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Research Article

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Phytochemical and pharmacognostical studies of the leaves of M*yxopyrum serratulum* A. W. Hill.

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ABSTRACT

Myxopyrum serratulum (Fam. Oleaceae) is commonly known as 'Chaturmallai' in Tamil and 'Chaturdharalata' in Sanskrit. In Ayurveda the leaves of the plant is used as astringent, acrid, sweet, thermogenic, anodyne, febrifuge and tonic. The whole plant has considerable Ethnobotanical uses in head ache, asthma, cough, fever, nerves, otitis, rheumatism and wounds. In order to ensure the use of genuine and authentic material in the preparation of herbal formulations, pharmacognostical and phytochemical methods of standardization of the plant has been carried out in the present work. Macroscopic, microscopic, powder microscopic and physico-chemical characters of the leaves of M. serratulum has been carried out. Preliminary phytochemical analysis and thin layer chromatographic studies have been performed on the various extracts of the leaves of M. serratulum. All these pharmacognostical and phytochemical studies can be used as a diagnostic tool for the correct identification of the plant and also to test adulteration if any.

Keywords: *Myxopyrum serratulum*, Ethnobotany, Pharmacognosy, Phytochemistry.

INTRODUCTION

In the modern world, people are becoming aware of the potency and side effect of synthetic drugs. There is an increasing interest in the natural remedies with a basic approach towards the nature. In India great deals of in depth knowledge exist among general public about the traditional use of herbal medicine. This is in addition to organized Indian system of medicine, Ayurveda, which has already gained world wide attention. To ensure the safety and efficacy of herbal medicines standardization and development of quality protocols for herbal medicines is extremely important. For the identification of medicinal plants and their constituents, WHO guidelines suggest the fingerprinting methods to meet the global standards of quality control of the herbal formulations [1].

Herbal formulations show the number of problems when quality control aspect is considered. This is because of nature of the herbal ingredients and different secondary metabolites present therein. It is also due to intrinsic and extrinsic factors (growing, harvesting, storage and drying processes) [2-4].

Myxopyrum serratulum (Oleaceae) commonly known as "Chaturamulla" is a large woody climbing shrub. The leaves are astringent, acrid, sweet, thermogenic, anodyne, febrifuge and tonic. They are useful in vitiated conditions of kapha and vata, cough, asthma, rheumatism, cephalalgia, nostalgia, consumption, fever, otopathy, neuropathy and cuts and wounds [5]. Iridoid glycosides were found in a related species study carried out on the leaves of *Myxopyrum smilacifolium* [6].

The present paper deals with the macroscopic and microscopic studies on the plant. Physico-chemical characters *viz*., loss of weight on drying, total ash, acid-insoluble ash, water-soluble ash have been determined. Fluorescence analysis of the leaves powder in various solvents has been carried out. Extractive values in petroleum ether (40°-60°C), benzene, chloroform, ethanol and water have been found out. Thin layer chromatography studies of the various extracts have been performed in different solvent systems and the Rf values have been determined. Preliminary phytochemical screening of the various extracts has been carried out.

EXPERIMENTAL SECTION

Materials and methods

The plant material was collected from Trivandrum District, Kerala in the month of september 2010. The plant was authenticated by Dr.V.Chelladurai, Research Officer (Botany). Central Council of Research in Ayurveda and Siddha, Government Siddha Medical College, Palayamkottai, Tamilnadu, India. A voucher specimen (MSU/PHAR/HER-139) has been preserved in the Herbarium of the Department of Pharmaceutical Chemistry, Manonmaniam Sundaranar University, Thirunelveli – 627 012.

Microscopic studies

Microscopical studies free hand sections of fresh leaves were cut cleared with chloral hydrate solution and water stained with safranin according to the prescribed methods. Photomicrographs were taken by Nikon digital camera [7, 8].

Physico chemical constants

Physico chemical constants such as the percentage of total ash content, acid insoluble ash, water soluble ash, water and alcohol soluble extractives and loss of weight on drying, were calculated based upon standard procedures prescribed in Indian Pharmacoeia [9].

Microscopic characteristics of powder drug

The microscopic characteristics of leaf powder were carried out in order to reveal various diagnostic characters of the leaves. A drop of hydrochloric acid and phloroglucinol was used to detect the lignified cells in the powder drug. Photomicrographs were taken by Nikon digital camera [10].

Fluorescence analysis

The fluorescence analysis of the powdered leaves was done by placing dry powdered leaves on a slide are observed by treating with several drops of different specified reagents. The observation of the developed colour was done with in one minute in order to avoid drying and resultant colour change [11]. For few tests, the sample of powdered drug after being placed on a slide and being treated with several drops of specified reagent was allowed to dry completely. Then the dried specimen was mounted in nitrocellulose allowed to dry and observed under UV lamp [12, 13].

Extraction of Plant Material

The leaves of *M. serratulum* were dried under shade and powdered. The dried powder (500 gm) was successively extracted using petroleum ether (40°- 60°C), benzene, chloroform, ethanol and water by using Soxhlet apparatus. The last trace of solvent was removed under reduced pressure by rotary evaporator. The dried crude extracts were used for the study [14].

Phytochemical Screening

The concentrated extracts were used for preliminary screening of various phytoconstituents *viz*. steroids and terpenoids, alkaloids, tannins and phenolic compounds, flavonoids, sugars and amino acids were detected by usual methods prescribed in standard tests [15, 16].

Thin layer chromatography

Thin layer chromatographic studies of the petroleum ether (40°- 60°c), benzene, chloroform, ethanol and water extracts were carry out in various solvents at 30°C using precoated silicagel G plate as adsorbent [17].

RESULTS AND DISCUSSION

Macroscopic characters

M.serratulum is a large shrub with quadrangular stems having obovate elliptic or elliptic leaves. The plant bears yellow flowers in axillary or terminal clusters and obovoid fruits Fig 1.

Fig 1: Macroscopic characters of *Myxopyrum serratulum* A. W. Hill

Microscopic studies

The microscopic characters of the leaf are presented in fig 2a and fig 2b. Microscopically upper epidermis is single layered with hexagonal structure in out line. The outer walls of which are cuticularished only covering trichomes emerge from the epidermal layer. A few stomata are seen on the upper epidermis. Epidermis is followed by mesocarp, made up of 2 to 5 layers of paranchymatous cells. Mesocarp is followed by continuous band of 3 to 5 layers sclerenchymatous fibres which are ensheated by a layer of parenchyma. In this region, vascular bundles are present and centre portion is occupied by thin-walled compactly arranged paranchymatous cells. The individual cells of which contain calcium oxalate crystals.

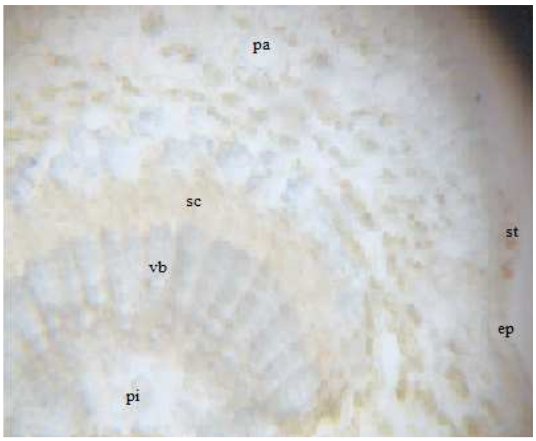

Fig 2a. Epicarp and mesocarp with vascular bundles

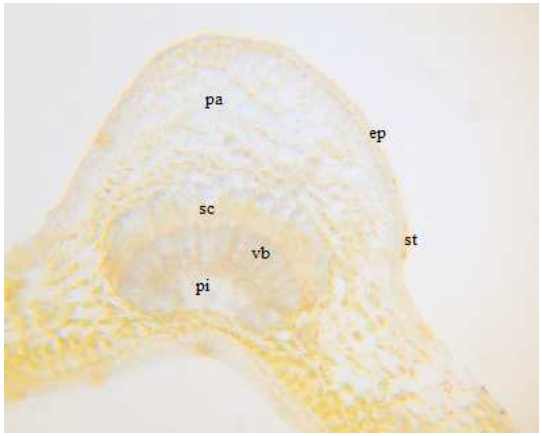

Fig 2b. Mid-rib

Fig 2c. Epicarp and mesocarp

Fig 2a: Microscopic characters the of leaves of *M. serratulum*. (ep) - epidermis; (pa) - paranchyma; (sc) - sclerenchyma; (vb) - vascular bundle; (pi) - pith; (st) - stomata

Powder microscopy

Microscopic characteristics of the leaf are depicted in Fig 3a – fig 3f. Microscopical characteristics of powdered drug reveals the presences of calcium oxalate, trichomes, epidermal cells, parenchyma, sclerenchyma and stomata.

Fig 3a. Calcium oxalate

Fig 3b. Epidermis

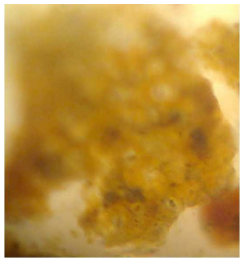

Fig 3c. Stomata

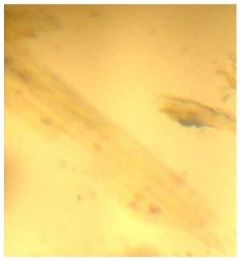

Fig 3d. Sclerenchyma

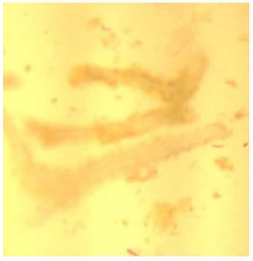

Fig 3e. Fibers

Fig 3f. Trichomes

Physicochemical constants determination

The physic-chemical characters are presented in Table 1. The observed loss of weight on drying for *M. serratulum* A.W. Hill leaves is 83% w/w. The total ash content value and water soluble ash value of powdered *M. serratulum* leaves are found to be more in crude drug. Ash value is a measure of the quality and purity of the crude drug. Alcohol and water soluble extractive values were determined to find out the amount of water and alcohol soluble compounds. The leaves showed more amounts of water soluble compounds than alcohol soluble compounds.

Fluorescence analysis

The results of fluorescence analysis are presented in Table 2. The colour emitted by the powdered leaves in the above tests were designated in terms of the two primary colours (red, yellow) three secondary colours (orange,green,purple) and one brown colour, which is mixture of three primary colours.

Phytochemical screening

Successive extraction of the leaves powder was carried out and ethanol extract was found to give a maximum extractive value (3.75%). In the phytochemical tests the petroleum ether, benzene, chloroform extract revealed the presence of alkaloids, triterpenoids, steroids. Ethanolic and aqueous extracts, showed the presence of carbohydrates, amino acids, proteins, tannins, saponins and steroids. The extractive values and results of individual tests for various phytoconstituents are presented in Table 3.

Thin layer chromatograpy

The best separation was achieved using benzene : chloroform (7:3), chloroform : ethanol (8:2) and Butanol :Acetic acid :Water (5:1:4) as mobile phase. After developing the plates were viewed under U-V light and in iodine chamber to locate the spots. The Rf values were calculated and presented in Table 4.

Table 1: Physico-chemical characters of the leaf powder of *Myxopyrum serratulum*

S. No.	Parameter	Values (%)
1.	Percentage of Foreign matter	< 2
2.	Loss of weight on drying	83
3.	Total ash content	11.1
4.	Water soluble ash	1.5
5.	Acid insoluble ash	1.0
6.	Extractive values Petroleum ether (40°- 60°c) Chloroform Benzene Ethanol Water	 0.75 1.00 2.50 3.75 5.00

Table 2: Fluorescence analysis of the leaf powder of the *Myxopyrum serratulum* its extracts

S. No.	Treatment	Ordinary light	Ultra-violet light
1.	Powder as such	Greenish-brown	Brown
2.	P+50% HCL	Greenish-brown	Greenish-yellow
3.	P+50% H_2SO_4	Dull Green	Green
4.	P+ con H_2SO_4	Brown	Dark brown
5.	P+ con HCL	Green	Yellowish-Green
6.	P+ con HNO_3	Reddish-brown	Yellow (fluorescence)
7.	P+ 50% HNO_3	Dull green	Brown
8.	P+1N NaOH in MeOH	Olive green	Yellowish-green
9.	P+1N NaOH	Brownish-yellow	Yellowish-green
10.	P+ H_2O	Yellowish-green	Green
11.	P+ KOH soln	Greenish-yellow	Green
12.	P+ $FeCl_3$	Dark green	Green
13.	P+ Acetic acid	Brown	Green
14.	P+ Iodine soln	Greenish-yellow	Dull green
15.	Extracts		
	Petroleum ether (40°- 60°C)	Dark green	Green
	Benzene	Green	Yellowish-green
	Chloroform	Green	Green
	Ethanol	Brown	Reddish-brown
	Water	Dark brown	Brown

Table 3: Preliminary phytochemical screening of the various extracts of the leaves of *Myxopyrum serratulum*

S. No.	Compound	Extracts				
		Petroleum ether (40-60)	Benzene	Chloroform	Ethanol	Water
1.	Alkaloids	-	-	-	-	+
2.	Triterpenoids	+	-	+	+	-
3.	Steroids	-	-	+	+	-
4.	Flavonoids	-	-	-	+	+
5.	Phenolic compounds	-	-	-	+	+
6.	Anthraquinones	-	+	-	-	-
7.	Saponins	-	-	-	+	+
8.	Tannins	-	-	-	-	-
9.	Reducing sugars	-	-	-	-	+
10.	Xantho proteins	-	-	-	-	+
11.	Amino acid	-	-	-	-	+
12.	Glycosides	-	-	+	+	-

Note : *'+' indicates presence '-' indicates absence*

Table 4: Thin Layer Chromatography of the leaves of various extracts of the *Myxopyrum serratulum*

S. No.	Extracts	Solvent system used	R_f values of the spots	
			In Iodine chamber	Under UV light (365 nm)
1.	Pet. ether (40-60^{o}C)	Hexane : Benzene (7:3)	0.90, 0.50, 0.15	-
		Benzene : Chloroform (7:3)	0.06, 0.10, 0.20, 0.30, 0.40, 0.50, 0.60, 0.70, 0.84, 0.88	0.4 (pink)
2.	Benzene	Benzene : Chloroform (7:3)	0.10, 0.20, 0.30, 0.35, 0.40, 0.50, 0.66, 0.88	-
		Chloroform : Ethanol (8:2)	0.16, 0.25, 0.40	
3.	Chloroform	Benzene : Chloroform (7:3)	0.08, 0.17, 0.26, 0.66, 0.88	0.55 (pink)
4.	Ethanol	Benzene : Chloroform (7:3)	0.08, 0.17, 0.26	-
		Butanol :Acetic acid :Water (5:1:4)	0.10, 0.20, 0.30, 0.60, 0.66, 0.87	
5.	Water	Butanol :Acetic acid :Water (5:1:4)	0.14, 0.40, 0.57, 0.70, 0.85	-

CONCLUSION

All these pharmacognostical and phytochemical studies can be used as a diagnostic tool for the correct identification of the plant drug and also to test adulteration if any.

Acknowledgement
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