLC of different extracts was developed with various solvent systems but the best solvent system in which spots were detected when solvent system, toluene: chloroform: ethanol (28.5: 57: 14.5) and

detection of spots was done in dragendoff's reagent with solvent system. In pet. ether extract three spots were detected in developed TLC of *sida rhombifolia* whose rf value from the range of 0.47-0.84, chloroform extract showed four spots whose rf value range from 0.21-0.78, acetone extract indicated three sports with rf value from the range of 0.10-0.42, ethanol extract of showed four sports with rf value range 0.37-0.84 and water extract showed two spots whose rf value range from 0.15-0.21 **(Table 12, Figure 4)**.

Qualitative chemical examination of extracts

The phytochemical screening of pet ether extract of *sida rhombifolia* were contains steroids, phenols and fixed oil while chloroform extract contains alkaloids and fixed oil. While acetone extract contains alkaloids, fixed oil and phenols. others ethanolic and aqueous extracts showed the presence of alkaloids, carbohydrate, protein, steroids, glycoside tannins, fixed oil, saponin, phenol, and alkaloids respectively **(Table 13)**.

Table 1: Organoleptic characters of leaves

S. No.	Organoleptic characters	*Sida rhombifolia*
1.	Size	2.5-5cm
2.	Surface characteristics, Texture	Obovate, Acute, Stellatly hair beneath
3.	Taste	Characteristics
4.	Color	Dark green
5.	Odor	Characteristic

Table 2: Organoleptic characters of stem

S. No.	Organoleptic characters	*Sida rhombifolia*
1.	Size	25-30 cm
2.	Surface texture	Soft and branched
3.	Color	Greenish
4.	Odor	Characteristic

Table 4: Stomatal index

Plant	Stomatal number (per mm^2)	Number of epidermal cells	Stomatal index (i= s/e+s x100)*
Sida rhombifolia	8	12	40

***Where: i = stomatal index, s= no. of stomata per unit area, e= no. of epidermal cells in the same unit area**

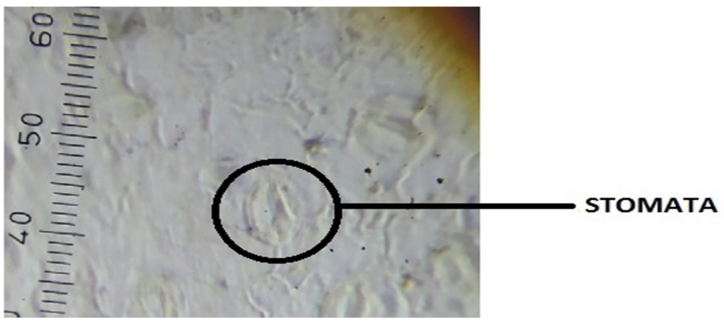

Figure 2: Structure of Stomata

Table 3: Microchemical Test

S. No.	Components	Test	Observation	Inference
1.	Cellulose	Iodine solution	Pale yellow	Cellulose present
		Iodine solution + Sulphuric acid	Bright blue	Cellulose present
2.	Chitin	Sudan red III	No red colour	Chitin absent
		Iodine solution + Sulphuric acid	No violet colour	Chitin absent
3.	Lignin	Iodine solution	Deep blue	Lignin present
		Iodine solution + Sulphuric acid	Brown	Lignin present
		Safranin	Red	Lignin present
		Phloroglucinol + Conc. Hcl	Stained red	Lignin Present
4.	Fixed oils and Fats	Chloroform	Soluble	Fixed oils & Fats present
5.	Suberin and Cutin	Iodine solution	Deep yellow	Suberin and Cutin present
		Iodine solution + Sulphuric acid	Deep brown	Suberin and Cutin present
6.	Tannins	Dilute ferric chloride solution	Greenish black	Tannins present
7.	Starch	Iodine solution	Blue	Starch present
		Water	Blue	Starch present
8.	Mucilage	Methylene blue	Deep blue	Mucilage present
		Iodine solution + Sulphuric acid	Violet	Mucilage present
9.	Proteins	Iodine solution	Yellow crystalloids	Proteins present
		Alcoholic picric acid solution	Yellow crystalloids	Proteins present
		Millon's reagent	Brick red colour	Proteins present
10.	Calcium oxalate crystals	Clear the section with chloral hydrate	Insoluble in acetic acid and caustic alkali	Calcium oxalates crystals present
11	Cellulose cell wall	Iodine & Sulphuric acid solution	Blue violet	Cellulose cell wall present
12.	Calcium carbonate	Acetic acid	Dissolve with acetic acid	Calcium carbonate present

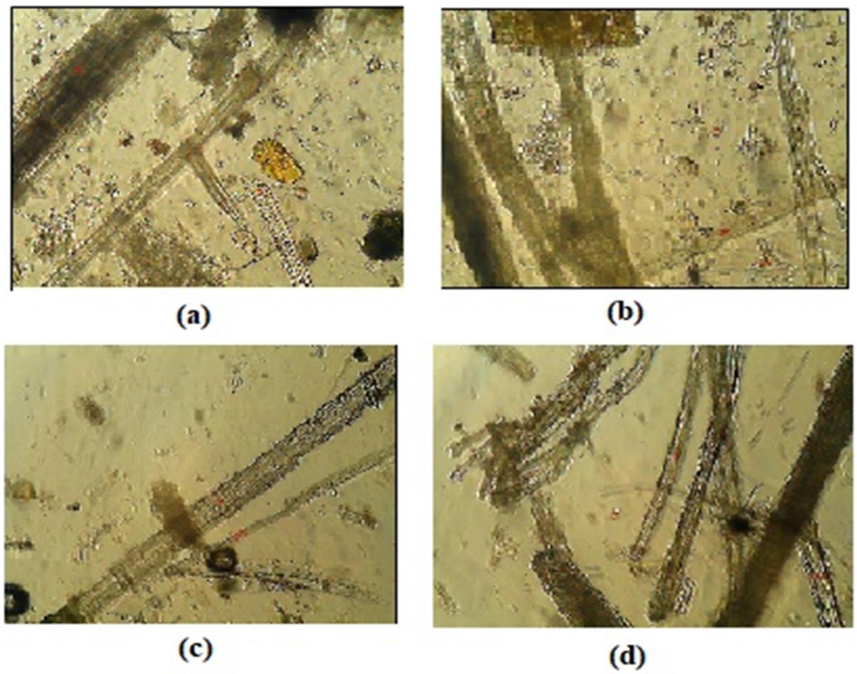

Figure 3: Powder microscopy of *sida rhombifolia*

Table 5: Observation of total ash

Plant	Weight of crucible(g) a	Weight of drug (g) b	Weight of crucible + weight of ash (g) c	Ash obtained (g) (c – a)	Total ash (mg /g)
Sida rhombifolia	20.550	2	21.640	1.09	109/2 = 54.50

Table 6: Determination of acid-insoluble ash

Plant	Weight of crucible (g)	Weight of drug (g)	Total ash (g) 'a'	Weight of crucible + weight of acid insoluble ash (g) 'b'	Acid insoluble ash obtained (g) 'a-b'	Acid insoluble ash (mg / g)
Sida rhombifolia	18.860	20.860	19.025	18.960	0.065	32.5

Table 7: Determination of water soluble ash

Plant	Weight of crucible (g)	Weight of drug (g)	Total ash (g) 'a'	Weight of crucible + Weight of water soluble ash (g) 'b'	Water soluble ash obtained (g) 'a-b'	Water soluble ash (mg /g)
Sida rhombifolia	21.625	23.625	21.850	21.825	0.025	12

Table 8: Determination of water matter

Plant	Weight of drug (g)	Weight of china dish (g)	Total initial weight (g) a	Constant weight after heating (g) b	Difference in weight (g) (a-b)	Loss on drying (mg / g)
Sida rhombifolia	4	65.60	69.60	68.90	0.7	700/4 = 175

Table 9: Swelling index

S. No.	Time	Readings of *sida rhombifolia* (ml)			Mean (ml)	Differ. for 4g plant material d=(b-a) (ml)	Swelling index for 1g (d/4) (ml)
		r_1	r_2	r_3	m_1		
1	Initially	25	25	25	25 (a)	1	0.25
2	After 3hours	26	26	26	26 (b)		

Table 10: Swelling index

Method	Drug (g)	Extract	Wt. of empty china dish(g)	Wt. of china dish + dried extract (g)	Difference (mg)	Extractable matter (mg/g)	Color of extract
Hot	4	Pet. Ether	50.28	50.46	180	45	Reddish Brown
	4	Chloroform	48.43	48.56	130	32.5	Brownish
	4	Acetone	53.04	53.23	190	47.5	Reddish brown
	4	Ethanol	50.75	50.76	10	2.5	Reddish brown
	4	Aqueous	53.90	54.16	260	65	Reddish brown
Cold	4	Pet. Ether	50.13	50.18	50	12.5	Yellow
	4	Chloroform	48.29	48.34	50	12.5	Yellowish green
	4	Acetone	52.94	53.01	70	17.5	Greenish
	4	Ethanol	50.40	50.46	60	15	Light Green
	4	Aqueous	53.66	53.85	190	47.5	Reddish brown

Table 11: Observation of height of foam

Test tube	1	2	3	4	5	6	7	8	9	10
Ratio of water: extract	9:1	8:2	7:3	6:4	5:5	4:6	3:7	2:8	1:9	0:10
Height of foam in *sida rhombifolia* (cm)	0	0	0	0	0	0	0	0.2	0.2	0.3

Table 12: TLC with solvent system toluene: chloroform: ethanol (28.5:57:14.5)

S. No.	Plant	Extract	Distance travelled by solute (cm)	Distance travelled by solvent (cm)	r_f value	Color of spot
1	*Sida rhombifolia*	Petroleum ether	9.5	4.5	0.47	Green
				7.5	0.78	Green
				8.0	0.84	Light green
2.	*sida rhombifolia*	Chloroform	9.5	2.0	0.21	Yellow
				4.5	0.47	Light yellow
				6.5	0.68	Light green
				5.0	0.78	Yellow
3.	*sida rhombifolia*	Acetone	9.5	1.0	0.10	Light yellow
				1.5	0.15	Green
				40	0.42	Light green
4.	*sida rhombifolia*	Ethanol	9.5	3.6	0.37	Yellow
				5.0	0.47	Light yellow
				6.5	0.68	Green
				4.5	0.84	Light green
5.	*sida rhombifolia*	Water	9.5	1.5	0.15	Light yellow
				2.0	0.21	Green

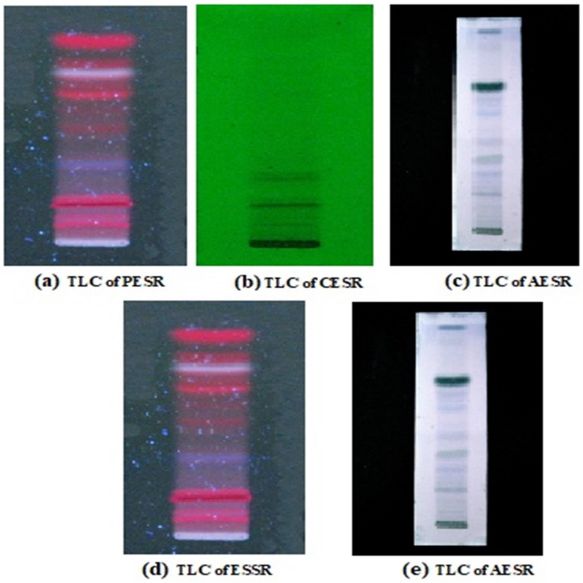

Figure 4: TLC profile of extract

Table 13: Inferences of qualitative chemical examination of extracts

S. No	Chemical constituents	Test	*Sida rhombifolia extracts*				
			Pet. ether	Chlorofom	Acetone	Ethanol	Aq.
1.	Alkaloids	Mayers test	–	+	+	+	+
		Dragendorff's test	–	+	+	+	–
		Wagners test	–	+	+	+	–
		Hagers test	–	+	–	+	–
2.	Carbohydrates	Molisch's test	–	–	–	+	–
		Benedict's test	–	–	–	+	–
		Fehling's test	–	–	–	+	–
3.	Glycosides	Modified borntragers	–	–	–	+	–
		Legal test	–	–	–	+	–
4.	Steroids	Salkowski's test	–	–	–	+	–
		Sulfur powder test	+	–	–	+	–
		Libermann burchard	+	–	–	–	–
5.	Resins	Acetone-water test	–	–	–	–	–
6.	Phenols	Ferric chloride test	+	–	+	+	–
7.	Tannins	Gelatin test	–	–	–	+	–
8.	Flavonoids	Alk. reagent test	–	–	–	–	–
		Zn-hcl acid reduction	–	–	–	–	–
		Shinoda test	–	–	–	–	–
9.	Fixed oils	Filter paper	+	+	+	–	–
10	Protenis	Millons test	–	–	–	–	–
		Ninhydrine test	–	–	–	+	–
		Biuret test	–	–	–	+	–

CONCLUSION

In this present study, Pharmacognostical and quality control parameters of *Sida rhombifolia* were studied. The morphological evaluations were done to ascertain the standard reference values for standardization of the plant materials, in which *sida rhombifolia* considers the parameters like macroscopic examination of leaves and stem, microchemical tests, stomatal number and stomatal index, powder microscopy, determination of total ash (acid insoluble and water soluble), loss of drying, swelling index, foaming index, determination of extractable matter, chromatographic studies, qualitative chemical examination. In the microscopy, the section study of the leaves of *Sida rhombifolia* shows the presence of xylem, fibers, trichomes, crystal, phylum, and anisocytic stomata is present.

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AUTHOR CONTRIBUTIONS

Conduct of the study, data collection, analysis and interpretation (Kumar Amit, Pathak Manish); Sample characterization (Chaudhary Ramesh); Sample testing

(Verma Vikrant); Manuscript preparation, design and review (Kumar Amit, Pathak M., Singh Lubhan)

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