Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

J P P Journal of Pharmacognosy and Phytochemistry

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2017; 6(4): 965-968
Received: 20-05-2017
Accepted: 21-06-2017

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Pharmacognostic and phytochemical studies on *Ferula sumbul* Hook. Roots

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Abstract
Ferula sumbul Hook. (Syn. *Ferula moschata* Reinsch.) commonly called Sumbul (Hindi) or Musk root (English), has been traditionally used for relieving anxiety, as a sedative in hysteria and other nervous disorders. Various studies were carried out on *F. sumbul* roots to evaluate the, macroscopic, microscopic, physico-chemical characteristics and thin layer chromatography (TLC) fingerprint profile using standard procedures. Roots were observed to be cylindrical, hairy with distinct odor. Microscopic evaluation revealed prominent cork cells, parenchymatous cells, vessels, prismatic calcium oxalate crystals and starch grains. Quantitatively, mean size of vessels, starch grains and calcium oxalate crystals were determined. Further, phytochemical screening of various extracts of the roots showed the presence of triterpenoids, flavonoids, coumarins, phenols, alkaloids, proteins and carbohydrates. The study was extended to develop fingerprint thin layer chromatogram. The data compiled herein is valuable for correct identification of *F. sumbul* roots.

Keywords: *Ferula sumbul*, microscopy, physico-chemical, thin layer chromatography, phytoconstituents

1. Introduction
Plants have been the backbone of the traditional system of medicine since time immemorial. Ancient literature has reported that many plants are therapeutically active and influence biological functions which may either be preventive or curative. Authenticated information is required for plants to be used as drugs. The most preliminary and essential step in developing a plant as drug is pharmacognostic information. This forms the scientific basis for correct identification and standardization of the crude plant drug based on macroscopic/microscopic as well as physico-chemical characteristics [1, 2].

The genus *Ferula* (Umbellifereae) has around 130 species widely distributed from Mediterranean region to Central Asia. *Ferula sumbul* (Syn. *Ferula moschata*) is one of the unexplored plants of this genus with only sporadic literature reports. Roots of *F. sumbul* have been traditionally used to relieve anxiety, as a sedative in hysteria, other nervous disorders and as a mild gastro-intestinal stimulant [3, 4]. *F. sumbul* roots, have been used in various disorders, and are different from the other species of the genus, which are usually resinous. Hence, the correct identification of the plant drug is essential to distinguish it from other similar rhizome drugs [5]. Present investigation was planned to study the pharmaognostic as well as phytochemical characters of *F. sumbul* roots.

2. Materials and methods
2.1 Collection and authentication of plant
Dried roots of *F. sumbul* were procured from RYM exports, Mumbai, India. These were authenticated by NISCAIR, New Delhi vide voucher specimen number NISCAIR/RHMD/Consult/2014/ 2483-62-2 dated 18/07/2014.

2.2 Macroscopic characters
Macroscopic characters included noting the shape, size, color, odor, taste and texture of *F. sumbul* roots.

2.3 Microscopic studies
2.3.1 Qualitative evaluation
Powdered drug, cleared in chloral hydrate and mounted in glycerin, was observed under a compound microscope. Various characteristic features of the drug were studied using photomicrographs. Photographs were taken with Nikon Labphoto 2 microscopic unit. Bright field was used for normal observations.

2.3.2 Quantitative evaluation [6]
Slides were prepared using the same procedure as for qualitative analysis. Eye piece was calibrated using stage micrometer. Using the calibrated eye piece, mean diameter of vessels, starch grains, and mean width of prismatic calcium oxalate crystals were determined based on 20 observations each.

2.4 Physico-chemical parameters
For physico-chemical evaluation, loss on drying, ash values viz, total ash, acid insoluble ash, alcohol and water soluble extractive values were determined in triplicate [7].

2.4.1 Loss on drying
Accurately weighed powdered plant drug (2 g) was placed in weighing bottle, and dried in oven at 105 ºC. Weight was checked at regular intervals until a constant weight was noted.

2.4.2 Determination of total ash
Powdered plant material (2 g) was taken in a tared silica crucible and incinerated at a temperature not exceeding 450 ºC until free from carbon. The resultant ash was cooled, weighed and the percentage yield was calculated.

2.4.3 Determination of acid insoluble ash value
The total ash obtained from 2 g of powdered plant material was boiled (5 min) with 25 ml of dilute hydrochloric acid, and the insoluble matter was collected on an ashless filter paper. It was washed with hot water, ignited and weighed. The percentage of acid insoluble ash was calculated with reference to drug.

2.4.4 Determination of alcohol soluble extractive value
Accurately weighed powder (5 g) of the plant material was taken and macerated with 100 ml of 95 % alcohol for 24 h. The contents were frequently shaken during first 6 h and allowed to remain. After 24 h, the extract was filtered. Filtrate (25 ml) was evaporated and dried to constant weight at 105 ºC.

2.4.5 Determination of water soluble extractive value
Water soluble extractive value was determined using the procedure described for alcohol soluble extractive, except that chloroform-water was used for maceration.

2.5 Phytochemical screening
Coarsely powdered *F. sumbul* roots (100 g) were successively Soxhlet extracted with petroleum ether, chloroform, and methanol. The marc was air dried and water extract was obtained by boiling with distilled water (1000 ml) for 2 h, filtered, concentrated and dried in an oven at 40-50 ºC. All the four extracts were screened for different classes of phytoconstituents [8].

2.6 Qualitative TLC fingerprinting
F. sumbul root (2 g) was macerated (30 min) and refluxed (30 min) with ethanol. The extract was concentrated, transferred to 5 ml volumetric flask, and the volume was made upto the mark. Two µl of ethanol extract was loaded on 20x20 cm silica gel G pre-coated aluminium plate, and the plate was developed using toluene: ethylacetate: formic acid (7.5:2.5:0.5). The developed plate was visualized after spraying with anisaldehyde-sulphuric acid reagent.

3. Results and Discussion
3.1 Macroscopic characters
Roots are dark brown in color, cylindrical, 5.5-12.0 cm long and 1.5-3.5 cm in diameter, surrounded with heavy tufts of hair; odor musky and camphoraceous; taste bitter. Fracture was brittle and the fractured surface was rough. Figure 1 shows the roots.

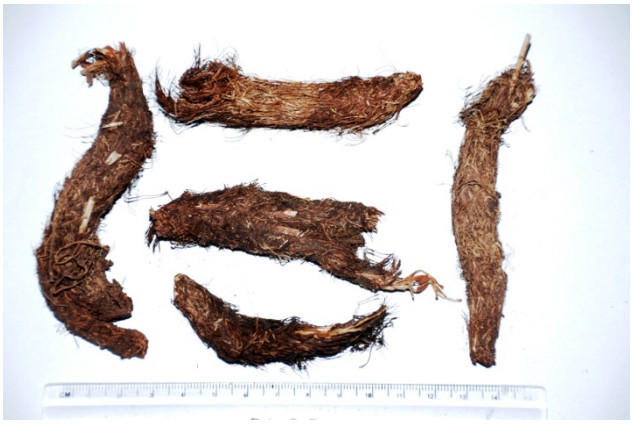
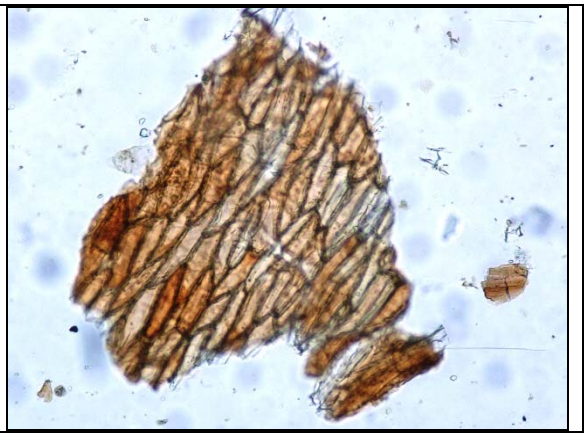
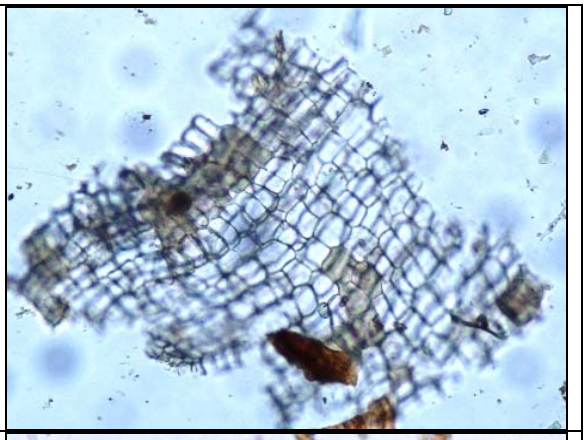
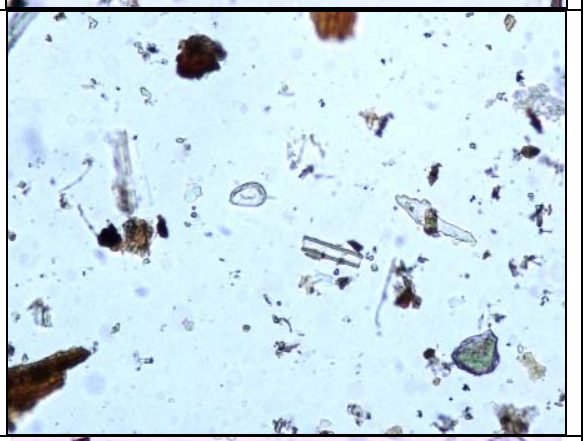
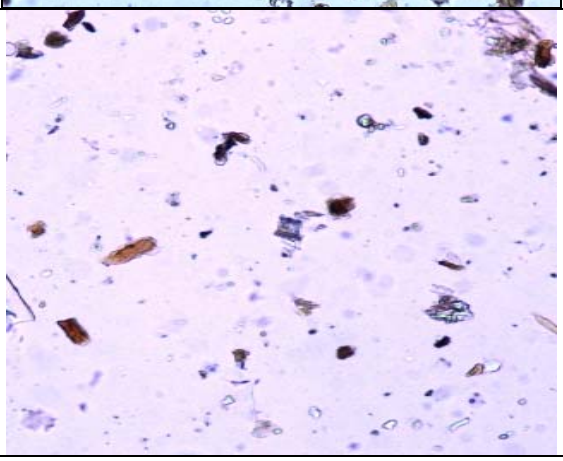


Fig 1: *Ferula sumbul* roots

3.2 Microscopic studies
3.2.1 Qualitative microscopic observations
Table 1 summarizes microscopic characters of *F. sumbul* root powder.

Table 1: Photomicrographs of microscopy of *F. sumbul* roots (100x)

Cork cells Thick layered, reddish brown in color	

Parenchyma cells Thin walled, polygonal, transparent	
Vessels Wide with reticulate thickenings, lignified	
Calcium oxalate crystals Abundant scattered prisms	
Starch grains Abundant, simple and compound.	

3.2.2 Quantitative evaluation

Mean size of vessels, starch grains and calcium oxalate crystals is presented in Table 2.

Table 2: Results of quantitative determinations of characteristic microscopic elements of *F. sumbul* roots

Microscopic element	Size range with mean (µm)
Vessels*	26.66-42.13-64.00
Starch grains*	5.33-6.66-10.66
Calcium oxalate crystals**	10.66-22.66-32.00

*Diameter, **Width

3.3 Physico-chemical parameters

Various parameters viz., loss on drying, ash and extractive values were determined. Mean values are presented in Table 3.

Table 3: Values for various physico-chemical parameters of *F. sumbul* roots

Parameter		Value (%w/w)
Loss on drying		8.01
Extractive value*	alcohol	4.59
	water	7.15
Ash value*	total ash	2.14
	acid insoluble ash	1.04

*Dry weight basis

3.4 Phytochemical screening

All the four extracts of *F. sumbul* roots showed different types of phytoconstituents as presented in Table 4.

Table 4: Results of Phytochemical screening of various extracts of *F. sumbul* roots

Class of phytoconstituents	Pet ether extract	Chloroform extract	Ethanol extract	Water extract
Fatty acids	+	-	-	-
Steroids	+	-	-	-
Triterpenoids	-	+	+	-
Flavonoids	-	+	+	+
Coumarins	-	+	+	+
Phenols	-	-	+	+
Alkaloids	-	+	-	-
Tannins	-	-	+	+
Proteins	-	-	+	+
Carbohydrates	-	-	+	+

+: present, -: absent

3.5 Qualitative TLC fingerprint profile

Thin layer chromatography is one of the most basic, essential and reliable parameters for establishing the identity of a plant drug. Many solvent systems were tried. Optimum resolution was obtained using toluene:ethylacetate:formic acid (7.5:2.5:0.5) on pre-coated plate after spraying with 0.5 % anisaldehyde. Figure 2 shows the fingerprint profile of *F. sumbul* roots.

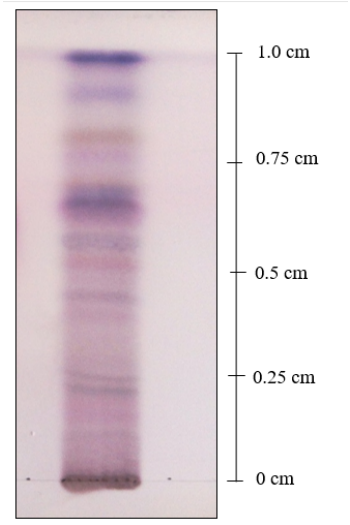


Fig 2: TLC fingerprint profile of *F. sumbul* roots

4. Conclusion

Present investigations on *F. sumbul* roots yielded a definite set of qualitative as well as quantitative parameters. The information generated during the present investigation will be of great use to ascertain the identity and quality of *F. sumbul* roots for future investigations.

5. Acknowledgement

The authors acknowledge University Grants Commission (UGC) for the financial support of this study.

6. References

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