

Pharmacognostical Standardization of Balamulachurna-Root Powder of *Sida rhombifolia* subsp. *retusa* (L.) Borssum

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ABSTRACT

Background: *Ksheerabala tailam* is well known Ayurvedic herbal formulation which is used in inflammations, pains and neurological disorders. The primary botanical source for *Ksheerabala tailam* is Balamulachurna. The literature refers to Balamulachurna as either of the root powders of *Sida cordifolia*, *Sida acuta*, *Sida cordata* or *Sida rhombifolia* subsp. *retusa*. To accomplish the clinical efficacy of *Ksheerabala tailam*, standardization of Balamulachurna is necessary. **Objectives:** The study is aimed to establish pharmacognostic standards of Balamulachurna with respect to *Sida rhombifolia* subsp. *retusa* root powder. **Materials and Methods:** Pharmacognostical standardization was carried out by focusing on organoleptic, macroscopic, microscopic, preliminary phytochemical evaluation, heavy metal assessments, fluorescence and HPTLC studies. **Results:** The roots macroscopic features were noticed as; an elongated taproot with few lateral roots, thick, woody, brown-coloured and bitter to taste. Organoleptic characteristics revealed the nature of the root powder as cream in colour with a pleasant odour and slightly bitter taste. The powder microscopic study specified the presence of fibres, fibre-sclereids, tracheids, vessel elements and calcium oxalate crystals. Physicochemical studies assessed total ash (7.5%), water-soluble ash (4.46%), alkalinity of water-soluble ash (0.4 ml) and acid-insoluble ash (3.3%). The preliminary phytochemical analyses revealed the presence of glycosides, alkaloids, phenols, flavonoids, tannins, terpenoids, and quinones. The HPTLC analyses exhibited various R_f values with the diversity of multiple phytoconstituents. **Conclusion:** The scientific data generated by the study is beneficial for adequately identifying and establishing standards for using *Sida rhombifolia* subsp. *retusa* as a primary drug for maintaining the quality and purity of the *Ksheerabala tailam*, an Ayurvedic formulation.

Keywords: *Ksheerabala tailam*, Balamulachurna, *Sida rhombifolia* subsp. *retusa*, Standardization, Pharmacognosy

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INTRODUCTION

India has an ancient, dynamic, and diversified cultural past. The concept of health and healing plays a significant role in this culture and tradition. India, rightly known as the botanical garden of the globe, is the world leader in producing the most medicinal herbs.^[1-5] According to estimates from the World Health Organization, 80% of people in developing nations like China and India still rely on traditional medicines, primarily plant-based medications, for their basic medical requirements.^[6-13] According to the National Medicinal Plants Board (NMPB), bala is the third-most-used medication in the Ayurvedic pharmaceutical sector. It is primarily collected from the wild.^[14] The literature

review referred to the roots of *Sida rhombifolia* subsp. *retusa* as Bala in Kerala.^[15] Bala is a common herbal remedy with a variety of applications. It is primarily employed in the form of powder with the name Balamulachurna (Bala: drug name; Mula: root; Churna: powder).^[14-17] It is also the primary component of *Ksheerabala tailam*, therapeutic oil made from *Sida rhombifolia* subsp. *retusa* (Figure 1). The musculoskeletal system is treated with this oil in cases of sickness as a rejuvenator, which promotes tissue recovery and relieves pain, stiffness, inflammations, and other adverse effects. It also nourishes the nerves, spinal cord, and brain. As a result, it is used to treat conditions like osteoarthritis, gout, poliomyelitis, facial paralysis, sciatica, and hemiplegia.^[14-17] The practice and demand for Ayurvedic medicine have rapidly expanded during the past few decades. Nowadays, in the same way, adulterations have increased with problems associated with their purity, quality, safety, and efficacy. The pharmacognostic standardization of crude drugs is the only source to stop adulteration and ensure the end products therapeutic efficacy



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without adulteration.^[17-22] The present study aims to standardize the Balamulachurna with the use of pharmacognostic tools.

MATERIALS AND METHODS

Collection of the material

Roots of *Sida rhombifolia* subsp. *retusa* was collected from hilly areas of Kottayam, Kerala and identified by comparing it with the herbarium specimen deposited at Sri Venkateswara University, Tirupati. The herbarium accession number is SVUBS1258.^[14,16,17]

Powder preparation

Shade-dried roots were sliced into small pieces and powdered using a grinding mill. The produced powder was passed through a #60 sieve and preserved in airtight containers for further study.^[16-19]

Macroscopic and organoleptic evaluation

The crude roots were examined with the naked eye for macroscopic characteristics. The organoleptic properties were observed and noted as per standard procedures.^[18-22]

Microscopic evaluation

The root powder was subjected to powder microscopy and maceration studies using the standard procedures.^[18-22]

Determination of physicochemical parameters

Physicochemical standards like pH of the water extract of root powder, ash, extractive and limit tests for heavy metals values were accomplished as per the standard procedures.^[14-22]

Powder analysis

Root powder was treated with different reagents to observe the colour changes.^[16-22]

Preliminary phytochemical screening

Preliminary Phytochemical screening tests were performed by standard methods.^[14,16-22]

Fluorescence studies

The fluorescence studies were done by treating the plant material with different reagents and observing colour changes under visible light and UV chambers.^[18-22]

High-performance thin-layer chromatography

The methanolic extract of root powder was applied manually in triplets on a TLC plate and subjected to a solvent system having toluene: ethyl acetate (6:4). It was saturated for 45 min in CAMAG® Flat Bottom Chamber of 10 x 10 cm. These plates were kept in the HPTLC instrument and analyzed under UV light at 366 nm and 254 nm; daylight and bands were observed.

The peaks area and height were recorded for the produced bands retention factor (R_f).^[14,16,19,20,22]

RESULTS

Macroscopic and organoleptic evaluation

Macroscopic characters

Roots are elongated taproots with a length of 20-25cm, and the diameter is 1 cm² to 1.5 cm². These were thick, hard to touch, brown-coloured and bitter to taste. Lateral roots were slender and few in number (Figure 1).

Organoleptic characteristics

The root powder is cream in colour, coarse fibrous powdery in nature, pleasant odour and slightly bitter taste (Figure 1 and Table 1). The root powder was subjected to powder microscopy, and noticed the presence of rosette crystals of calcium oxalate, starch grains, collenchyma cells and fibres (Figure 2: A, B, C, D).

Microscopic characters

Powder microscopy

Root Maceration

The dried root was subjected to maceration and following elements were noticed. (Figure 2: E and F).

Fibres

Fibres are primarily narrow with thick walls and narrow lumen, 545 mm long and 12 mm wide (Figure 2: D, E and F).

Fibres – Sclereids

These cells resemble the fibres but have a wider lumen shorter than the fibres, 250-450 m long, and 25 m thick (Figure 2: E and F).

Tracheids

They were elongated, thick-walled cells with tapering ends; lateral wall pits well developed, bordered and abundant; 210 m long (Figure 2: E and F).

Vessel elements

They are narrow, cylindrical, and relatively long. Perforation plate is simple, mostly oblique, one-sided tailed or non-tailed, tail short, thick and 220-260 m long (Figure 2: E and F).

Physicochemical parameters

The pH, ash and extractive values are depicted in Table 2 and Table 3.

In heavy metal analysis, Arsenic (As) and Lead (Pb) content were found to be less than 2 to 20 ppm respectively.

Powder analysis^[7-10]

Root powder was treated with different reagents like distilled water, 5% aqueous NaOH and 60% aqueous sulphuric acid, and the powder was pressed between filter paper for 24 hr. The results are documented in Table 4.

Fluorescence analysis

Fluorescence study is an essential tool for the standardization of powder drugs. Results in the form of colour variations were obtained by treating the plant powder with different reagents like NaOH, HNO₃, H₂SO₄, FeCl₃, HCl, picric acid and acetic acid under ordinary light and ultraviolet (UV) light at 254 and 366 nm (Table 5).

Preliminary phytochemical screening

This study was accomplished for qualitative screening through methanolic and water extracts (Table 6).

HPTLC screening

Root extracts of the samples were spotted in triplets on the silica gel "G" plate (Figure 3). These plates were developed utilizing the mobile phase as toluene: ethyl acetate (6:4). Various spots were identified under UV light at 366 nm (Figure 3: A), 254 nm (Figure 3: B), under Iodine vapours (Figure 3: C) and derivatized with Anisaldehyde sulphuric acid (Figure 3: D). Different R_f values are summarized in Table 7. The densitograms were developed for the root powder of *Sida rhombifolia* subsp. *retusa* plant (Figure 3: E). TLC studies of the root powder showed six major spots under U.V. light (366) nm with the R_f values as; 0.36 (red), 0.54 (red), 0.57 (blue), 0.71 (red), 0.86 (blue) and 0.99 (red); under Iodine, vapours shows one spot at R_f value 0.71 (brown) and under visible region after derivatizing with anisaldehyde sulphuric acid shows one spot at R_f value 0.71 (purple).

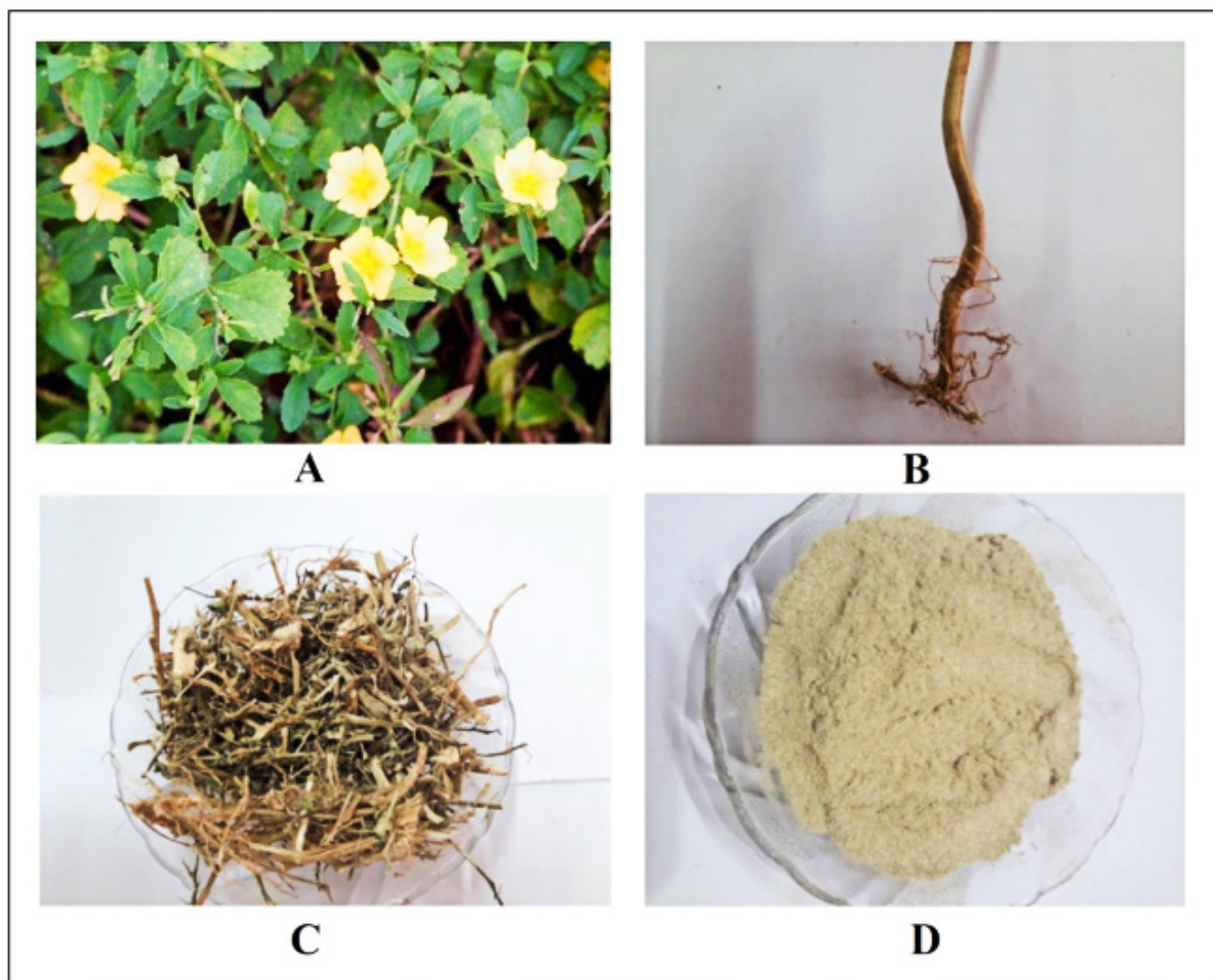


Figure 1: A. Morphology of the plant; B. Fresh roots; C. Dried roots; D. Root powder.

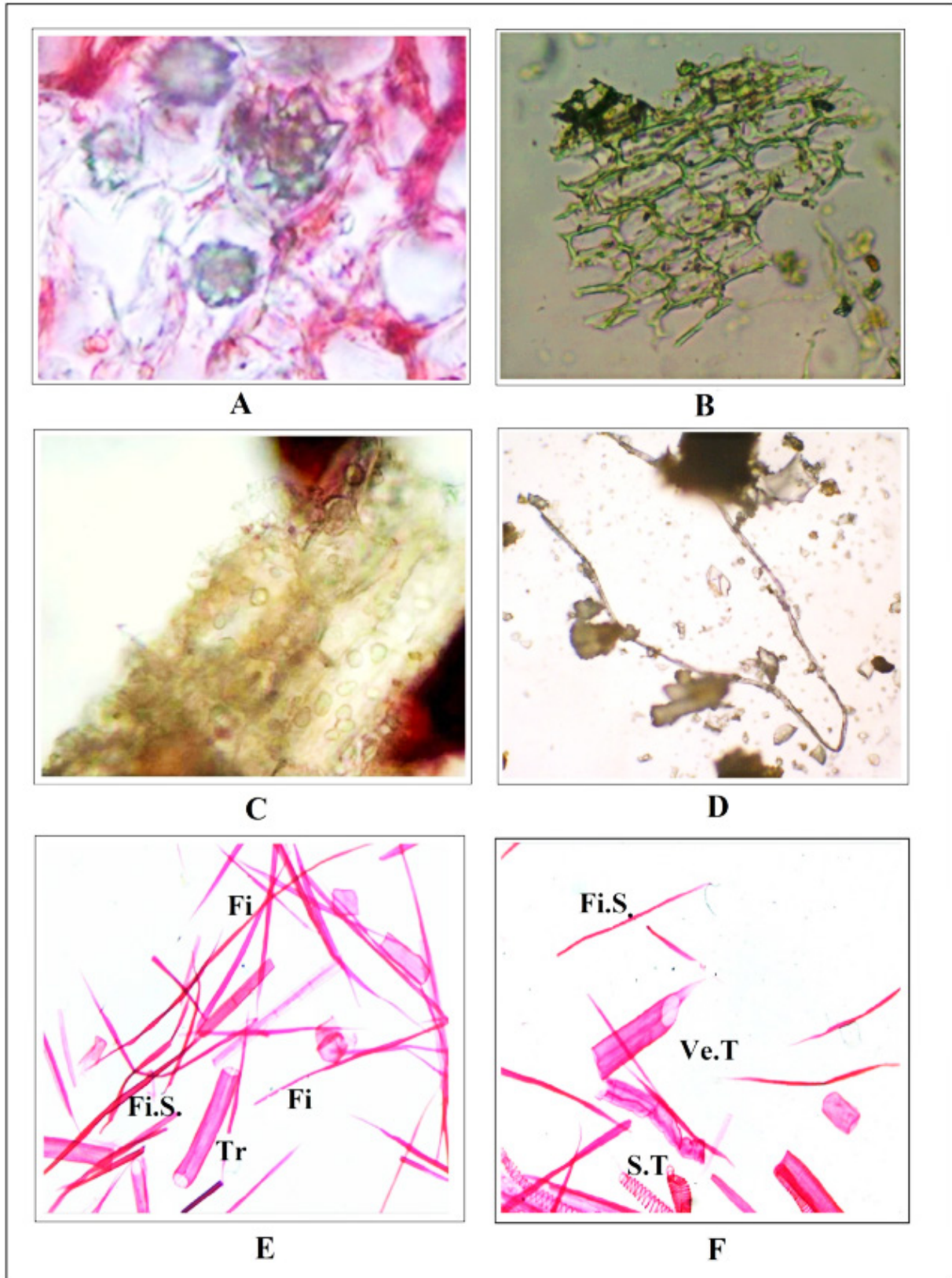


Figure 2: A. Rosette crystals of calcium oxalate identified through powder microscopy; B. Collenchyma cells (powder microscopy); C. Starch grains on parenchymatous region (powder microscopy); D. fibres (powder microscopy); E and F maceration studies: Fi: Fibres; Fi.S.: Fibres – Sclereids; Tr: Tracheids; Ve.T.: Vessel elements with short tapering ends; S.T.: Spiral thickening on tracheary elements.

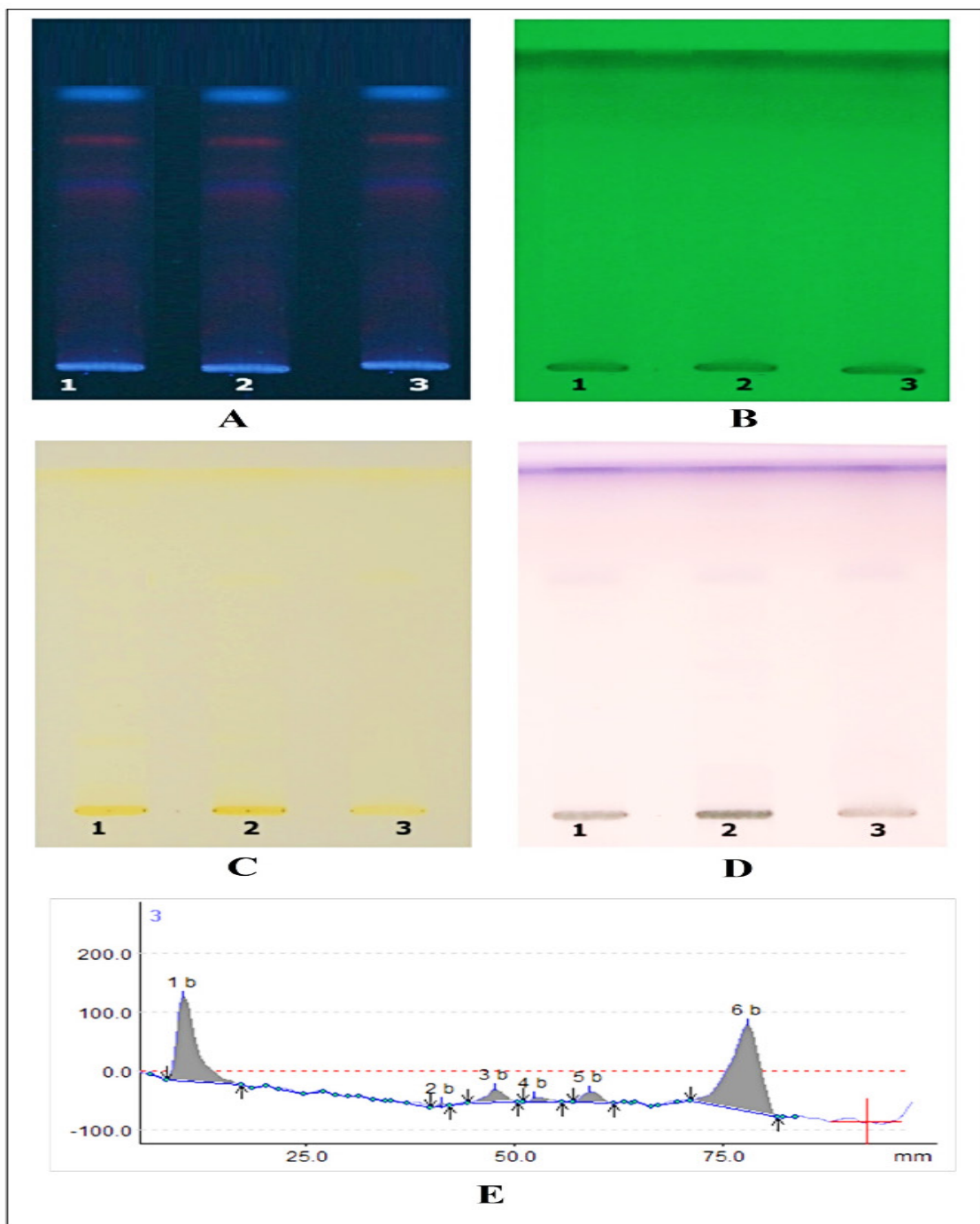


Figure 3: A. TLC chromatogram under UV light at 366 nm; B. TLC chromatogram under UV light at 254 nm; C. TLC chromatogram under Iodine vapors with visible region; D. TLC chromatogram derivatized with anisaldehyde sulphuric acid; E. Densitogram showing the separation of peaks of root powder at UV 366nm.

DISCUSSION

Ayurveda is the most widely used system in the Traditional Indian system of medicine. In Ayurveda system of Medicine, *Ksheerabala tailam* is a compound formulation used to treat various diseases. The primary botanical source for this formulation is Balamulachurna,^[14-17] root powder of *Sida*

rhombifolia subsp. *retusa*. The root powder is subjected to different pharmacognostic standardization methods to maintain the quality of the formulation without any adulteration.

The observations from organoleptic, macroscopic, microscopic, physico-chemical, heavy metal assessments and fluorescence studies revealed some specific results for the drug plant, *Sida*

Table 1: Characteristics.

Colour	Appearance	Odour	Taste
Cream	Fine powder	Pleasant	Slightly bitter

Table 2: pH and ash values.

pH	Total ash (%)	Water soluble ash (%)	Alkalinity of water soluble ash (ml)	Acid insoluble ash (%)
7.2	7.5	4.46	0.4	3.3

Table 3: Extractive values.

Alcohol soluble extract (% w/w)	Water soluble extract (% w/w)	Hexane soluble extract (% w/w)	Chloroform soluble extract (% w/w)
4.72	5.04	1.1	0.93

Table 4: Powder analysis.

Treatment	Observation
Powder treated with water	Non-sticky
Powder shaken with water	Foam like froth
Powder treated with 5% aqueous NaOH	Pale Brown
Powder treated with 60% aqueous H ₂ SO ₄	Dark Brown
Powder pressed between filter paper for 24 hr	No oil stain

Table 5: Fluorescence analysis.

Experiments	Visible / Day light	UV Light	
		254 nm	366 nm
Drug powder	Cream in colour	Pale Brown	Brown
Drug powder + 1 N NaOH (aq.)	Light Brown	Brown	Blackish Brown
Drug powder + 1 N NaOH (alc.)	Light Brown	Yellowish Brown	Pale Green
Drug powder + 1 N HCl	Brown	Black	Black
Drug powder + 50% H ₂ SO ₄	Dark Brown	Yellowish Brown	Greenish yellow
Drug powder + 50% HNO ₃	Orange	Yellowish Brown	Black
Drug powder + Picric acid	Green	Yellowish Green	Green
Drug powder + Acetic acid	Brown	Pale Brown	Black
Drug powder + Ferric chloride	Dark olive (green)	Yellowish Brown	Black

Table 6: Qualitative phytochemical screening of methanol and aqueous extracts of root powder.

Name of the Compound	Alkaloids	Flavonoids	Terpenoids	Steroids	Tannins	Phenols	Glycosides	Coumarins	Quinones
Aqueous extract	+	+	+	-	-	+	-	-	+
Methanolic extract	+	+	+	+	+	+	+	-	+

Table 7: Peak list of root powder at 366nm UV.

Peak no	Y-Pos	Area	Area (%)	Height	R _f value
1	10.3	395.58	35.20	141.14	0.02
2	41.2	5.51	0.49	5.75	0.45
3	47.6	54.32	4.83	21.03	0.54
4	52.5	21.35	1.90	8.19	0.60
5	59.1	46.46	4.13	18.29	0.70
6	78.1	600.73	53.45	144.39	0.96

rhombifolia subsp. *retusa*, which clearly differs from the other botanical sources of Bala like; *Sida cordifolia*, *Sida acuta* and *Sida cordata*.^[14-17]

The qualitative analysis of preliminary phytochemical screening from methanolic extracts revealed that the plant is a good source of glycosides and flavonoids, followed by alkaloids, phenols, tannins, terpenoids, and quinones.

HPTLC is an essential tool to standardize the product by developing fingerprint profiles, quantifying phytoconstituents and determining impurities. The R_f values produced by HPTLC analysis are specific for *Sida rhombifolia* subsp. *retusa* and ultimately differs from *Sida cordifolia*, *Sida acuta* and *Sida cordata*.^[14-17]

CONCLUSION

The study on Balamulachurna with the scope of *Sida rhombifolia* subsp. *retusa* root powder successfully established the pharmacognostic standards. The morphological, microscopical, physicochemical, preliminary phytochemical and chromatographic parameters of this controversial drug would definitely support the identification of the species. It is also helpful in determining its purity and quality standards during the preparation of Ayurvedic formulations. Further, this type of data could be employed amicably in evaluating pharmacopoeial standards and adulteration in terms of quality assurance of drugs with techniques like HPTLC.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

SVUBS: Sri Venkateswara University Botany Section;
UV: Ultraviolet; **HPTLC:** High-Performance Thin-layer Chromatography; **pH:** Potential of Hydrogen.

SUMMARY

The present study is aimed to standardize Balamulachurna concerning *Sida rhombifolia* subsp. *retusa*. The root powder of *Sida rhombifolia* subsp. *retusa* was subjected to various standardized screenings like organoleptic, microscopic, physico-chemical, preliminary phytochemical, fluorescence and HPTLC studies. Organoleptic characteristics revealed specific characteristics of the powder like colour, taste and Odour. Microscopic studies explored the presence of rosette crystals of calcium oxalate, starch grains, collenchyma cells, fibres, tracheids and vessel elements. Physico-chemical screening disclosed the inorganic composition and other impurities present along with the drug. The root powder is a good source of flavonoids and glycosides disclosed by preliminary phytochemical screening. The majority of the results obtained from the above screenings are specific to this plant compared with other botanical sources. The findings of the study are helpful in future analysis for proper identification and authenticity of the drug, Balamulachurna.

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