ORIGINAL ARTICLE

Pharmacognostic Standardizations of Traditionally used Hepatoprotective Plant *Fraxinus Micrantha*

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Abstract

Aim: In recent scenario, there is a renewed interest in herbal remedies because they are supposed to be safe and are considered as green medicine. The wrong identification and adulteration of a plant material lead to its ineffectiveness. This problem is solved by evaluation the pharmacognostic parameters of medicinal plants. *Fraxinus micrantha* is one of the ashes of olive family Oleaceae, found in Asia mainly in India and Nepal. It has been traditionally used in the treatment of liver disorders. **Material and Methods:** The present study was designed to evaluate the pharmacognostic parameters and to develop a monograph for the authentication of this plant. Pharmacognostic parameters *of F. micrantha* bark (macroscopy, microscopy, physicochemical parameters, phytochemical screening, and development of thin-layer chromatograms) were evaluated. **Results and Discussion:** Macroscopy showed that the outer surface of bark was grayish brown with patches, inner surface light brown in color, odor is characteristic, having a sweet taste and splintery fracture. Transverse section of the bark under scanning electron microscope showed long vessels with embedded starch grains and a thin long fiber. Clusters of prismatic calcium oxalate crystals, thick-walled cork cells, long thin fiber, and vessels embedded with starch grains were detected in powder microscopy. Physicochemical characters represented total ash 6.34%, acid insoluble ash 1.4%, water-soluble ash 2.2%, ether soluble extractive 0.14%, chloroform soluble extractive 0.36%, alcohol-soluble extractive 1.3%, water-soluble extractive 2.2%, and loss on drying was 5.6%. Preliminary phytochemical screening of extracts (successive extraction) showed the presence of lipids and steroids in hexane extract, alkaloids in chloroform extract. Methanol extract gave positive tests for alkaloids, glycosides, and flavonoids. Aqueous extract showed the presence of carbohydrates. The thin-layer chromatography of *F. micrantha* showed the presence of one spot in hexane extract, seven spots in chloroform extract and also seven spots in methanol extract. These studies are helpful in the authentication of *F. micrantha* and maintaining its therapeutic efficacy. **Conclusion:** All the above parameters can be employed for the authentication and identification of traditionally used medicinal plant *F. micrantha* and will help to minimize the chances of adulteration. To the best of our knowledge, this is the first study of its kind on *F. micrantha,* and therefore, it is of much importance for further research on this plant.

Key words: *Fraxinus micrantha*, pharmacognostic evaluation, scanning electron microscopy, standardization

INTRODUCTION

The demand of herbal drugs is increasing very fast as these drugs have negligible side effects and considered as green medicine. Furthermore, the increased incidences of harmful effects of synthetic medicines to humans and environment emphasize the attention toward herbal medicines. The major advantages of herbal medicines are their availability, economic and have less or no side effects but the major disadvantage is their adulteration.[1] The high effectiveness of herbal drugs increased their demand which further leads to the scarcity of these drugs. Therefore, to meet the increased demand for the drug is easily adulterated. Therapeutic efficacy of herbal plant or

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drug highly depends on its quality and quantity of chemical constituents present.[2] The incorrect identification of a plant material leads to its ineffectiveness, and people start losing their interest in herbal medicine.[3] The most common error in the identification of plant is that a common vernacular name is given to two or more entirely different species.[4] All these problems can be solved by evaluating the pharmacognostic parameters of medicinal plants, and it is very essential fact to lay down pharmacognostic specifications of medicinal plants. Pharmacognostic standardization ensures the identity of plant and parameters which will help to detect adulterants and ensures reproducible quality of herbal products which will lead to safety and efficacy of natural products.[5]

Therefore, taking in view the above deliberations the pharmacognostic standardization of *Fraxinus micrantha*, a traditionally used hepatoprotective plant was carried out.

MATERIALS AND METHODS

Plant material

The dried bark of *F. micrantha* was procured from Sri Venkateswara University, Tripura, and the identity of the plant was confirmed by Dr. K. Madhava Chetty, Assistant professor, Department of Botany, Sri Venkateswara University, Andhra Pradesh. A voucher specimen number 711 has been deposited in the same department's herbarium.

Pharmacognostic Evaluation

Macroscopy

Characteristics such as color and shape of the plant material were observed with the naked eye or with the aid of a magnifying lens. Dried powdered plant material was subjected to organoleptic evaluation such as color, taste, odor, and fracture.

Microscopy

Histology

Histology of transverse section of *F. micrantha* bark was carried out using scanning electron microscope (Zeiss, EVO/LS10). Small sections of stems were cut and placed on stubs of scanning electron microscope and visualized.

Powder microscopy

Dried powdered material was obtained by grinding. The powdered material was placed on a glass slide and cleared with chloral hydrate (Unichem Laboratories) and then mounted in glycerine (Thermo Fisher Scientific India Pvt., Ltd.). Prepared glass slides were observed under the microscope (Magnus Ltd.) for the evaluation of microscopic features of the powdered plant material.[6]

Physicochemical Evaluation

Ash values

Ash mainly represents the inorganic matter present in the plant material. The amount and composition of ash remaining after incineration of plant material vary considerably according to the part of the plant, age, treatment, etc. The constituents of the ash also vary with time and area.[7] Percentage of ash with reference to the air-dried drug was calculated. Acid-insoluble ash and water-soluble ash were calculated with reference to total ash.[6]

Extractive values

The extracts obtained by exhausting crude drugs are indicative of approximate measures of their chemical constituents. Varieties of chemical compounds are available in crude drugs having variable properties. Various solvents are used for extraction of desired chemical compounds from the plant material.[8]

Loss on drying

About 10 g of powdered plant material, without preliminary drying, was placed in a tarred evaporating dish. The plant material was then dried at 105°C for 5 h and then weighed again. This process of drying and weighing was continued at the interval of each 1 h until the difference between two successive weighing corresponds to not more than 0.25%.[6]

Fluorescence analysis

A small quantity of dried and finely powdered *F. micrantha* bark was placed on a clean glass slides and 1–2 drops of freshly prepared reagent solutions were added and mixed by gently tilting the slide. Then, the slides were placed inside the ultraviolet (UV) chamber and viewed in daylight, short (254 nm), and long (365 nm) ultraviolet radiations. Change in color was observed by application of different reagents.[6]

Extraction

The plant material was coarsely powdered and subjected to successive soxhlet extraction using different solvents in increasing order of their polarity, namely petroleum ether, chloroform, methanol, and finally the marc was digested with water to obtain an aqueous extract. Each extract was concentrated using rotary evaporator (IKA, Works INC., North America) and stored at low temperature.[9]

Phytochemical screening of extracts

The four extracts prepared from the powdered *F. micrantha* bark were subjected to preliminary phytochemical screening using standard methods for different classes of phytoconstituents using specific standard reagents.[10,11] The

phytochemical screening helps in identifying the chemical constituents belonging to a particular class as each class has its own pharmacological importance.

Thin-layer chromatographic (TLC) fingerprinting of extracts

Thin-layer chromatography is a method for analyzing mixtures and determining the number of components present in it. It is also used for the identification and to check the purity of a compound. The identification of separated compounds can be achieved on the basis of their retention factor (R_f) values.

RESULTS

Pharmacognostic evaluation

Macroscopic evaluation

Macroscopy of *F. micrantha* bark was carried out and the characteristics observed were briefed in Table 1 and Figure 1.

Microscopy

Histology

Transverse section of the bark was cut down, placed on the stubs and observed under scanning electron microscope and it depicted the characters of the plant showing in Figure 2.

Powder microscopy

Photographs of powder microscopy are shown in Figure 3.

Physical parameters

The air-dried powdered bark of *F. micrantha* was subjected to the study of physical parameters and the results obtained are shown in Table 2.

Fluorescence analysis

Fluorescence analysis of the dried powdered bark of *F. micrantha* was carried out by observing it under the daylight, short UV (254 nm), and long UV (365 nm) in an ultraviolet chamber. The colors observed in different solvents under different ultraviolet radiations are summarized in Table 3.

Percentage yield of different extracts

The percentage yield of each of the foSr extracts, i.e., petroleum ether extract, chloroform extract, methanol extract, and aqueous extract was calculated, and the results obtained are reported in Table 4.

Table 1: Organoleptic features of *F. micrantha* bark

Characters	Dried bark
Color	Outer surface: Grayish brown with patches Inner surface: Light brown
Odor	Characteristic
Taste	Sweet
Fracture	Splintery

F. Micrantha: *Fraxinus Micrantha*

Table 2: Physical parameters of *F. micrantha* bark

Sr. No.	Physical parameters	% Value obtained
1.	Total ash	6.34
2.	Acid-insoluble ash	1.4
3.	Water-soluble ash	2.2
4.	Ether soluble extractive value	0.14
5.	Chloroform soluble extractive value	0.36
6.	Alcohol soluble extractive value	1.3
7.	Water-soluble extractive value	2.2
8.	Loss on drying	5.6

F. micrantha: *Fraxinus micrantha*

Figure 1: *Fraxinus micrantha* bark

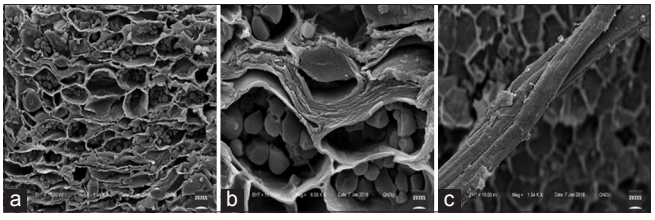

Figure 2: (a) Vessels, (b) starch grains embedded in vessels (c) fiber

Table 3: Results of fluorescence analysis of dried powdered bark of *F. micrantha*

Experiment	Visible/Daylight	UV light (254 nm)	UV light (365 nm)
Powder as such	Brown	Dark brown	Light brown
Powder+50% HNO_3	Orange brown	Black	Dark brown
Powder+1N HCl	Pale yellow	Black	Light brown
Powder+conc. H_2SO_4	Dark brown	Black	Dark brown
Powder+NH_3	Dark brown	Black	Dark brown
Powder+I_2 solution	Dark brown	Black	Dark brown
Powder+40% NaOH	Pale yellow	Black	Pale yellow
Powder+10% $AgNO_3$	Pale yellow	Black	Pale yellow
Powder+5% $FeCl_3$	Pale yellow	Dark purple	Light brown
Powder+10% $MgSO_4$	Pale yellow	Dark purple	Light green
Powder+5% $CaCl_2$	Pale yellow	Black	Light green
Powder+5% $CuSO_4$	Pale yellow	Dark brown	Light green

F. micrantha: *Fraxinus micrantha*, UV: Ultraviolet

Table 4: Percentage yield of different extracts of *F. micrantha* bark

S. No.	Extract	Yield (g)	Color	Physical appearance
1.	Petroleum ether	4.48	Yellowish	Fatty
2.	Chloroform	9.34	Dark green	Semi solid mass
3.	Methanol	13.45	Green	Sticky mass
4.	Aqueous	8.76	Dark brown	Sticky mass

F. micrantha: *Fraxinus micrantha*

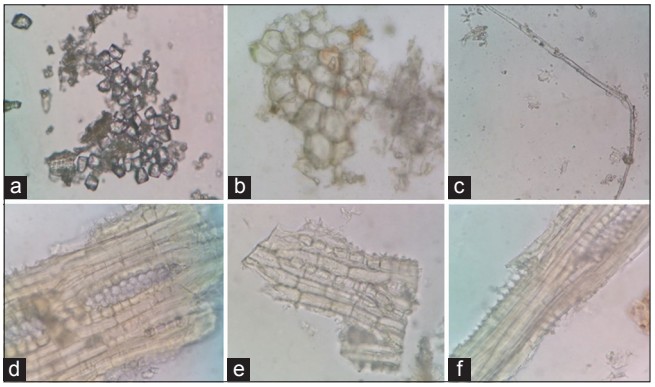

Figure 3: Powder microscopy of *F. micrantha* showing (A) clusters of calcium oxalate crystals (b) cork cells (c) fiber (d) starch grains embedded in vessels (e) epidermal cells (f) vessels

Preliminary phytochemical screening

The different extracts prepared from the powdered bark of *F. micrantha* were subjected to preliminary phytochemical tests to check the presence of a particular class of components present in them. The results of phytochemical screening are compiled in Table 5.

TLC fingerprinting of extract

The different extracts, namely hexane, chloroform, and methanol extracts of *F. micrantha* bark were subjected to thin-layer chromatography to develop the chromatographic pattern of various chemical constituents present in each extract. The chromatogram developed for hexane extract, chloroform extract, and methanol extract is shown in Figure 4 and R_f values are calculated for each spot as given in Table 6.

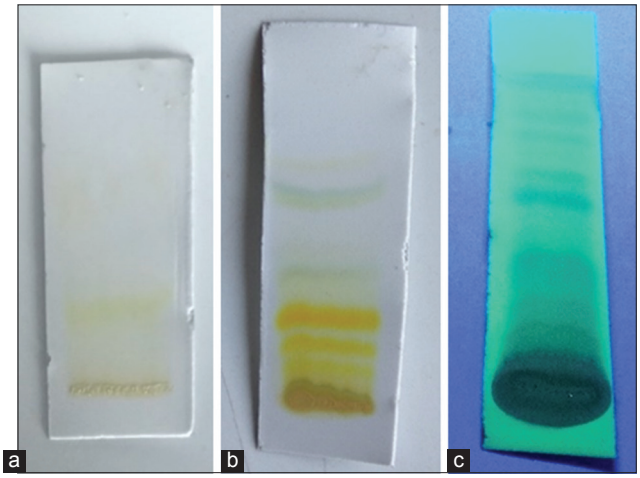

Figure 4: Thin-layer chromatogram of *Fraxinus micrantha* (a) hexane extract (b) chloroform extract (c) methanol extract

DISCUSSION

The quality control and safety profile have become an important issue in industrialized and developing countries due to non-availability and limited data on medicinal plants. Both the consumers and health-care professionals need authoritative information on the safety and efficacy of

Table 5: Results of preliminary phytochemical screening of *F. micrantha* bark extracts

Sr. No.	Phytochemical constituents	Pet ether extract	Chloroform extract	Methanol extract	Aqueous extract
1.	Alkaloids				
	Mayer's test	-	+	+	-
	Hager's test	-	+	+	-
	Wagner's test	-	-	+	-
	Dragendorff's test	-	-	+	
2.	Saponins				
	Frothing test	-	-	-	-
3.	Carbohydrates				
	Molisch's test	-	-	-	+
	Fehling's test	-	-	-	+
4.	Glycosides				
	Borntrager's test	-	-	+	-
	Legal's test	-	-	+	-
5	Flavonoids				
	Alkaline reagent test	-	-	+	-
	Shinoda test	-	-	+	-
6.	Steroids and triterpenoids				
	Liebermann's test	+	-	-	-
	Liebermann-Burchard test	+	-	-	-
7.	Test for lipids				
	Solubility test	+	-	-	-
	Sudan IV test	+	-	-	-

F. micrantha: *Fraxinus micrantha*

Table 6: Results of TLC fingerprinting of extracts

Extract	Mobile phase	No. of spots	R_f value
Hexane extract	Hexane :ethyl acetate 6:4	1	0.35
Chloroform extract	Chloroform :ethyl acetate 7:3	7	0.8, 0.77, 0.57, 0.48, 0.37, 0.25, 0.14
Methanol extract	Hexane :ethyl acetate 5:5	7	0.86, 0.87, 0.68, 0.60, 0.42, 0.29, 0.21

TLC: Thin-layer chromatographic

medicinal plants.[12] Therefore, the WHO emphasized the standardization studies of medicinal plants which help in the preparation of pharmacopeial standards, quality control profile, and herbal monographs.[6]

The present study was carried out to establish the standardization parameters and diagnostic characters of *F. micrantha*. The outer surface of bark was grayish brown with patches while the inner surface was light brown in color, having sweet taste, characteristic odor, and splintery fracture. Scanning electron microscopy showed the presence of vessels, starch grains embedded in vessels and a fiber. Powder microscopy showed the presence of prismatic calcium oxalate crystals, cork cells, thin long fiber, starch grains embedded in vessels, and epidermal cells and vessels.

Physicochemical parameters were carried out for the standardization of plant material. The powdered plant material of *F. micrantha* showed that total ash value was 6.34%, acid insoluble ash was 1.4%, water-soluble ash was 2.2%, ether soluble extractive was 0.14%, chloroform soluble extractive was 0.36%, alcohol-soluble extractive was 1.3%, water-soluble extractive was 2.2%, and loss on drying was 5.6% [Table 3]. Preliminary phytochemical screening of extracts showed the presence of lipids and steroids in hexane extract, alkaloids in chloroform extract. Methanol extract gave positive tests for alkaloids, glycosides, and flavonoids. Aqueous extract showed the presence of carbohydrates. The thin-layer chromatography of *F. micrantha* showed the presence of one spot in hexane extract, seven spots in chloroform extract and also seven spots in methanol extract. Thus, the standardization process can be done by series of stepwise pharmacognostic studies as above. These studies are helpful in the authentication of plant material

and act as a reference for correct identification and to find adulterants in particular plant material and maintaining the efficacy and quality of herbal drugs.

CONCLUSION

All the above parameters can be employed for the authentication and identification of traditionally used medicinal plant *F. micrantha*. This study will also help to minimize the chances of adulteration of this traditionally important medicinal plant. To the best of our knowledge, this is the first study of its kind on *F. micrantha,* and therefore, it is of much importance for further research on this plant.

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