ORIGINAL ARTICLE

Pharmacognostical and physicochemical evaluation of Agasti leaf

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ABSTRACT

Sesbania grandiflora (L.) Pers., commonly known as Agasti, is widely used in Ayurveda for the treatment of diseases and for processing of various formulations in *Rasashastra*. It is used for its astringent, antihistaminic, anxiolytic, anticonvulsive and febrifugal activities. Moreover, because of its edible nature, the leaves and pods are used as flavoring items in the cuisine of South India. A detailed investigation of fresh and powder of leaves of Agasti was carried out. The diagnostic characters of this plant include stomatal characters, presence of resins, oil globules, appressed epidermal hair and mucilage cells. Physicochemical studies revealed loss on drying (0.6%), total ash (10.75%), acid insoluble ash (0.045%), alcohol-soluble extractive (21.7%), and water-soluble extractive (30.72%). Preliminary analysis for the presence of various functional groups revealed the presence of alkaloids, saponins, phenols and proteins. Thin layer chromatographic study of the alcoholic extract showed the presence of five, six and seven spots in short UV, long UV and after spraying developing reagent, respectively. The information generated by this particular study will provide relevant pharmacognostical and physicochemical data needed for proper identification and authentication of leaves of this particular species.

Key words: Pharmacognostical study, physicochemical study, thin layer chromatography

INTRODUCTION

Sesbania grandiflora (L.) Pers. (Fabaceae), known as Agasti or swamp pea, is an important medicinal plant and native to many Asian countries including India. It is a small, erect, quick-growing, short-lived and soft-wooded tree which grows to a height of 10 m. It has a diameter of 25 cm, is sparsely branched, has straight cylindrical stem with white soft wood. The bark is light gray, corky, and deeply furrowed. The leaves are pinnate, 15–30 cm long, with 16–30 pairs of linear oblong leaflets. Racemes are 2.5 cm long with two to four white to pink, pendulous flowers. The corolla is 7–9 cm long and pods are 50–60 cm long.[1] The bark, leaves, gums, and flowers are considered medicinal. They are used as diuretic, emetic, emmenagogue, febrifuge, laxative, and tonic.[2] It is also used for treating nyctalopia[3] and a variety of refractive ocular disorders. Extracts of various parts show anxiolytic, anticonvulsive, cytoprotective and hemolytic effects.[5] In *Rasashastra*, it is used for processing of various formulations. The tender leaves, green fruit, and flowers are eaten alone as a vegetable or mixed into curries or salads in various parts of South Asia.[6] As it is a fast growing tree, it combines well with agriculture (agroforestry) in areas where trees are not normally grown and becomes an important fuel wood source. Tender portions serve as cattle fodder.[7] Pharmacognostical evaluation of leaves, which are the most important useful part, is not available in literature. Therefore, a detailed investigation of fresh as well as powder of leaves of Agasti was carried out using pharmacognostical and physicochemical parameters.

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MATERIAL AND METHODS

Fresh leaves of Agasti (bearing pink inflorescence) were collected in the month of January from the botanical garden of the Institute of Ayurvedic Pharmaceutical Sciences, Jamnagar.

Pharmacognostical evaluation of fresh drug including histochemical studies were carried out by taking free hand sections.[8] Powder microscopy of shade-dried powder was carried out. Photomicrographs were taken using Swift Ives Camera Lucida. Leaf constants and quantitative microscopic methods were carried out using ocular and stage micrometer, C Baker, London, and Abbe's drawing apparatus.[9] Physicochemical constants,[10] organic analysis, fluorescence studies and thin layer chromatography (TLC) were carried out from shade-dried powder. Voucher herbarium specimen along with voucher crude drug sample is preserved in the Pharmacognosy Lab, IPGT and RA, Gujarat Ayurved University, Jamnagar. Botanical identification was carried out by using various floras.[11]

Results

Macroscopic characters

Leaf is compound and pari-pinnate with an average length of 15–25 cm. It is narrow with numerous leaflets which are opposite in arrangement. Single leaflet is 2–4 cm long and 10–15 mm in breadth, linear, oblong, mucronate, deciduous, stipulus lanceolate or setaceous deciduous. On an average, in a mature compound leaf, there are 20–30 paired leaflets [Figures 1 and 2].

Microscopic characters

Surface preparation

The stomata are anisocytic with three subsidiary cells around the guard cells in the lower epidermis, whereas both anisocytic and anomocytic stomata are seen in the upper epidermis. The trichome covering is unicellular with a conical bulbous base, thick walled, appressed to the epidermis [Figures 3 and 4]. The upper epidermis shows single-layered, barrel shaped cells, whereas lower epidermis shows somewhat barrel to oval shaped cells. Polygonal, thin-walled parenchymatous cells were seen on powder microscopy [Figures 5 and 6]. On

Figure 1: Compound leaf with pink inflorescence

Figure 2: Dimensions of fresh leaf (4 × 1.5 cm)

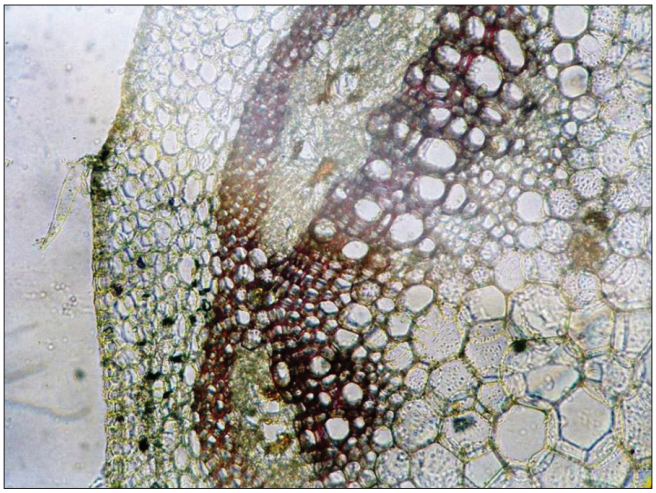

Figure 3: Petiole showing vascular bundles and resin and mucilage containing cells with presence of appressed trichome

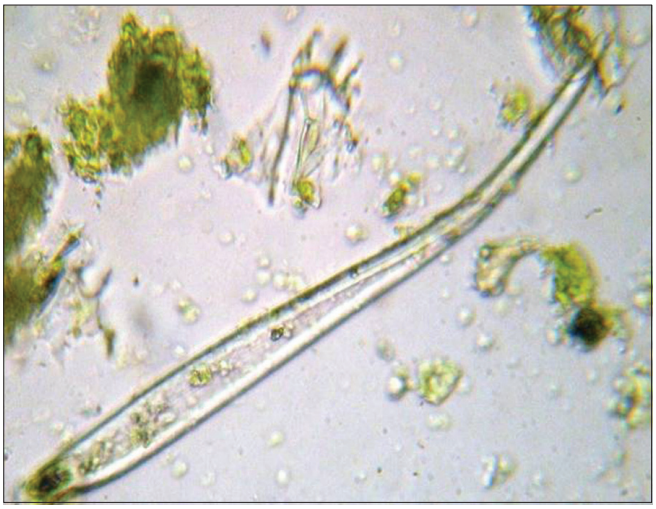

Figure 4: Powder microscopy showing conical based appressed trichomes

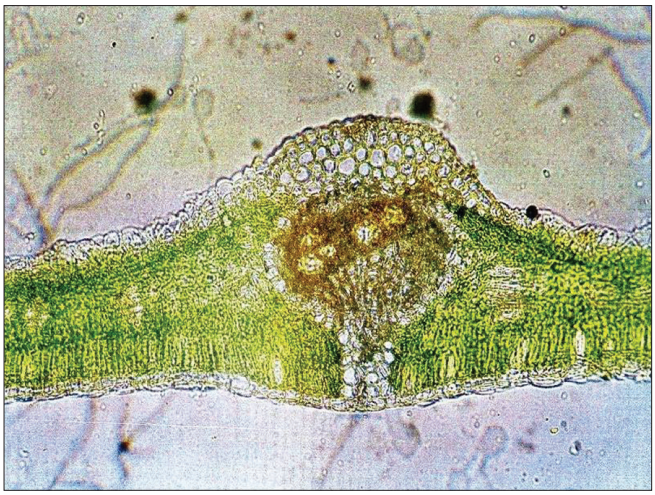

Figure 5: TS of leaflet showing upper and lower epidermis along with palisade cells

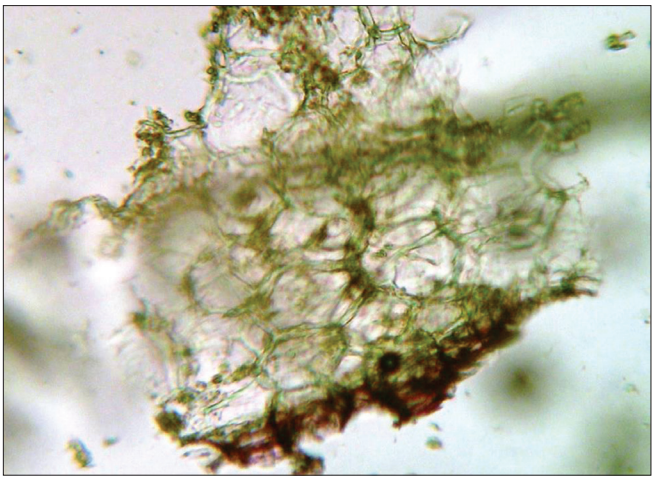

Figure 6: Powder microscopy showing epidermal cells

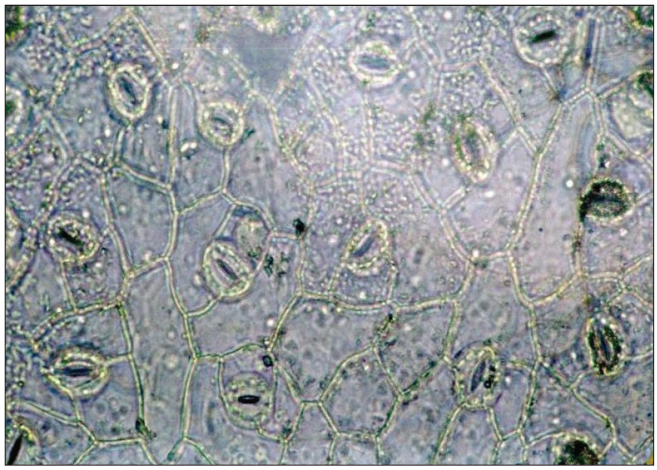

Figure 7: Lower epidermis showing anisocytic stomata (20×)

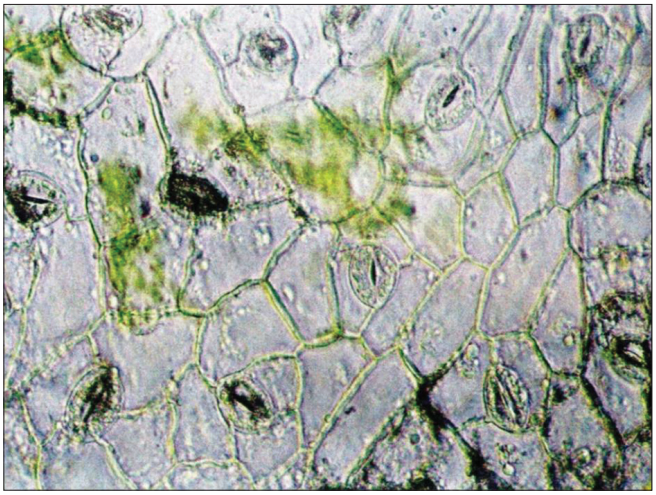

Figure 8: Upper epidermis showing anisocytic stomata (20×)

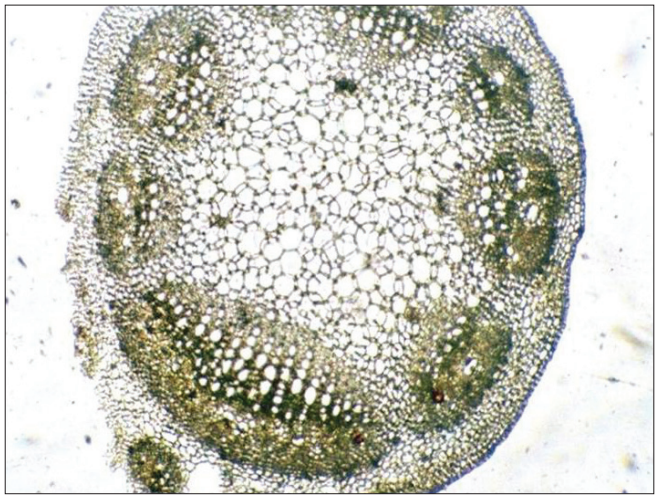

Figure 9: Main rachis ground plan

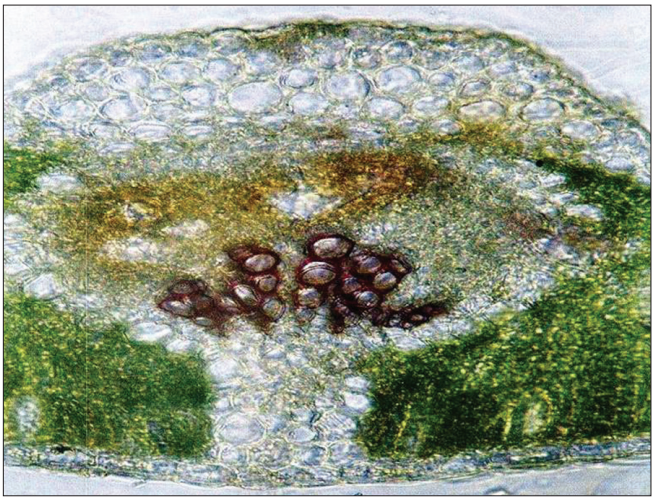

Figure 10: TS of leaflet through midrib along with vascular bundles

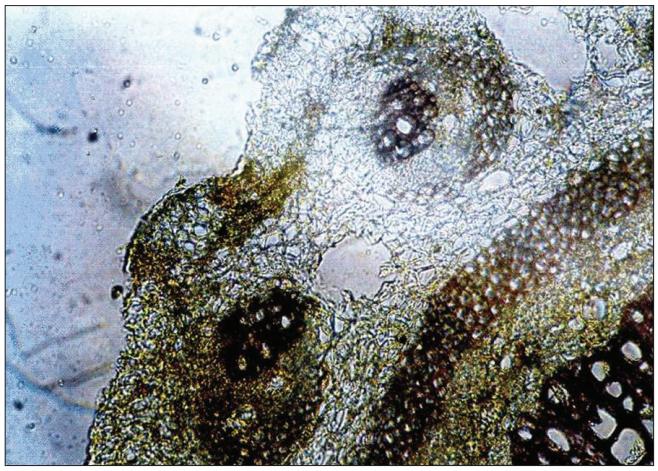

Figure 11: TS of petiole with extravascular bundles (20×)

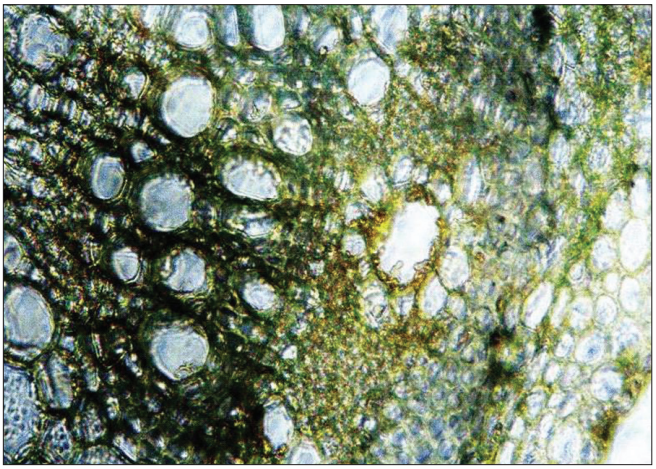

Figure 12: TS showing the presence of mucilage cells

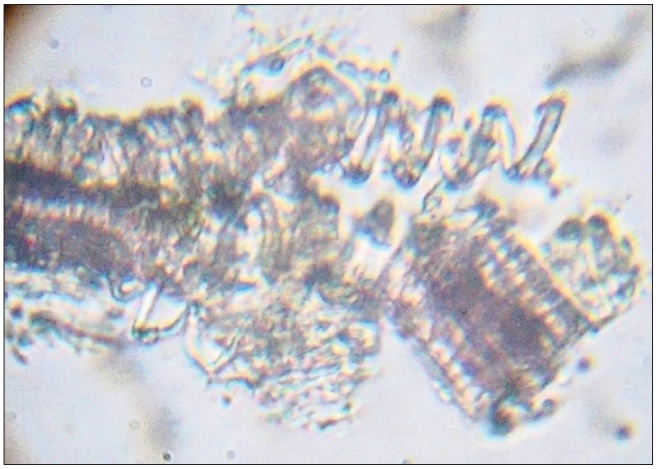

Figure 13: Powder microscopy of leaf showing the presence of spiral vessels

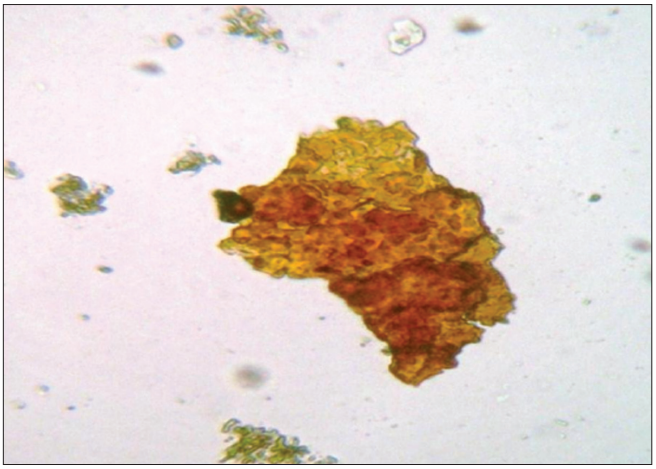

Figure 14: Powder microscopy showing tannin contents

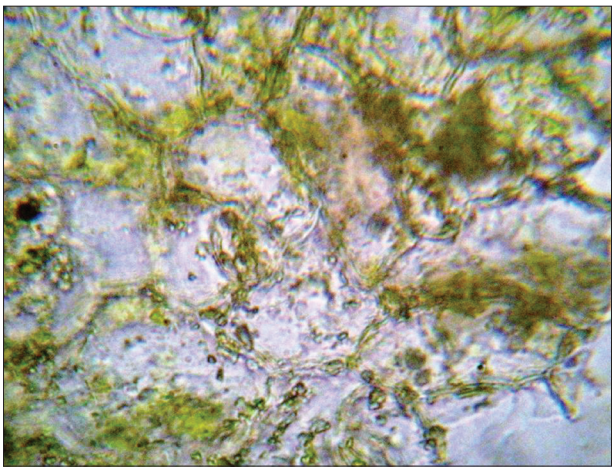

Figure 15: Powder microscopy showing the presence of epidermal cells with chloroplasts

an average, the vein islet number is 20–23, stomatal index is 15–20 and palisade ratio is 7–8 in upper epidermis and 5–6 in lower epidermis.

Table 1: Organoleptic characters of powder of *Sesbania grandiflora*

Character	Observation
Color	Light green
Texture	Coarse
Taste	Bitter (Tikta) with mucilage
Smell	Characteristic

Transverse section of leaflet

The upper epidermis has a thick cuticle with single-layered, barrel shaped cells. Lower epidermis is also single layered with stomata and compactly arranged with unicellular simple epidermal hairs [Figures 3, 5, 7–9]. The mesophyll is differentiated into palisade and spongy parenchyma. Palisade cells are seen below the upper epidermis and are formed into two layers [Figures 5 and 10]. The cells are compactly arranged, long and tubular with chloroplasts. Spongy parenchyma forms rest of the tissue, with rounded cells, which vary in shape and are loosely arranged and enclosing small air spaces with

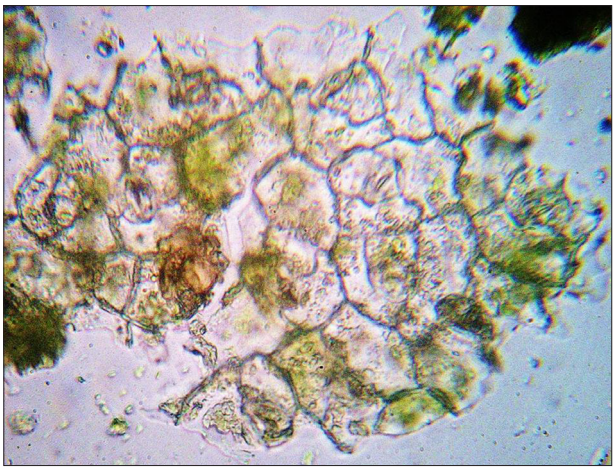

Figure 16: Powder microscopy showing resin cells and anisocytic stomata

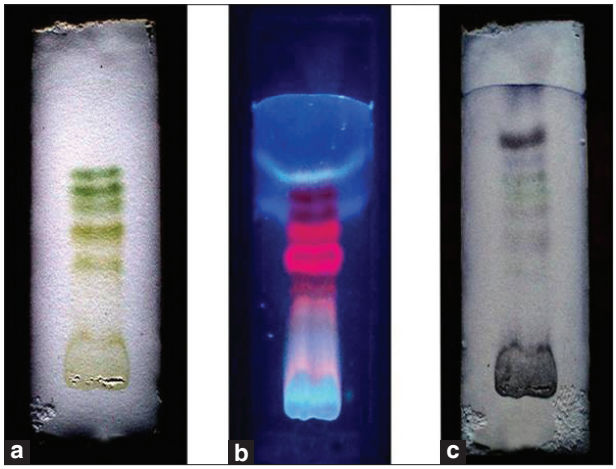

Figure 17: Methanol soluble extractive chromatograms (a) Short UV, (b) Long UV (c) After spraying anisaldehyde in H_2SO_4

Table 2: Ultraviolet analysis of leaf powder of *Sesbania grandiflora*

Treatment	Visible light	UV light	
		Short wave (254 nm)	Long wave (365 nm)
Powder as such	Light green	Mild dark green	Gray
In methanol	Fluorescent green	Fluorescent green	Dark reddish orange
In methanol and NaOH	Dark green	Blackish dark green	Blackish green
In ethanol	Green	Dark green	Orange
In ethanol and NaOH	Dark green	Dark green	Dark greenish orange
In dilute HCl	Yellowish green	Parrot green with yellowish tinge	Grayish green

Table 3: Preliminary qualitative analysis of *Sesbania grandiflora* leaf powder for the presence of various functional groups

Material	Reagent	Functional groups	Observation	Result
Alcoholic extract of powder of dried leaves	Dragendorff's reagent	Alkaloids	Brown ppt.	Present
	Neutral $FeCl_3$	Phenols	Violet color	Present
	Dil. $FeCl_3$	Tannins	Blue color	Present
	Cold conc. H_2SO_4	Cynogenic glycosides	No color change	Absent
	Biuret reagent	Proteins	Violet color	Present
	Benedict's reagent	Carbohydrates	Yellow ppt.	Present
	Shaking in test-tube	Saponins	Frothing with honeycomb appearance	Present

Table 4: TLC analysis of methanolic extract of *Sesbania grandiflora* leaf powder

Conditions	Methanol extract	
	No. of spots	R_f value
Short UV (254 nm)	5	0.52, 0.61, 0.65, 0.70, 0.78
Long UV (366 nm)	6	0.46, 0.52, 0.61, 0.65, 0.70, 0.78
After spraying anisaldehyde in H_2SO_4	7	0.46, 0.56, 0.67, 0.70, 0.78, 0.83, 0.93

numerous chloroplast secretory cavities containing oil and mucilage cells [Figures 11 and 12].

The vascular tissue consists of one large vascular bundle in the midrib. Each vascular bundle is conjoint, collateral, closed and surrounded by parenchymatous bundle sheath that extends toward both lower and upper epidermis. Metaxylem is situated toward lower epidermis and protoxylem toward upper epidermis. The phloem of the vascular bundle is directed toward lower epidermis. Parenchymatous bundle sheath consists of resin cells and tannin containing cells [Figures 3 and 9].

Powder microscopy

Organoleptic characters are shown in Table 1. Anisocytic stomata, simple epidermal hairs, dark yellowish brown tannin fragments, light yellowish resinoids, oil globules, mucilage cells, spiral vessels and epidermal cells were observed under the microscope [Figures 6, 13–16].

The moisture content[12] was 0.6%, total ash[13] 10.75%, acid insoluble ash[14] 0.045%, alcohol-soluble extractive[15] 21.7%, while the water-soluble extractive[16] was found to be 30.72%. Fluorescence studies of shade-dried powdered drug in different media were carried out under visible light and UV light of long and short wavelengths.[17] When the powdered drug treated with different reagents was observed under UV and ordinary light, it emitted various color radiations [Table 2] which helped in identifying the drug in powder form. Physicochemical analysis for the presence of various functional groups was carried out on the methanol soluble extractive.[18] The results are shown in Table 3.

The same extract was examined by TLC using the solvent system of Toluene:Ethyl acetate in a ratio of 8:2. The developed plate was observed under 254 and 366 nm. Anisaldehyde in H_2SO_4 vapors was used as derivatization for visualization and the developed plate was incubated at 105°C and observed under daylight[19] [Figure 17]. The observations are tabulated in Table 4. Phytochemical and chromatographic studies revealed that the plant essentially contains saponins, tannins and phenols.

Discussion and Conclusion

Pharmacognostical evaluation of *S. grandiflora* (L.) Pers. leaves provided specific parameters that will be useful in scientific evaluation, identification and authentication of the drug. Stomatal characters, presence of resins, oil globules, appressed epidermal hairs and mucilage cells are important demarcating characters.

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