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Pharmacognostic, phytochemical and physiochemical studies of *Mimusops Elengi* Linn stem bark (Sapotaceae)

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ABSTRACT

In recent years there has been rapid increase in the standardization of selected medicinal plant of potential therapeutic significance. Despite the modern techniques, identification of plant drug by pharmacognostic study is more reliable. Mimusops elengi belongs to family Sapotaceae, commonly known as Spanish cherry is one such tree native to the Western Ghat region of the peninsular India. By looking the high traditional use of the plant Mimusops elengi Linn, the present investigation was undertaken for research with the purpose of drawing the pharmacopoeial standards for this species. The present study deals with pharmacognostical parameters for the bark of Mimusops elengi which mainly consists of macromorphology and microscopical characters, physio-chemical constants and phytochemical screening. This information will be of used for further pharmacological and therapeutical evaluation of the species and will assist in standardization for quality, purity and sample identification.

Keywords: *Mimusops elengi*, Pharmacognostic study, Phytochemical screening, Standardization.

INTRODUCTION

Medicinal plants have a long-standing history in many indigenous communities and continue to provide useful tools for treating various diseases. The practices of traditional medicine are based on hundreds of years of belief and observations, which predate the development and spread of modern medicine. [1] Today, there is widespread interest in herbal drugs. This interest primarily stems from the belief that herbal medicines are safe, inexpensive and have no adverse effects. [2] Medicinal plants are moving from fringe to main stream use with a greater number of people seeking remedies and health approach. [3] It is no wonder that the world's one-fourth population i.e. 1.42 billion people, are dependent on traditional medicines for the treatment of various ailments. [4] However a key obstacle, which has hindered the acceptance of the alternative medicines in the developed countries, is the lack of documentation and stringent quality control. There is a need for documentation of research work carried out on traditional medicines. With this backdrop, it becomes extremely important to make an effort towards standardization of the plant material to be used as a medicine. The process of standardization can be achieved by stepwise pharmacognostic and phytochemical studies. These studies help in identification and standardization of the plant material. Correct identification and quality assurance of the starting materials is an essential prerequisite to ensure reproducible quality of herbal medicine which will contribute to its safety and efficacy. [5] *Mimusops elengi* linn commonly known as Bakul and Spanish cherry, belonging to Sapotaceae family is considered as a sacred plant among Hindus and has obtained important place in religious texts as well as in ancient Sanskrit literature. Its fragrant flowers are celebrated in the Puranas and even placed amongst the flowers of the Hindu paradise. [6] A small to large ornamental evergreen tree found in the Deccan Peninsula and Andaman Islands and frequently

cultivated in gardens for the sake of its fragrant flowers; it is grown also as an avenue or shade tree throughout the greater parts of India. [7] In the traditional Indian system of medicine, the ayurveda and various folk system of medicine, *Mimusops elengi* possess several medicinal properties such as astringent, tonic and febrifuge etc. [8,9] Chemical studies have shown that, Bark contain Taraxerone, taraxerol, spinasterol, sodium salt of betulinic acid and urosolic acid, Fatty acid esters of alpha-spinasterol. [10, 11] Preclinical studies have shown that the bark possess Anti-anxiety, Antihyperlipidemic, Antiulcer Anticonvulsant, Anti-inflammatory, analgesic, antipyretic, Antioxidant, Cytotoxic, Antidiabetic, Diuretic and Hypotensive activities. [12] The current article describes some pharmacognostical, physicochemical and phytochemical characteristics studied. The main objective of this study is to supplement constructive information with regards to its identification, Characterization and standardization of plant *Mimusops elengi.*

MATERIALS AND METHODS

Collection of sample:
The fresh bark of *Mimusops elengi* was collected in the month of November from Sangavi, Pune District, Maharashtra state, India. The plant was identified and authenticated by Agharkar Research Institute, Pune and a voucher specimen was deposited with a voucher specimen sample No. S/B-105. The fresh bark was removed and dried in shade for 20 days. The fresh bark was used for the study of macromorphological and microscopical characters; whereas the dried bark powder was used for determination of powder microscopy, physicochemical characterization and phytochemical analysis.

Macrmorphologcal Description:
The stem bark of *Mimusops elengi* was subjected to macroscopic studies which comprised of organoleptic characteristics *viz*. color, odour, appearance, taste, shape, texture, fracture, etc. of the drug. These parameters are considered as quite useful in quality control of the crude drug and were evaluated as per standard WHO guidelines. [13-15]

Microscopic characteristics:
Fresh barks of *Mimusops elengi* were selected for the microscopical studies. Microscopic sections were cut by free hand sectioning. Numerous temporary and permanent mounts of the microscopical sections of the bark specimen were made and examined microscopically. Histochemical reactions were applied with staining reagents on transverse sections and on bark powder by reported methods. [13-17] Photomicrographs of the microscopical sections were taken with the help of MOTIC Digital Microscope, provided with MOTIC IMAGE PLUS 2.0 software.

Powder characteristics
Preliminary examination and behavior of the powder with different chemical reagents was carried out and microscopical examination was carried out as per reported methods. [16, 17]

Micrometry
Quantitative microscopy of the transverse sections and bark powder were performed to determine the size and dimensions of tissues, cells and cell contents. [18, 19]

Physicochemical Evaluation:
Analysis of Physicochemical constants of the powder bark has been done to evaluate the quality and purity of the drug. Various physicochemical parameters like Moisture contents, foreign organic matters, Ash values and Extractive values were calculated as per WHO guidelines. The information collected from these test was useful for standardization and obtaining the quality standards. [20, 21]

Florescence Analysis:
Many herbs show fluorescence when the cut surface or powder is exposed to UV light and this can be useful in their identification. The fluorescence character of the plant powders (40 mesh) was studied both in daylight and UV light (254 nm and 366 nm) and after treatment with different reagents like sodium hydroxide, hydrochloric acid, nitric acid and ferric chloride etc.[22,23]

Phytochemical Investigations:
The qualitative chemical tests carried out for the identification of the nature different phytoconstituents present in the powered crude drug. The tests were carried out by using standard conventional protocols. [13, 14]

RESULT AND DISCUSSION

Macrmorphologcal Description:
Mimusops elengi is a small to large evergreen tree reaching a height of about 20 meters and 1 meter in circumference. The organoleptic evaluation of the bark and bark powder revealed that the both were brownish black or grayish black in color, with characteristics odour and astringent taste.[15,18] Fig. 1 explains the extra features observed in bark of the plant material. The results of macromorphology were depicted in Table 1.

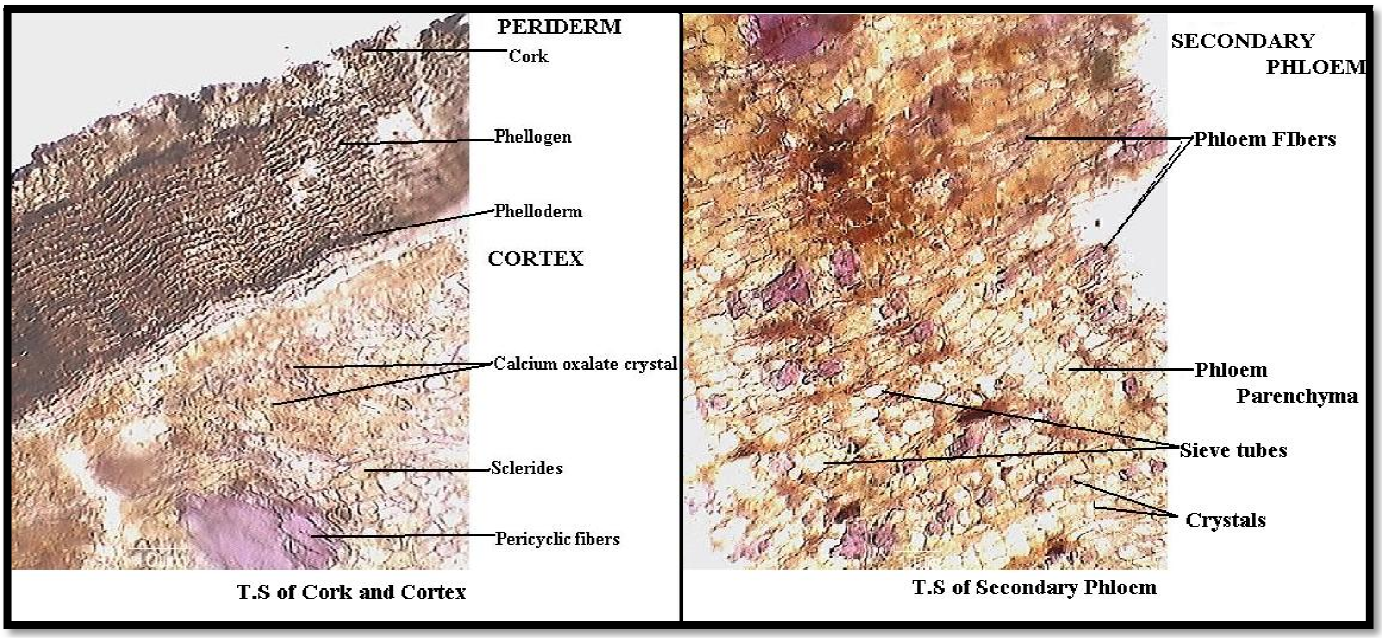
Figure 1: Macromorphology of *Mimusops elengi* bark

Table 1: Macrmorphologcal Description

S N	Characters	Observation
Organoleptic Characters		
1.	Colour	Black or Grayish black
2.	Odour	Characteristic
3.	Taste	Astringent
Quantitative Macromorphology		
4.	Length	02-10 cm
5.	Thickness	05-10 mm
Extra Features		
6.	Shape	Curved or Channelled
7.	Texture	Rough
8.	Fractures	
	Inner Surface	Longitudinally Fissured
	Outer Surface	Fibrous and Longitudinally Striated

Microscopic characteristics:
The transverse section of the bark shows the typical anatomical characteristics as a outer layer of periderm followed by cortex and secondary phloem region. The cork region was found to be well defined. The cork cells are rectangular and compactly arranged in 5 to 6 layers which were found to be filled with brown coloured cellular contents.[13,14] Phellogen is compactly arranged in 2 to 3 layers, Phellogen and phelloderm are indistinguishable. The cork originates in the sub epidermis or second layer of cortex. The cortex region shows the presence of simple calcium oxalate crystals, starch and sclerides. They are solitary, appears brown in color. Within the cortex region, bundle of lignified Pericyclic fibers were also present. Pericycle is represented by a discontinuous ring consisting of thick walled fibers and supported by some parenchyma types of cells.[16-18] They are found in a group of 2 to 10 (Fig. 2). The secondary phloem is a wide zone of tissue composed of sieve tubes, companion cells, phloem parenchyma, alternating with strands of phloem fibers transverse by phloem rays (Fig. 3).

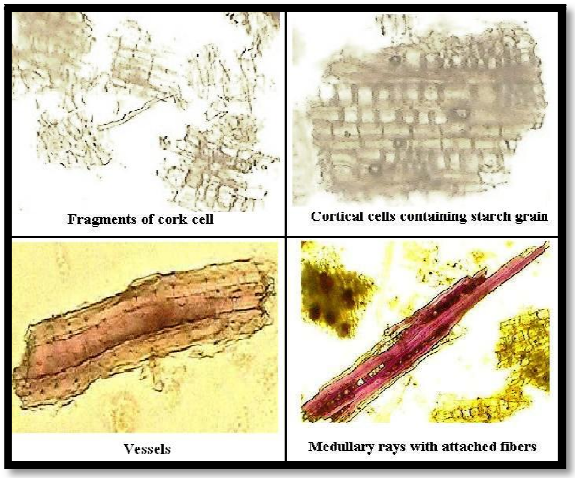
Figure 2: T.S of *Mimusops elengi* bark



Powder characteristics

Powder bark is brown, non aromatic, astringent. The microscopic examination of the powder shows fragments of cork cells, Vessels and fibers of various shapes and thickness, tannin cells, stone cells, solitary crystals and other cell contents (Fig. 3).[18]

Figure 3: Powder Characteristics of Mimusops *elengi* bark



Micrometry:

The results of micrometric characters of tissues, cells and cell contents were depicted in Table 2. Measurements of different cells are frequently necessary for the quantitative identification of closely allied substances. In most cases, these allied substances are mixed with the original drugs as adulterants and substituent's.[19-21] Thus, the adulterants and/or substituent's present in crude drugs can be distinguished by this way with the aid of optical microscopy (Fig.4).

Figure 4: Micrometry of Mimusops *elengi* bark

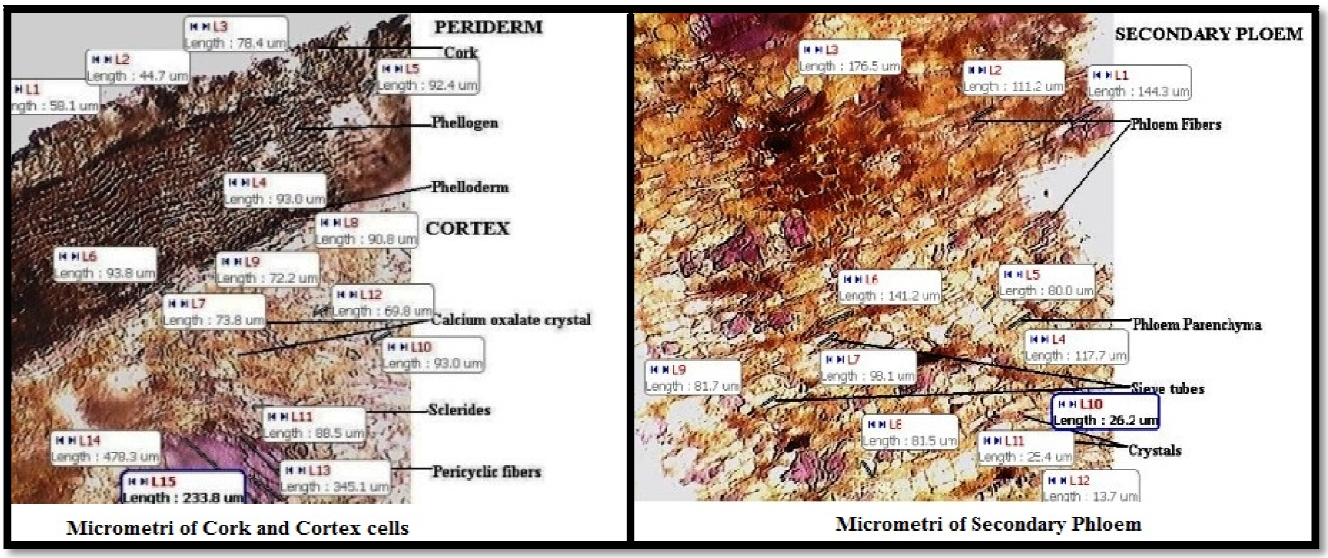


Table 2: Micrometry of Some Cells

S. N.	Type of cells	Length in micrometer (µm)
1	Cork cells	60.40±16.96
2	Phellogen	93.06±00.70
3	Phelloderm	78.93±10.30
4	Sclerides	83.76±12.30
5	Pericyclic fibres	352.4±122.4
6	Phloem fibres	144.0±32.65
7	Phloem Parencyma	112.9±30.87

Values are expressed as mean ± SEM

Physicochemical Parameters:

The results of the physicochemical constants of raw material lie within the limit which is mentioned in Table 3; this signifies that the quality and purity of raw material was good enough; the results of foreign organic matter denote presence of any organism, part or product of an organism, other than that named in the specification and description of the herbal material concerned;[19,21] which was found to be 0.33 ±0.28, it indicates that their may be present of part or product of an organism in very less amount. Insufficient drying favors spoilage by molds and bacteria and makes possible the enzymatic destruction of active principles. Not only the ultimate dryness of the drug is important, equally important is the rate at which the moisture is removed and the condition under which it is removed thus the determination of moisture content also provide the method of preparation of drug; [18,19] and it is observed that the moisture content of the drug was found to be 03.86 ±0.72 which signify that the drug is properly dried and properly stored. The total ash is particularly important in the evaluation of purity of drugs, i.e. the presence or absence of foreign matter such as metallic salts or silica.[20,21] An analytical result for total ash was found to be 5.16 ±0.28. The amount of acid-insoluble siliceous matter present was 0.16 ±0.28. The water soluble ash was found to be 2.46± 0.05; this parameter is used to detect the presence of material exhausted by water whereas the value for Sulphated ash was found to be 2.70 ±0.34 which is within fairly wide limit. As the ash values of the crude drugs lies with in the fair limit which signify its quality and purity and gives idea about the total inorganic content. The water soluble extractive values indicated the presence of sugar, acids and inorganic compounds;[19,21] the water soluble extractive value found to be 05.00 ±0.50 and the alcohol soluble extractive values indicated the presence of polar constituents like phenols, alkaloids, steroids, glycosides, flavonoids.[20,21] The alcohol soluble extractive was found to be 7.10 ± 0.76 which signify that the large amount of constituents of bark was soluble in alcohol than water.

Table 3: Physicochemical parameters

S. N.	Parameters	Bark Powder
1.	Foreign organic matter (% w/w)	0.33 ±0.28
2.	Moisture content(LOD) (% w/w)	03.86 ±0.72
Ash Values		
3.	Total ash (% w/w)	5.16 ±0.28
4.	Acid insoluble ash (% w/w)	0.16 ±0.28
5.	Water soluble ash (% w/w)	2.46± 0.05
6.	Sulphated ash (% w/w)	2.70 ±0.34
Extractive values		
7.	Water soluble extractive value (% w/w)	05.00 ±0.50
8.	Alcohol soluble extractive value (% w/w)	07.10 ±0.76

Values are expressed as mean ± SEM

Fluorescence analysis:

The results of fluorescence analysis were expressed in Table 4. Fluorescence study is an essential parameter for first line standardization of crude drug. In fluorescence the fluorescent light is always of greater wavelength than the exciting light. Light rich in short wavelengths is very active in producing fluorescence and for this reason ultraviolet light produces fluorescence in many substances which do not visibly fluoresce in daylight.[22,23]

Table 4: Fluorescence analysis of Powder

S N	Solvents Treatment	Visible light	Short UV (254 nm)	Long UV (366 nm)
1.	Drug + Distilled water	Light yellow	Dark brown	Dark Black
2.	Drug + Pet. Ether	Reddish brown	Blackish green	Blackish brown
3.	Drug + Chloroform	Light brown	Dark brown	Dark Black
4.	Drug + Methanol	Yellowish brown	Greenish black	Dark brown
5.	Drug + Conc. HCl	Dark brown	Brownish black	Off white
6.	Drug + Conc. HNO_3	Ceramic yellow	Black	Reddish brown
7.	Drug + Conc. H_2SO_4	Greenish brown	Black	Violet black
8.	Drug + Picric acid	Yellowish Brown	Greenish black	Ceramic yellow
9.	Drug + Ammonia solution	Mud brown	Greenish black	Ceramic brown
10.	Drug + 10% Sodium hydroxide	Dark brown	Ceramic green	Brownish Black
11.	Drug + Ferric chloride	Greenish black	Yellowish brown	Blackish green

Preliminary Phytochemical Screening:

The preliminary phytochemical investigations of powdered bark was performed which shows the presence of Tannins and Phenolic derivatives, Steroids, flavanoids and Saponins type of major secondary metabolites which revealed their potent therapeutic activity.[14] The results of the screening were express in Table 5.

Table 5: Preliminary Phytochemical Screening of Powder

S. N.	Parameters	Observation
1	Carbohydrates	+
2	Proteins	+
3	Glycosides	+
4	Flavonoids	+
5.	Volatile oil	-
6.	Alkaloids	-
7.	Tannins	+
8.	Steroids	+
9.	Terpenoids	+
10.	Saponin	+

+ indicates presence

CONCLUSION

Standardization is essential measure for quality, purity and sample identification. Macromorphology and microscopy along with the Quantitative analytical microscopy is one of the simplest and cheapest methods to start with for establishing the correct identity of the source materials. Physiochemical and Chemical analysis of bark confirm the

quality and purity of plant and its identification. Here the information collected was useful for further pharmacological and therapeutical evaluation along with the standardization of plant material.

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