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Original research article

Pharmacognostical Analysis and *In Vitro* Antioxidant Activity of *Scoparia dulcis* L.

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Abstract:
Scoparia dulcis L. is widely used in the traditional system of medicine for treating liver ailments. In the present study the detailed pharmacognostical evaluation of *Scoparia dulcis* Linn. whole plant (Scrophulariaceae), including morphology, microscopy, physicochemical, phytochemical screening and *in vitro* antioxidant activity of 70% ethanol extract. Microscopy of different plant part was done by performing transverse sections and longitudinal sections, which were identified by the different staining reagents and dyes. Physicochemical constants were done for whole plant; it includes ash value, extractive value and moisture content. Phytochemical screening was done for aqueous and 70% ethanolic extract in maceration and soxhletion, results revealed the presence of alkaloids, glycosides, carbohydrates, phenolic compound, flavonoids, saponins, proteins, and amino acids. Ethanolic extract were tested for their in vitro 1, 1- Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity. These study includes parameters to establish the authenticity of *S. dulcis* and can possibly help to differentiate the drug from its other species and this may be attributed to its free radical scavenging potential, which in turn may be attributed to the presence of 2° metabolites.

Introduction

Scoparia dulcis Linn. (Scrophulariaceae) commonly known as mithipatti (Hindi), sarakkotthini (Tamil), kallurukki (Malayalam), bon-dhonya (Bengali), sweet broom weed or ghoda tulsi is a large glabrous or pubescent shrub with smooth or lenticellate branches; leaves elliptic to oblong or obovate, fruit a purplish black berry. The plant grows throughout India, in hedges or neglected places. The plant is astringent, sweet, cooling, diuretic, and constipating. It is useful in vitiated conditions of pitta, burning sensation, ophthalmodynia, diarrhea, skin eruption, and obesity.[1,2,3,4]

S. dulcis is an annual herb widely distributed in tropical and subtropical regions. In these regions, fresh or dried S. dulcis plants have traditionally been used as remedies for stomach troubles,[3] hypertension,[5] diabetes,[6] bronchitis,[7] and as analgesic and anti-pyretic agents.[8] A number of different principles such as scoparic acid A, scoparic acid B, scopadulcic acid A and B, scopadulciol, and scopadulin[9] have been shown to contribute to the observed medicinal effect of the plant.

These compounds were found to possess various biological activities such as inhibition of herpes simplex virus replication, gastric H+, K+-ATPase activation, antitumor activity, etc.[10] In a study of the anti-diabetic effect of S. dulcis, a glycoside named ammelin was obtained from fresh plants, which relieved other ailments accompanying diabetes, liver diseases, such as pyorrhea, eye troubles, joint pain, susceptibility to cold, etc., within a very short period of time. In spite of the numerous medicinal uses attributed to this plant, pharmacognostical and pharmacological information about this plant has not yet published.[11]

Materials & Methods:

Collection and authentication

Scoparia dulcis L. (whole plant) was collected from the campus of Manipal college of Pharmacy, Udupi, Karnataka, and authentified by Dr. Sreenath, senior lecturer, Department of Botany, Bangalore University, Bangalore. A specimen sample of the same was presented in the herbarium section of the department for future reference (voucher no. BUB 12005).

Organoleptic Study

The organoleptic study indicates the external characters like colour, odour and taste.

Morphology of *S. dulcis*

Morphological characteristics are referred to the evaluation of herbs by color, odor, taste, size, shape, and special features like touch, texture, etc., it is a technique of qualitative evaluation based on the study of morphological and sensory profiles of herbs.

Microscopy of *S. dulcis*

The microscopy of S. dulcis plant was carried out using digital microscope attached with computer system (Besto-1688). The plant parts were sliced fine by using microtome for its transverse section and longitudinal section; the finest sections were selected for the study. The transverse section was stained with phloroglucinol and HCl for staining the lignified tissues and mounted with glycerin and observed under microscope. The sections were examined under microscope using ×10 eye piece and objective pieces 10, 40, and ×100. For leaf constants ×5 eye pieces and ×10 objective piece were used.

Leaf constants

The specification of leaf constants mentioned in quantitative microscopy includes:[12]

- Vein-islet number and veinlet-termination
- Stomatal number and stomatal index
- Palisade ratio

The main importance of these values lies in identifying the species, whether the powder is authentic or adulterated.

Method of determination of vein-islet number and veinlet-termination number*:*

Three to four cut portions of the leaf from the central region of lamina of 6 sq. mm size were boiled in methanol in a test tube. Each of these portions was kept on a slide in methanol with lower portion facing upward so that veins were prominent on the lower surface and a small drop of glycerin was added. 5× eye piece and power objective 10× were used. Stage micrometer was focused and camera Lucida was fixed. A black sheet was placed on the side of the microscope where camera Lucida was fixed. Then using a stage micrometer 1 mm square was drawn. Image of the leaf was super-imposed on the square on the black sheet. Vein-islet and vein-termination were traced and counted. The vein-islet and vein-termination, including those intersected by the bottom and left side of the square were included but those intersected by the top and right side were excluded. Six such groups were counted.

Method determination of stomatal number and stomatal index

The leaf was peeled in such a way that both the upper and lower epidermis were separated. The peeled leaf part was taken in a test tube containing about 5 ml of methanol and heated on a water bath until the fragments were rendered transparent. Then the piece was taken on to a watch glass, to this a few drops of ethanol was added and washed with water. It

was then stained with few drops of safranin, washed immediately with water and mounted with the help of glycerin. Microscopy of the stained piece was done by using compound microscope (5x + 10x), equipped with a camera Lucida. The epidermal cells and stomata were drawn on the black sheet and counted within the 1 mm square. The cells and stomata in more than half portion outside the square were not counted.

Method for determination of palisade ratio
Small pieces of leaves of the lamina were taken and boiled in methanol until the leaf pieces became transparent. Then by using camera Lucida, four adjacent cells of upper epidermis were traced. Then focused (×10) on the palisade layer and traced off the palisade cells beneath the four epidermal cells which were already traced. The palisade cells which were 50% more on the inside of the epidermal walls were also traced. Six such determinations were done.

Physicochemical constant
Physicochemical constants, such as the percentage of total ash, water soluble ash, acid-insoluble ash, moisture content (on the basis of dry weight), water and alcohol soluble extractives were determined.[13]

Phytochemical extraction
Extraction was carried out by two methods maceration and soxhlet extraction.

Maceration (Cold extraction) 500 g of the shade dried coarse powder of plant of S. dulcis whole plant was macerated with 1.5 l of 70% ethanol and water separately and was kept at room temperature for 5 days. After 5 days, the solution was filtered and solvent was evaporated under reduced pressure, the residue was weighed and percentage of yield was calculated with reference to air-dried crude powdered materials. The extract (residue) was stored in a refrigerator for further study.

Hot successive extraction (Soxhlet) 500 g of the shade dried coarsely powdered S. dulcis whole plant (500 g) was subjected to continuous hot extraction with 70% ethanol and water individually. The extracts were filtered, dried and percentage of yield was calculated with reference to crude air-dried powder.

Phytochemical screening
Preliminary phytochemical studies were carried out on water and methanolic extracts obtained from both maceration and soxhlet extraction. Various phytoconstituents, namely alkaloids, carbohydrates, glycosides, saponins, proteins, amino acid, phenolic compounds and flavonoids were detected. [14,15]

1, 1-Diphenyl-2-picrylhydrazyl [DPPH] scavenging assay
The assay was carried out in 96 well micro titre plates. The extract (100 µl) and the standard (100 µl) were serially diluted with double distilled water. DPPH (100 mM, 100 µl) solution was added to all the wells except the blank and incubated at room temperature for 20 min. The absorbance was measured at 490 nm using ELISA reader (BIORAD - 550).[14,15]

Results and Discussion
Morphological characteristics revealed, leaf was simple and pinnatified, green color having characteristic odor and sweet taste. Stem was woody, outer surface was rough, odor characteristic, taste sweet, color green and fracture irregular and fibrous. Fruit had smooth outer surface, green color, odor characteristic and taste astringent [Figure1a--f].

Figure 1a-f: Morphology

The leaf of the plant, both upper and lower surface contained anomocytic stomata. It contained unique sessile glandular trichome [Figure 2a--c]. Stem contained starch grain and calcium oxalate crystals in cortex region. Its vascular bundle contains spiral vessel, tracheid, fiber, etc., The epidermis of stem had uniseriate, multicellular, glandular trichomses [Figures 3a--e and and 4a--f].

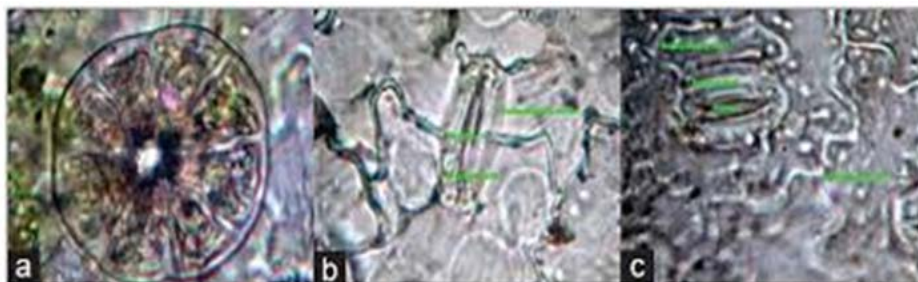

Figure 2a-c: Leaf microscopy

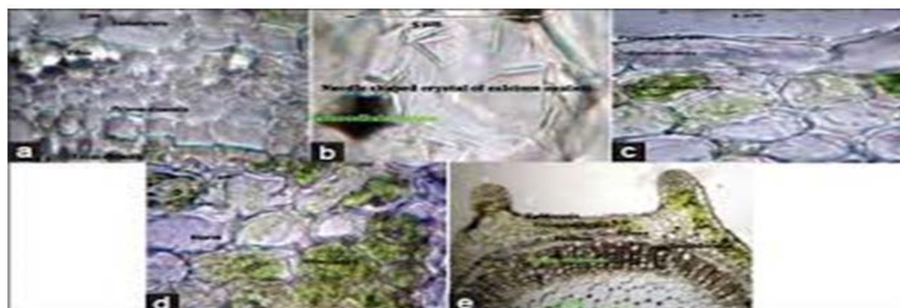

Figure 3a-e: Stem Microscopy

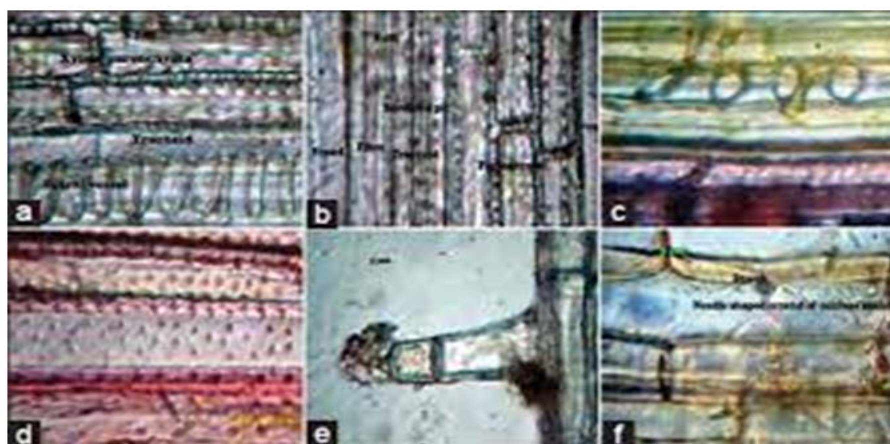

Figure 4a-f: Stem Microscopy

The root showed the presence of periderm containing brownish matter. The phellogen shows two rows of cells encircling the single layer of phelloderm. The cortex consisted of 4-6 layers of tangentially oblong and radially compressed parenchyma cells. Some of the parenchyma cells contained starch grains. The phloem consisted of sieve elements and companion cells. The xylem was lignified and consists of xylem elements such as parenchyma and trachieds. Cambium separates the xylem and phloem region. Medullary rays in the phloem region are non-lignified whereas lignified in the xylem region [Figures 5a-d and 6a-i].

Figure 5a-d: Root Microscopy

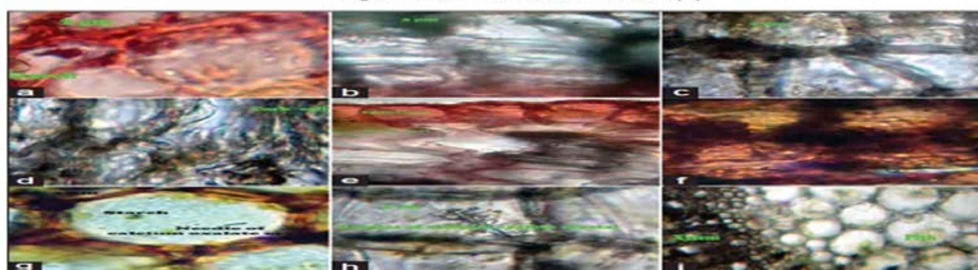

Figure 6 a-i: Root Microscopy

The organoleptic study indicates the external characters like colour, odour and taste. The results of the present study are indicated in Table 1.

Table 1: Organoleptic study of the sample of root and shoot

S.No.	Sample	Colour	Odour	Taste
1	Root	Yellowish green	Pleasant	Bitter
2	Shoot	Light green	Pleasant	Bitter

Leaf constants like, vein-islet number, veinlet termination number, stomatal number, number of epidermal cells, stomatal index, and palisade ratio were monitored and tabulated [Table 1]. The finding showed range of vein-islet number was between 11.5 and 14.6, ranges of Veinlet termination number was between 16.1 and 20.4. The ranges of stomatal number was between 76 and 79 (upper surface) and 82-86 (lower surface), range of epidermal cells were between 477 and 532 (upper surface) and 435-491 (lower surface), stomatal index was 12.5 ± 2 (upper surface) and 14.3 ± 2 (lower surface) and ranges of palisade cells was between 6 and 9 [Table 2].

Table 2: Leaf Constant of *S. dulcis*

Constants	Ranges
Vein-islet no.	11.5-14.6
Veinlet termination no.	16.1-20.4
Stomatal no.	76-79 (upper surface) 82-86 (lower surface)
Number of epidermal cell	477-532 (upper surface) 435-491(lower surface)
Stomatal index	12.5 ± 2 (upper surface) 14.3 ± 2 (lower surface)
Palisade ratio	6-9

The extractive value result had minor differences according to the extraction processes employed. 70% ethanolic extract was found to be higher in maceration and Soxhlet apparatus extraction in comparison to water extracts [Table 3].

The plant showed total ash 21.3%, water soluble ash 14.3% and acid insoluble ash 15.2%. Its moisture content on dry weight basis was found to be 13.4% [Table 3].

Table 3: Physical Constant & Extractive value of *Scoparia dulcis* L.

Pharmacognostical analysis	Percentage w/w yeild
Extractive value	
Maceration	
Water soluble	11.3
Alcohol soluble	12.3
Soxhlet extraction	
Water soluble	15.3
Alcohol soluble	13.3
Ash value	
Total ash	21.3
Water soluble	14.3
Acid insoluble	5.2
Moisture contents	13.4
Foreign Organic Matter	1.2

Preliminary phytochemical study found all the tested phytoconstituents in both extracts. The saponin test was found more prominent in water extract than in ethanolic extract. Phytochemical test suggested that *S. dulcis* has prominent phenolic and carbohydrate content as compared to other tested phytoconstituents [Table 4].

Table 4: Preliminary phytochemical tests for Scoparia dulcis

	Flavonoids	Phenolics	Steroids	Alkaloids	Glycosides
Ethanolic extract (Maceration)	+	+	-	+	+
Ethanolic extract (Soxhlet Extraction)	+	+	+	+	+
Aqueous extract(Maceration)	+	+	+	+	+
Aqueous extract(Soxhlet Extraction)	+	-	-	+	-

1,1- Diphenyl-2- Picryl Hydrazyl (DPPH) radical scavenging assay

The results of DPPH assay of ethanolic and aqueous extract are given in. Among the ethanolic extract showed an highest DPPH free radical scavenging activity (IC_{50} 11.3 ± 3.1 µg /ml), followed by ethanolic extract (Maceration) (IC_{50} 67.4 ± 6.0 µg /ml), ethanolic extract (Soxhlet) (IC_{50} 47.9 ± 2.9 µg /ml), aqueous extract (Maceration) (IC_{50} 132.4 ± 0.7 µg /ml), aqueous extract (soxhlet) (IC_{50} 116.3 ± 0.2 µg /ml), fraction 3_8 (IC_{50} 76.3 ± 0.3 µg /ml). The standard vitamin C showed an IC_{50} value of 31.2 ± 0.4 µg/ml [Table 5].

Table 5: *In vitro* antioxidant activity of *S. dulcis* extracts

Name of the sample	IC_{50} in µl
	DPPH assay
Ethanol Extract (Maceration)	67.4 ± 6.0
Ethanol Extract (Soxhlet)	47.9 ± 2.9
Aqueous (Maceration)	132.4 ± 0.7
Aqueous (Soxhlet)	116.3 ± 0.2
Vitamin C	31.2 ± 0.4

Values are mean ± SD, n=3.

Note: In the DPPH and NO scavenging assays, the IC_{50} refers to concentration of the test sample required to scavenge 50% of DPPH or NO radicals. In reducing power assay the IC_{50} refers to concentration of the test sample required to increase the reducing power by 50%.

Conclusion

The macroscopy, microscopy, and study of physical constants of the plant *S. dulcis* Linn. were performed. The pharmacognostical investigations provide this authentic data to evaluate this herb for its standardization and authentication in future. The *in vitro* antioxidant activity study of these extracts confirmed that, only 70% ethanolic extracts (maceration & soxhlet) DPPH scavenging potential. Among these extracts showing DPPH scavenging activity, ethanolic extract shows the highest potency.

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