Research Article

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MARKER PARAMETERS FOR PHARMACOGNOSTICAL EVALUATION OF CONTROVERSIAL PLANTS VIS-A-VIS MOORVA

Sevantika Rotti [1]*, P. Subrahmanya [2]

[1] Ph. D Scholar, Alva's Ayurveda Medical College, Moodbidri, Karnataka, India

[2] Professor and HOD, Alva's Ayurveda Medical College, Moodbidri, Karnataka, India

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***Corresponding author**

E-mail: sevantika.m.galagali@gmail.com

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ABSTRACT

The plant Moorva is found as Jwarahara plant as mentioned in Brihatrayees, Laghutrayees and Nighantu literatures of Ayurveda. Moorva is a controversial plant and many plants are being used in the name of Moorva. Pharmacognosy research study becomes an important role in identification of controversial plants. The reasons for controversy could be either different plants of different region are often known by common name, several names for one plant or commercial substitution in non-availability of classical drug with local drug. The genuine basic raw material is very much essential for good quality medicines. Present study aims to define standards for identifying three source plants of Moorva botanically and chemically. Morphological evaluation carried out by qualitative evaluation based on morphological and sensory profile. Microscopical evaluation done for histological characters by using microscope and micro photography By Chemo-microscopy chemical method, evaluation is done with powders of plants. Preliminary phytochemical screening is done by qualitative chemical tests for establishing chemical profile. Chromatography study is performed by adsorption chromatography method. Three plants showed different cell structures, cell contents and different physical standards and phytochemicals. TLC shows presence of different phytoconstituents with different bands. Differentiation in cell, cell content, presence of phytoconstituents and different bands with TLC suggests, the three source plants have their different diagnostic value. Three source plants of Moorva have different marker parameters.

Keywords: Antipyretic, *Chonemorpha macrophylla*- G. Don, *Clematis triloba*- A. St. Hill, Controversy, Moorva, *Maeruaarenaria*- Hook f and Thoms, Jwara, Pharmacognosy, TLC.

INTRODUCTION

Basic approach towards Pharmacognosy of source plants of Moorva is made here which include study of External Morphology, Study of internal cell structures, organization and study of tissues like vascular, dermal and ground, becomes a key in identification of the correct species of the plant. Hence Pharmacognostic characters including botanical description, distribution, macroscopical and microscopical characters, study of powdered drugs has been carried out. Evaluation of preliminary; Phytochemicals in order to identify the constituents of plants, qualitative chemical tests are carried out. Physicochemical tests are carried out in order to assess purity and strength of plants, which enable to detect substitutions and adulteration. Thin Layer Chromatography study carried out, which can be a necessary tool for identification and purity of drugs by comparison with Chromatogram of Standard drugs.

MATERIAL AND METHODS

Morphological evaluation carried out by qualitative evaluation based on morphological and sensory profile of the drug. Microscopical evaluation is done for histological characters, achieved by using microscope and micro photography. Various reagents and stains were used to distinguish cellular structures. By Microchemistry chemical method, evaluation is done with powders of plants.

Preliminary phytochemical screening and physicochemical tests are done by qualitative chemical tests for establishing chemical profile. Chromatography study is performed based on adsorption chromatography method

Macroscopical study of *Clematis triloba* A. St. Hill

It is an extensive climber, often found trailing amongst gross. whole plant except the older stems seraceo-villious or tomentose, stems sulcate, leaves simple or ternate, entire or shallowly 1-7 lobed the lobes usually mucronate blades, 1-2 inch long, ovate, acute to orbicular, base rounded, cuneate or cordate, petioles slender, twiny 3/4th to 3 inch long, petiolules 1/4th to 3/4th inch long flowers white more than axillary corymbose panicles, bracts foliaceous, ovate, acute. Sepals 4-6, stellate and spreading, pubescent outside, glabrous inside, oblong or obovate terminated by tomentosemucro; stamens 1/4th to 3/8th long; Filaments ligulate, glabrous, connective not produced; achenes ovoid, compressed, silky villous with long feathery tails. Flowers are seen during September to November months.

It is very common through Deccan; seen in konkan. kartrizghat near Pune, Dongargaon near Ahmednagar, hills near Pune, Mawal district of Pune. The dried stem of *Clematis triloba* is light brown in colour having ridges and furrows with presence of bark and is fibrous inside.[1]

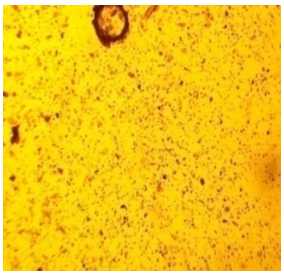
Clematis triloba

Microscopical study of *Clematis triloba* A. St. Hill

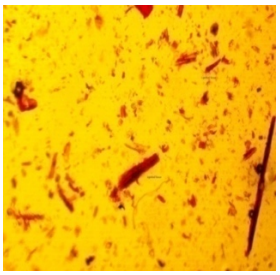
T.S of stem shows following structures it is made up of single layered epidermis without any epidermal out growths. Hypodermis is made of parenchymatous tissue. Cortex is made up of parenchyma. Endodermis is single layered. Vascular bundles are conjoint collateral open type. Xylem is facing towards the center and phloem facing towards periphery. In between cambium is present. Bundle cap is present above the phloem tissue which is made up of sclerenchyma. Large pith is present which is made up of parenchyma tissue. This undergoes a secondary growth by cork cambium and fascicular cambium. Due to the activity of cork cambium it gives phelloderm outside which is made of dead cells below secondary cortex. Due to the activity of fascicular cambium it cuts and gives rise to secondary phloem outside and secondary xylem inside. Secondary xylem consists of more amount of conjunctive tissue which is made of sclerenchymatous tissue. More no of secondary xylem is found and primary xylem pushing towards center. (Figure 1)[2]

Powder Microscopy

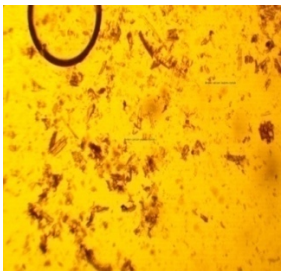
Yellow colored powder. Powder was fibrous with Sclerenchyma, spiral variety. Lignified broken tissue was prominently visible. Starch and calcium oxalate crystals was seen. Phloem fibers contain tannin.[3]



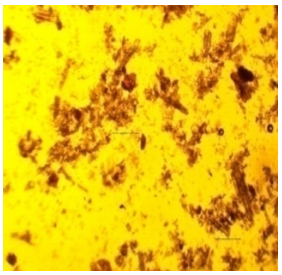
Starch



Lignified tissue



Calcium oxalate Crystals



Tannin

Macroscopical study of *Maerua arenaria*– Hook f and Thoms

It is a large woody climber with terminal corymbose of greenish yellow flowers. Leaves simple and entire, varying from broadly ovate to oblong and from acute to obtuse. It is found in deccan and Carnatic districts from Godavari southwards.

Wet root of *Maerua arenaria* is pink in colour sweet in taste and fleshy inside. Light brown colour is noticed when dried. Piliferous layer outside and fibrous inside.[4]

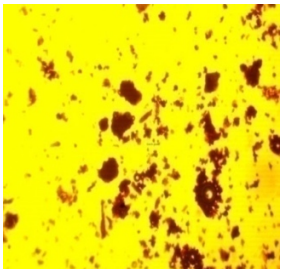
Maerua arenaria

Microscopical study of *Maerua arenaria*– Hook f and Thoms

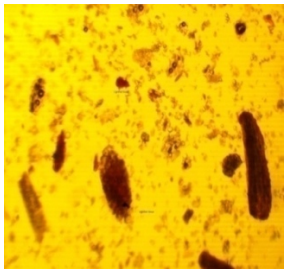
T. S. of *Maerua arenaria* root shows normal secondary growth in which cork cambium undergo the activity to form cork outside and forms the secondary cortex inside. Secondary cortex made up of parenchymatous cells. In the epidermis there is formation of lenticels. The cambium undergoes the activity to form the secondary xylem inside which is made up of sclerenchymatous cells. Outside it gives rise to phloem tissue. In between the secondary xylem and phloem, there is a formation of secondary medullary rays. The primary xylem tissue pushing towards center, large number of secondary xylem tissue is formed, and Pith is absent. (Figure 2 and 3)[5]

Powder Microscopy

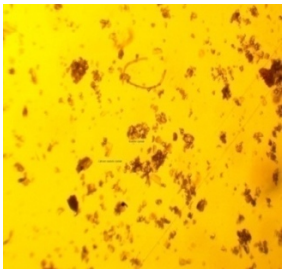
Yellowish powder with prominent cork, fiber with spiral, pitted etc. verities and lignified pigmented stone cells are visible in clusters. Starch grains are plenty clearly visible. Calcium oxalate crystals Rosette and prism variety. Cork tissue contains tannin.[6]



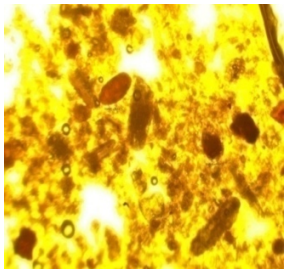
Starch



Lignified tissue



Calcium oxalate and Rosette Crystals



Tannin

***Chonemorpha macrophylla*-** G. Don

It is found in Western Ghats, at low levels and western cotes, in moist forests. It is a large climber with milky juice. Leaves are large nearly orbicular, fulvous tomentose beneath, very large fragrant white flowers. The corolla lobes often 2-inch-long and 1.5 inch broad, lobes sharply twisted to the left, salver shaped and follicles about a foot long.

Colour of root of *Chonemorpha macrophylla* is woody brown with presence of ridges and furrows. Lenticels are noticed on the surface.[7]

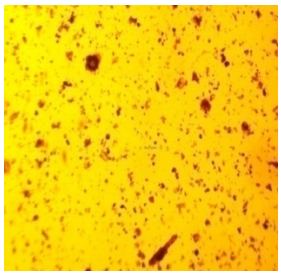
Chonemorpha macrophylla

Microscopical study of *Chonemorpha macrophylla*- G. Don

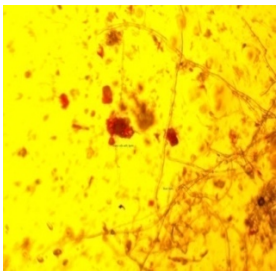
T.S of *Chonemorpha macrophylla*, stem is divided into epidermis, cortex and stele. Epidermis is made of single layer with epidermal hairs. Hypoderms is situated below the epidermal layer which is made up of parenchymatous tissue. 5-6 layers of cortex is present inner most layer of cortex is endodermis which is single layered. Stele consists of pericycle, vascular bundle and pith. Pericycle is made up of single layered parenchymatous tissue. Vascular bundles are conjoint collateral and open type. Small pith is present. It also undergoes normal secondary growth. Cork cambium cuts and gives rise to cork outside and secondary cortex inside. Cambium cuts and gives rise to more amount of secondary xylem tissue towards inside and small amount of phloem towards outside. Primary xylem tissue pushing towards the center. (Figure 4 and 5)[8]

Powder Microscopy

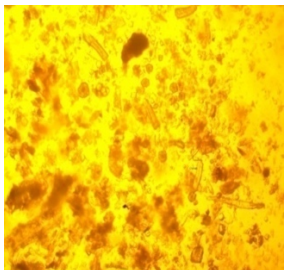
Brown colored powder with prominent cork, vascular fibers with spiral, pitted etc. verities and lignified pigmented stone cells are visible in clusters. Starch grains are plenty clearly visible. Calcium oxalate crystals are rod variety. Epidermis contains tannin.[9]



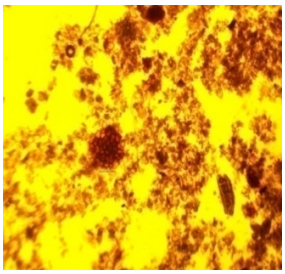
Starch



Lignified tissue

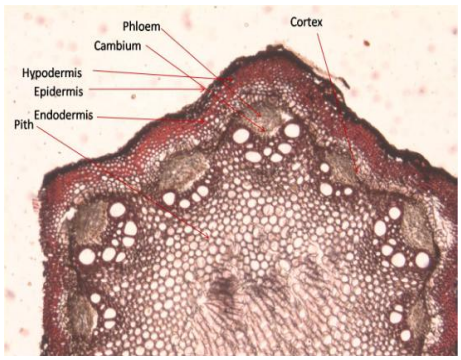


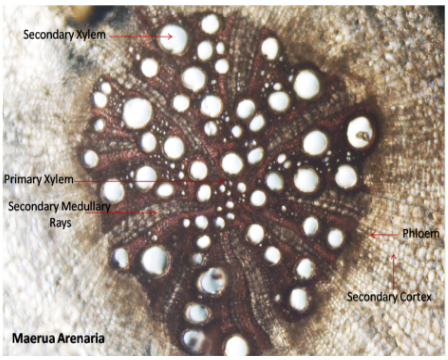
Calcium oxalate Crystals



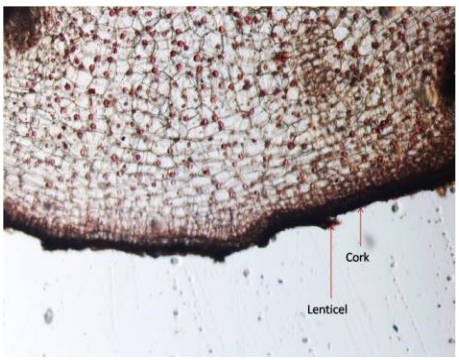
Tannin

RESULTS

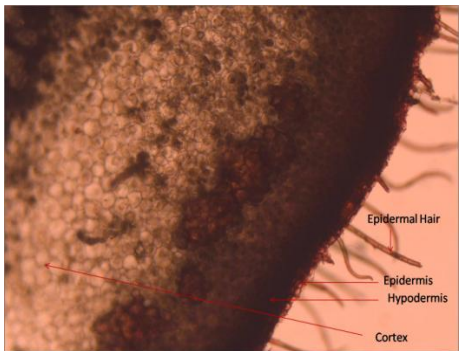


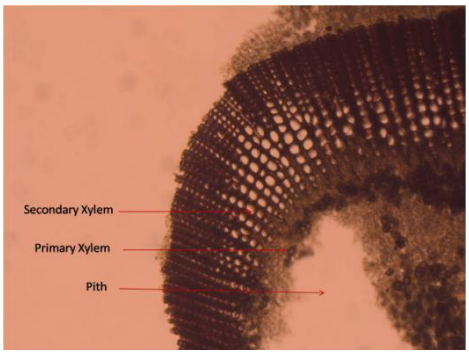


Transverse Section of *Clematis triloba* stem



Transverse Section of *Maerua arenaria*





Transverse Section of *Chonemorpha macrophylla* Stem

Preliminary phytochemical and physicochemical analysis of 3 plants

Physicochemical Analysis

Analysis	*Clematis triloba*	*Maerua arenaria*	*Chonemorpha macrophylla*
Foreign matter	1.6%	1.0%	1.2%
Moisture content	14.0%	10.4%	20.0%
Ph	6.53	4.75	7.15
Water soluble extractive	39.88%	56.0%	42.08%
Alcohol soluble extractive	32.04%	23.2%	22.2%
Total ash	6.8%	7.8%	14.1%
Water soluble ash	31.0%	49.0%	37.0%
Acid insoluble ash	62.0%	24.0%	69.0%[10]

Phytochemical analysis

The following tables indicate the presence or absence of metabolites in the methanol and aqueous extracts.

Methanolic Extract

Tests	*Clematis triloba*	*Maerua arenaria*	*Chonemorpha macrophylla*
Carbohydrates	+	+	+
Glycosides	-	+	-
Polysaccharides	-	-	-
Tests for Proteins			
Free amino acids	-	-	-
Bradford test	+	+	+
Tests for alkaloids			
Dragendroff's test	+	+	+
Mayer's test	+	+	+
Tests for steroids			
Liebermann-Burchard test	+	-	+
Salkowski's test	-	-	-
Triterpenoids	+	+	+
Tests for flavonoids			
Test 1	-	-	-
Shinoda test	-	-	-
With sodium hydroxide	+	-	+
Tests for Tannins			
$FeCl_3$ test	-	-	+
Dilute HNO_3 test	-	-	+
Test For Lipid	+	+	+
Test for Oils	+	-	-
Test for saponins	-	+	-[11]

Aqueous Extract

Tests	*Clematis triloba*	*Maerua arenaria*	*Chonemorpha macrophylla*
Carbohydrates	+	+	+
Glycosides	-	+	-
Polysaccharides	-	-	-
Tests for Proteins			
Free amino acids	-	-	-
Bradford test	+	+	+
Tests for alkaloids			
Dragendroff's test	+	+	+
Mayer's test	+	-	+
Tests for steroids			
Liebermann-Burchard test	-	-	-
Salkowski's test	-	-	-
Triterpenoids	+	+	+
Tests for flavonoids			
Test 1	-	-	-
Shinoda test	-	-	-
With sodium hydroxide	+	-	+
Tests for Tannins			
$FeCl_3$ test	-	-	+
Dilute HNO_3 test	-	-	+
Test for Lipid	+	+	+
Test for Oils	+	-	+
Test for saponins	+	+	+

Thin Layer Chromatography[12]

Solvent used: Butanol : Aceticacid : Water (4:1:5)

Rf values in TLC of *Clematis triloba*

Solvent front-17 cm

Samples	Solute Front (cm)	Rf values of bands with spraying reagent
Water	2	0.117
	2.9	0.17
	4.2	0.264
Methanol	5.6	0.329
	11.7	0.688

Rf values in TLC of *Maerua arenaria*

Solvent front-13 cm

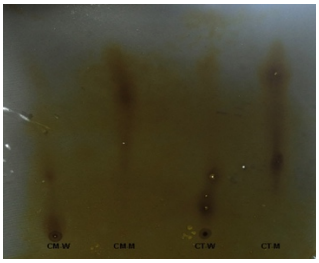
Sample	Solute Front (cm)	Rf values of bands with spraying reagent
Water	1.5	0.115
Methanol	1.3	0.1
	2.4	0.184
	6.8	0.523

Rf values in TLC of *Chonemorpha macrophylla*

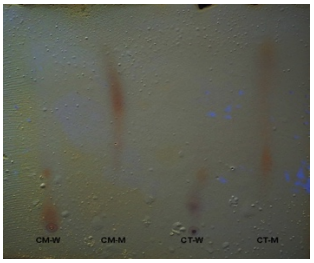
Solvent front-17 cm

Samples	Solute Front (cm)	Rf values of bands with spraying reagent
Water	0.9	0.0529
	4.2	0.247
Methanol	5.1	0.3
	6.7	0.394
	9.8	0.576
	12	0.705

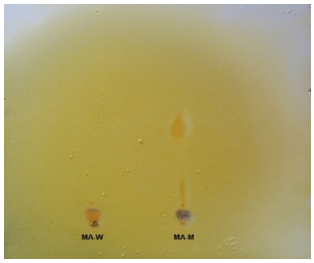
TLC Photographs



Clematis triloba



Maerua arenaria



Chonemorpha macrophylla

DISCUSSION

Identification of the plants can be done by following with findings of cell structures and cell contents of three plants by the study of Transverse section and Powder microscopy.

Transverse section of *Clematis triloba* has large pith whereas *Chonemorpha macrophylla* is with small pith. Epidermal outgrowth is absent in *Clematis triloba* and present in *Chonemorpha macrophylla*. Presence of lenticels is special feature seen in *Maerua arenaria*. Vascular fibers are of spiral variety in *Clematis triloba* whereas in *Maerua arenaria* and *Chonemorpha macrophylla* they are spiral and pitted.

Powder microscopy show lignified broken tissue prominently seen in *Clematis triloba*. Starch grains in *Clematis triloba* are occasionally seen whereas in *Maerua arenaria* and *Chonemorpha macrophylla*, plenty of them are seen. *Clematis triloba* shows presence of calcium oxalate crystals and *Maerua arenaria* shows rosette and prism variety of calcium oxalate crystals and in *Chonemorpha macrophylla* they are rod shaped.

Saturated tannin present in phloem fibers in *Clematis triloba,* the same is present in cork in case of *Maerua arenaria* and in *Chonemorpha macrophylla* it is deposited in epidermis.

As a part of qualitative evaluation, when three plants are subjected for Physicochemical evaluation, the Foreign organic matter for all the three plants is within standard limits. The Moisture content of *Maerua arenaria* is lowest among all the three plants, which is 10.4%, whereas in *Clematis triloba* and *Chonemorpha macrophylla*, it is 14.0% and 20.0% respectively. The pH, in case of *Maerua arenaria* and *Clematis triloba* are acidic in nature with the values 4.75 and 6.53 respectively whereas in case of *Chonemorpha macrophylla* its value is 7.15, which is slightly alkaline in nature.

Extractive values for three plants are highest in aqueous media and lowest in alcoholic media. The Total Ash, Water soluble and Acid insoluble ash in case of *Clematis triloba* being 6.8%, 31.0% and 62.0% respectively. They are 7.8%, 49.0% and 24.0% in case of *Maerua arenaria* and 14.1%, 37.0% and 69.0% in *Chonemorpha macrophylla.*

All the three plants show presence of carbohydrates in aqueous and methanolic extracts. Glycoside present in *Maerua arenaria* with both extracts. Protein is present in all three plants with both extracts. Alkaloids present in three plants with both extracts. Steroids present in *Clematis triloba* and *Chonemorpha macrophylla* with methanolic extract. Triterpenoids present in all three plants with both extracts. Flavonoids present in *Clematis triloba* and *Chonemorpha macrophylla* in both extracts. Tannin present in *Chonemorpha macrophylla* in both extracts. Lipid present in all plants with both extracts. Oil is present in *Clematis triloba* in both extracts, whereas in *Chonemorpha macrophylla* it is present in aqueous extract. Saponin is present in all three plants with aqueous extract whereas in *Maerua arenaria,* it is present even in methanolic extract.

In case of *Clematis triloba*, Thin Layer Chromatography shows three different bands with aqueous extract and two bands with methanol. *Maerua arenaria* shows one band with aqueous extract and three different bands with methanolic extract. *Chonemorpha macrophylla* shows two bands with aqueous extract and four bands with methanolic extract, which are indicative of different Phytoconstituents of three plants.

CONCLUSION

Morphological details are known by observing the plant with naked eye. Thus, sensory characters are elicited. Microscopical examination provides several diagnostic characters. Transverse section shows peculiar characteristic arrangement of tissues of plants which provides a key to differentiate the plants. The powder microscopy also provides the presence of some diagnostic elements like fibers, vessels and cell deposits like starch grains etc. on the basis of which plants are identified.

Plants contain variety of chemical compounds that act upon the body and can be used to treat the diseases. Detection of such active compounds may help in explaining probable mode of action of plants.

A thin layer chromatography provides a chromatogram with the help of which, the plant is identified by comparison with the chromatogram of the standard drug.

Thus, the study fulfils in defining standards for identifying three controversial source plants of Moorva, thereby fulfilling the aim of study.

Abbreviations

CT: *Clematis triloba* A. St. Hill.
MA: *Maerua arenaria*- Hook f and Thoms
CM: *Chonemorpha macrophylla*- G. Don
A: Aqueous extract
M: Methanolic extract

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