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Phyto- pharmacognostic Analysis Of *Ayurvedic* Medicine *Bala* (*Sida Cordifolia* Linn.)

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Abstract:

Bala (*Sida cordifolia* Linn.), of Family *Malvaceae* is very important plant since long in traditional medicine of India. The drug is given various vernacular names i.e. *Kharethi* (Hindi), *Baladana* (Gujarati), *Chikana* (Malayalam) and *Simaka* (Panjabi) etc. the drug is attributed *Balya*, *Kantikarka*, *Grahi*, *Vrishya*, *Ojhovardhaka*, *Stambhana*, *Brihmana*, *Sothahara*, *Rasayana* and *Hridya* properties in different *Ayurvedic* classical texts. So, to evaluate the pharmacognostic and preliminary phytochemical standards of whole plant of *Bala* various methods including microscopy, physio-chemical contents and phytochemical estimation including quantitative analysis were done to determine the diagnostic features for the identification and standardization of intact and powdered drug. The organoleptic examination of the intact and powdered materials of whole plant of *Bala* revealed greenish yellow colour, odourless odour and slightly bitter taste. It showed presence of carbohydrate, alkaloids, amino acids, protein, saponin, and tannins in aqueous and methanol extracts. This study will be helpful to ensure the purity, safety and efficacy of the drug.

Keywords: *Bala*, Pharmacognostic, Phytochemical, *Balya*, *Kantikaraka* and *Sida cordifolia* (Linn.)

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INTRODUCTION:

Bala (*Sida cordifolia* Linn.), of family *Malvaceae* is distributed throughout the hotter parts of India and fairly common in various provinces in country like Uttar pradesh, West Bengal, Karnataka, Andhra pradesh, Tamilnadu, Rajasthan and Kerala etc. It is a common weed which grows in waste palaces¹. *Bala* is known as *Bariyara* locally, *Kharethi* in Hindi, *Bedela* in Bengali, *Chikana* in Malyalam, *Baladana* in Gujarati, *Simaka* in Punjabi and Country mallow in English². The drug is used as ethano-medicine by tribals of India for aphrodisiac, diuretic, analgesic, anti-inflammatory, emollient and cardio-tonic in different *Ayurvedic* classical texts. *Bala* is mentioned as having *Madhura Rasa*, *Laghu - Snigdha - Pichchhila Guna*, *Shita Virya*, *Madhura Vipaka* and *Vatapittahara Karma* etc. and attributed *Balya*, *Kantikaraka*,

Grahi, *Vrishya*, *Ojhovardhaka*, *Stambhana*, *Brihmana*, *Shothahara*, *Rasayana* and *Hridya* properties. The historical evidence of *Bala* is traced from *Vedic* period, *Samhita* period and ancient *Nighantu* period to current modern texts. After medieval period, various types of *Bala* are described by *Nighantus* under title of *Baladvaya*, *Balatraya* and *Balachatushtaya*. Also, various *Sida* species are added as adulteration and substitute in market samples of *Bala*. Hence, authentication of the *Bala* (*Sida cordifolia* Linn.) on macroscopic and microscopic level is the need of hour. This, study is aimed for the same.

MATERIALS AND METHODS:

Microscopic, physio-chemical and phytochemical study including quantitative analysis of *Bala* (*Sida cordifolia* Linn.)

were done to determine the diagnostic features for the identification and standardization of intact and powdered drug. All the standard references of procedures were followed from authentic books and sources during the study.

Bala plants were collected from Jaipur district, Rajasthan state. Specimens were dried by keeping them between the folds of old newspapers. It is necessary to change these papers at regular intervals, until the plants are well dried. The dried specimens were pasted on the herbarium sheets of standard size with proper labelling. The authentication of plants material collected for study was done at botany department, University of Rajasthan, Jaipur vide reference number RUBL211726 as *Bala* (*Sida cordifolia* Linn.) and belong to family *Malvaceae*. After identifying the plant, for study purpose whole plant of *Bala* was washed with running water and kept for drying under shade. The procured dried parts were powdered, labelled, packed and subjected for organoleptic and other analytic studies.

Taxonomic classification of *Bala*³:

Kingdom – Plantae – Plants

Subkingdom – Tracheobionta –Vascular plants

Superdivision – Spermatophyta - Seed plants

Division – Magnoliophyta – Flowering plants

Class – Magnoliopsida – Dicotyledons

Subclass – Dilleniidae

Order – Malvales

Family – Malvaceae – Mallow family

Genus – *Sida* (Linn.) – fanpetals

Species – *Sida cordifolia* (Linn.)

Vernacular Names⁴:

English - Country mallow

Hindi - *Bariyaar, Khiratee, Khareti, Bariar, Bariyara, Kharenti*

Bengali - *Swetberela, Brela, Bala, Bedela, Barila*

Gujarati - *Mahabala, Khapat, Bala, Kharatee, Baladana*

Malayalam - *Kurunthott, Vellurum, Kathuram, Katturam*

Marathi - *Chikana, Khiranti*

Punjabi - *Kowar, Simak, Kharent, Kharyati, Kharanhatee*

Botanical description⁵:

Habitat: - *Bala* is distributed throughout the hotter parts of India and fairly common in various provinces in country like Uttar pradesh, West Bengal, Karnataka, Andhra pradesh, Tamilnadu, Rajasthan and Kerala. It is a common weed which grows in waste places.

Morphology: - Shrubby, branched, softly hairy and with much stellate, hair nearly all over and sub-persistent.

Leaves: - 1-2 inch long, cauline and ramal, alternate, stipulate, cordate and not acuminate and petioles are 1/2 to 1.5 inch long.

Pedicels: Solitary or few together, hort. Some up to ¼– 3/4 inch long jointed much above the middle.

Calyx: - ¼ to 3/4 inch long, lobes ovate, acute.

Corolla: - Slightly exceeding the calyx, yellow.

Fruit: - ¼ to 1/3 inch in diameter, (Flowering and Fruiting - October – February)

Seed: - Smooth, flattened, reniform and brown or black.

Carpels: - 7 to 10 strongly reticulated ciliate on the upper margins. The two dorsal margins almost scabrid, awns 2 nearly as long as the carpels, linear, retorsely scabrid and hairy.

Root: - Occurs in variable sized pieces, 5 to 15 cm long with few lateral slender rootlets of smaller size, tap root branched at the tip, outer surface buff to greyish – yellow minutely striated or smooth, odourless, taste slightly bitter.

Chemical Constituents⁶: -

Root: - C₂₈ phyto-ecdysones viz, sidasterone A, sidasterone B, carboxylated tryptamines, quinazoline alkaloids, sympathomimetic amines, β-phenethylamine. β-sitosterol, acylsteryglycoside sitoindoside, ephedrine, S-(+)-Nb-methyltryptophan methylester, hypaphorine, vasicinone, vasicine, vasicinol, Ψ ephedrin, choline, betaine, phytosterol, resin acids.

Seed: - Proteins, steroids, resin, resin acid, mucin, phenethylamine, ephedrine pseudoephedrine, fatty oil, potassium nitrate, linoleic acid, malvalic acid, sterculic acid and coronaric.

Aerial parts: - Palmitic, stearic, hexacosanoic acids and β-sitosterol.

Pharmacognostic study:

Pharmacognostic study was carried on the basis of morphological characters such as colour, odour, taste etc.

Physiochemical Parameters:**Determination of Moisture Content⁷:**

Moisture content was determined by placing weighed sample of 5 g of drug in oven at 105° for 5 hours, and calculated weight of sample for every 30 minute, until the weight of the sample came out to be constant, no variation of weight was recorded. This sample was allowed to cool at room temperature in a desiccator for 1 hour before weighing.

Determination of pH⁸: The pH value of an aqueous liquid may be defined as the common reciprocal of the hydrogen ion concentration expressed in gram per liter. It practically means the quantitative indication of the acidity or basic nature of a solution.

The pH of a given solution is measured by using digital pH meter. First Standardized the pH meter. Tablets of different pH were taken and each tablet was dissolved in 100 ml of distilled water to prepare solutions of different pH. The instrument was switched on and left for

some time until required different pH solutions appeared. Buffer solution was taken in the beaker and the electrode was dipped in it. Same procedure was repeated for the other buffer solution after washing the electrode thoroughly with distilled water. The sample was taken (10% aqueous solution) and electrode was dipped in it and the value of pH was noted.

Determination of Extractive values⁹:

Alcohol Soluble Extractive: - 5 g coarsely powdered air dried drug was macerated with 100 ml of Alcohol of the specified strength in a closed flask for twenty-four hours. It was then continuously shaken for six hours using rotary shaker and allowed to stand for eighteen hours. The content was filtered using filter paper. The filtrate was transferred to a pre-weighed flat bottomed dish and evaporated to dryness on a water bath. Then, the dish was kept in oven at 105°, to constant weight and weigh. The percentage of alcohol-soluble extractive was calculated with reference to the air-dried drug.

Water Soluble Extractive: - 5 g coarsely powdered air dried drug was macerated with 100 ml of water of the specified strength in a closed flask for twenty-four hours. It was then continuously shaken for

six hours using rotary shaker and allowed to stand for eighteen hours. The content was filtered using filter paper. The filtrate was transferred to a pre-weighed flat bottomed dish and evaporated to dryness on a water bath. Then, the dish was kept in oven at 105°, to constant weight and weigh. The percentage of water-soluble extractive was calculated with reference to the air-dried drug.

Determination of Ash value¹⁰:

Total Ash: - Weighed accurately 2 g of the air-dried drug in a silica dish and incinerated at a temperature not exceeding 450° until free from carbon. Then, cooled and weighed. Percentage of ash value was calculated on the basis of air - dried drug.

Acid Insoluble Ash: - Boiled the total ash with 25 ml of 2M hydrochloric acid for 5 minutes, collected the insoluble matter in a Gooch crucible or on an ash less filter paper, washed with hot water, ignite, cool in a desiccator and weighed. Calculate the percentage of acid - insoluble ash with reference to the air - dried drug.

Water – Soluble Ash: - Boiled the total ash for 5 minutes with 25 ml of water; collected the insoluble matter in a Gooch's Crucible or on an ash less filter paper, Washed with hot water and ignite for 15 minutes at a

temperature not exceeding 450 C. Subtract the weight of the insoluble matter from the weight of the ash; the difference in weight represented the water – soluble ash. Calculate the percentage of water – soluble ash with reference to the air - dried drug.

Preliminary Phytochemical Screening:

Phytochemical examinations were carried out for all the extracts as per the standard methods.

Tests for Carbohydrates:

Molisch's Test: - 2 ml of test Solution was taken in a test tube and 2 ml of the Molisch's reagent was added and shaken carefully and then about 1ml. of conc. H₂SO₄ is poured from side of the test tube and allowed to stand for one 1 minute. A purple colour ring at the junction of the two layers, if formed, indicated the presence of Carbohydrate.

Benedict's test: - It is used for reducing sugars and composed of mainly copper sulphate and sodium hydroxide. To the 4 ml of aqueous solution of drug, 1 ml of Benedict's solution was added and heated almost to boiling. Solution appears green, yellow, orange, red or brown colour in order of increasing concentrations of simple sugar in the test solution.

Fehling solution test: - It is generally used for reducing sugars and composed of two solutions, which are mixed in situ. Fehling solution A composed of 0.5% of copper sulphate whereas Fehling solution B composed of Sodium Potassium Tartarate. Equal volumes of Fehling A and Fehling B solutions were mixed (1 ml each) and 2 ml of aqueous solution of drug was added followed by boiling for 5-10 minutes on water bath. Formation of reddish brown coloured precipitate due to formation of cuprous oxide indicates presence of reducing sugar.

Tests for Alkaloids: -

Dragondroff's reagent test: - 2 ml of test Solution was taken in a test tube in which 2 ml of the Dragondroff's reagent (Mixture of Potassium Iodide and Bismuth sub nitrate solution) was added. An orange precipitate if formed indicated presence of alkaloids.

Wagner's Test: - Drug solution was taken in a test tube in which few drops of Wagner's reagent (dilute Iodine solution), formulation of reddish-brown precipitate.

Hager's Test: - A saturated aqueous solution of picric acid was employed for this test. When the test filtrate was treated with this reagent, an orange yellow precipitate was obtained which indicates the presence of alkaloids.

Test for Amino acids: -

Ninhydrin test: - The Ninhydrin test is used to detect the presence of alpha-amino acids and proteins containing free amino groups. Protein solution when heated with ninhydrin molecules, it gives characteristic deep blue or pale yellow colour due to the formation of complex between two ninhydrin molecule and nitrogen of free amino acid.

Tests for Proteins: -

Biuret test: - A few mg of the residue was taken in water and 1 ml of 4% sodium hydroxide solution was added to it, followed by a drop of 1% solution of copper sulphate. Development of violet or pink colour indicates the presence of proteins.

Xanthoprotic test: - A small quantity of test sample was taken with 2 ml of water and 0.5 ml of concentrated nitric acid was added to it. Development of yellow colour indicates the presence of proteins.

Millon's test: - A small quantity of test sample was taken and 2 to 3 ml of millon's reagent was added. The white precipitate slowly turning to pink, indicate the presence of proteins.

Test for saponin: -

Foam test: - A small quantity of the test sample was taken in a test tube and shaken

vigorously with a small amount of sodium bicarbonate and water. A stable, characteristic honeycomb like froth indicates the presence of saponin.

Test for glycosides: -

Borntrager's test: - 1 ml of Benzene and 0.5 ml of dilute ammonia solution was added to the extract and was observed for the formation of reddish pink colour.

Test for Phenolic Compound: - The extract was taken in water and warmed; to this 2 ml of ferric chloride solution was added and observed for the formation of green and blue colour.

Test for Steroids: -

Salkowski reaction: - Few mg of extract was taken in 2 ml of chloroform and 2 ml of concentrated sulphuric acid was added from the side of test tube. The test tube was shaken for few minutes. The development of red colour indicates the presence of steroids.

Test for Tannins: -

Ferric chloride solution: - A 5 percent solution of ferric chloride in 90 % alcohol was prepared. Few drops of this solution were added to a little of the above filtrate. Appearance of dark green or deep blue colour indicates the presence of tannins.

Lead acetate: - A 10 percent w/v solution of basic lead acetate in distilled water was added to the test filtrate. Development of precipitate indicates the presence of tannins.

Potassium Dichromate: - A solution of potassium dichromate was added to the filtrate. Appearance of dark colour indicates the presence of tannins.

Thin Layer Chromatography (TLC): -

Thin layer chromatography is a tool for separation and identification of chemical constituents. Thin layer chromatography is a technique in which a solute undergoes distribution between two phases, a stationary phase acting through adsorption and a mobile phase in the form of a liquid. The adsorbent is a relatively thin, uniform layer of dry finely powdered material applied to a glass, plastic or metal sheet or plate. Glass plates are most commonly used. Separation may also be achieved on the basis of partition or a combination of partition and adsorption, depending on the particular type of support, its preparation and its use with different solvent.

Identification can be effected by observation of spots of identical R_f value and about equal magnitude obtained,

respectively, with an unknown and a reference sample chromatographed on the same plate. A visual comparison of the size and intensity of the spots usually serves for semi-quantitative estimation.

Chromatography plates: - T.L.C. plate coated with 0.25 mm layer of silica gel 60 F₂₅₄ with fluorescent indicator was used. (Each plate dimension is 10 cm long and 2 cm width)

Activation of pre-coated Silica gel 60 F₂₅₄: - Plates were dried in hot oven at 105⁰ C for one and half hour.

Preparation of mobile solution: - Chloroform : Methanol (7:3)

Preparation of test solution: - 4 g powdered drugs were extracted with 100 ml of ethanol (90%) in a Soxhlet's apparatus consecutively three times. Extract was filtered and concentrated to 10 ml.

Sample application: - Samples were applied with the help of capillary 1 (one)

cm above the base of T.L.C. plate. Then it was dipped in mobile solution. T.L.C. plate was removed from the mobile solution immediately after the spot reached the 1 (one) cm below the top of the T.L.C. plate.

Visualization: - Anisaldehyde sulphuric acid spray.

R_f Value: - Measured and recorded the distance of each spot from the point of its application and calculated R_f value by dividing the distance travelled by the spots by the distance travelled by the front of the mobile phase.

Observations and results:

The different pharmacognostic parameters were studied and evaluated in order to standardize the drug. The results of pharmacognostic parameters i.e. microscopic study, physicochemical parameters, phytochemical analysis have been cited in below.

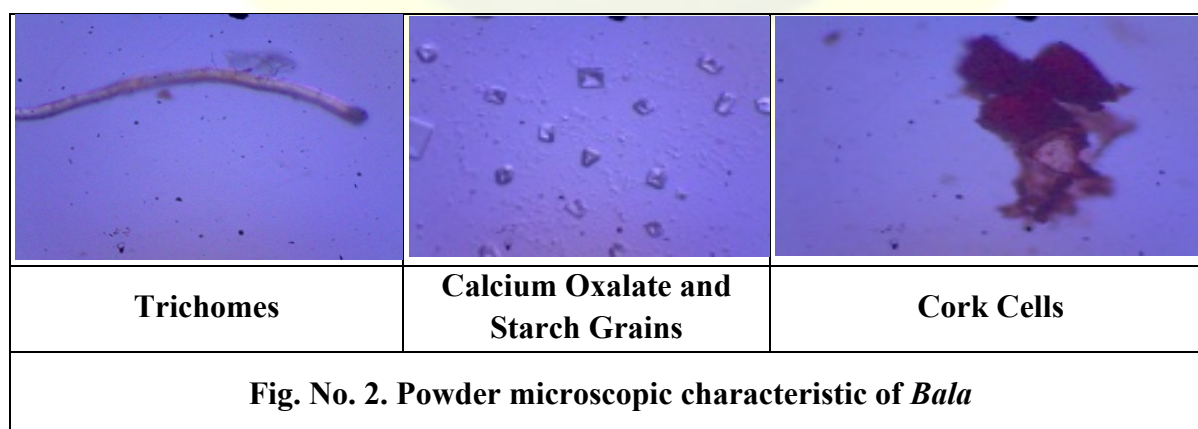


Fig. 1. Powder of whole plant of *Bala*

Table 1. Macroscopic examination of whole plant of *Bala* powder

S. No.	Observed	<i>Bala - Sida cordifolia</i> (Linn.)
1.	Colour	Greenish yellow
2.	Odour	Odourless
3.	Taste	Slightly bitter

Powder microscopic study of *Bala*: - In powder microscopy, structure like cork cells, trichomes, calcium oxalate crystals and starch grains were seen.



Physicochemical parameters:

In this study, moisture content, pH, extractive value (alcohol soluble extractive value and water soluble extractive value) and ash values (total ash, acid insoluble ash and water soluble ash) were determined below-

Table 2. Physiochemical analysis of whole plant of *Bala* powder

S. No.	Physiochemical parameter	Results % w/w
1.	Moisture content	3.94
2.	pH value	5.9
3.	Water soluble extractive value	11.48
4.	Alcohol soluble extractive value	3.67
5.	Total ash	5.67
6.	Acid insoluble ash	1.56
7.	Water insoluble ash	4.32

Phytochemical analysis:

Phytochemical are nutritive plant chemicals that have protective or disease preventive properties. A plant cell produces two types of metabolites- primary metabolites involved directly in growth and metabolism (carbohydrates, lipids and proteins etc.) and secondary metabolites not involved in metabolic activity (alkaloids, phenolics,

sterols etc.) but act as defence chemicals. The preliminary phytochemical investigations of aqueous and alcohol extract of whole plant of *Sida cordifolia* (Linn.) were performed which reveals the presence of carbohydrates, alkaloids, amino acids, saponin, glycosides, steroids and tannins. The results of the screening were expressed in Table 3.

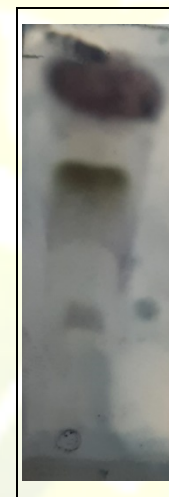
Table 3. Phytochemical analysis of whole plant of *Bala*

Name of test	<i>Sida cordifolia</i> (Linn.)	
	Aq.	Al.
(+)= Positive and (-)= Negative		
Carbohydrate test		
Molish test	+	+
Benedict test	+	+
Fehling test	+	+
Alkaloids test		
Dragendorff test	-	+
Wagner's test	-	-
Hager's test	-	-
Amino acids		
Ninhydrine test	+	-
Proteins		
Biuret test	-	-
Xanthoprotic test	+	+
Millon's test	+	-
Saponine		
Foam test	+	-
Glycosides		
Borntrager's test	-	-

Phenolic compound		
Phenolic test	+	+
Steroids		
Salkowaski reaction	-	-
Tannins		
FeCl ₃ test	-	+
Lead acetate test	+	+
Pot. Dichromate test	-	-

Table 4. Thin Layer Chromatography of whole plant of *Bala*

Sample	<i>Bala (Sida cordifolia Linn.)</i>
R_f value	0.16, 0.28, 0.50, 0.65, 0.76, 0.86, 0.96



**Fig. 3. Thin Layer Chromatography of whole plant of *Bala*
(*Sida cordifolia Linn.*)**

DISCUSSION:

Bala (Sida cordifolia Linn.) is slightly bitter in taste, odourless in odour and greenish yellow in colour. Powder microscopic study of whole plant powder of *Sida cordifolia* (Linn.) revealed tracheid, calcium oxalate, starch grain and cork cells after observation under microscope. Loss on drying is a water holding property of test

substance. Moisture content and pH value was found to be 3.94% and 5.9. Extractive value is directly relative to strength or potency of drug which estimates in different solvents. Water soluble extract value was found in sample 11.48% and alcoholic extractive value was found 3.67%. Ash value is the indicator of the presence of inorganic and earthy matter in

the plant. The higher ash value is suggestive of thermo – non labile / heat stable or inorganic constituents. The total ash value in sample 5.67%, the acid insoluble content which indicates the presence of siliceous matter and heavy metals in sample 1.56%. Water soluble ash estimates the inorganic water soluble salt in sample 4.32%. Qualitative analysis of inorganic matter showed the presence of carbohydrate, alkaloid, tannin, protein, amino acid, phenolic compound and saponin in *Bala* powder. Thin layer chromatography establishes the phytochemical fingerprint profiling in drug for identity.

CONCLUSION:

The plant *Sida cordifolia* (Linn.) is well-known *Ayurveda* plant. After performing

the work, it was found that the phytochemical screening confirmed the presence of various phytochemical constituents such as carbohydrates, alkaloids, amino acids, saponin and tannins. Different physicochemical parameters such as total ash, acid insoluble ash, water soluble ash, water soluble extract, alcohol soluble extract, loss on drying value and R_f value was observed. These values can be useful to detect adulteration. All studied standardization parameters like pharmacognostic study, phytochemical screening and physicochemical parameters provide the knowledge in the identification and authentication of whole plant of *Sida cordifolia* (Linn.).

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