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Research Article

PHARMACOGNOSTIC EVALUATION OF BARK AND SEEDS OF *MIMUSOPS ELENGI* L

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ABSTRACT

Pharmacognostic evolution of *Mimusops elengi*'s bark and seed powder for extractive value, fluorescence analysis, ash analysis, treatment with strong acids, phytochemical and element analysis. Highest extractive value for seeds and bark were found with water, with more or less same residual nature. Under UV light seed gives fluorescent green colour after treating it with iodine and ferric chloride. Total ash content was highest in bark (12.5 %) as compared to seed (6.0 %). Water-soluble ash was more or less similar in both bark and seed. Seed powder showed clearly distinct dark reddish orange colour when treated with concentrated H_2SO_4. Alkaloids were absent in seeds and bark, while Tannin was present only in bark. Elemental analysis by XRF reveal presence in order of Ca>K>S>Fe>Sr>Ti in bark, and in seed Ca>K>S>Mn>Fe>Cu>Sr.

Key words: Pharmacognostic, Extractive value, Phytochemical, *Mimusops elengi*, Element

INTRODUCTION

It is documented that 80% of the world's population has faith in traditional medicine, particularly plant drug for their primary health care[1]. The number of reports of patients experiencing negative health consequences caused by the use of herbal medicine has increased in recent year. Analysis and studies have revealed a variety of reasons for such problem. One of the major cases of reported adverse events are directly linked to the poor quality of herbal drug and raw medicinal plant materials[2]. Thus the traditional medicine required intensive and urgent investigation in the next few years from botanical, chemical, and biological perspective, particularly for the diseases in developing world, and also validated and standardized traditional medicinal agents must become a critical component for sustainable global health care [3].

Recently, many international authorities and agencies, including the World Health Organization, European Agency for the Evaluation of Medicinal Products and European Scientific Cooperation of phytomedicine, US Agency for Health Care Policy and Research, European Pharmacopoeia Commission and Department of Indian System of Medicine have started creating new mechanisms to induce and regulate quality control and standardization of botanical medicine. For ayurvedic medicine and other traditional medicines, newer guidelines of standardization are required, and thus pharmacognostic evaluations of medicinal plant/herbal formulation promoted by WHO. This will be a major step in the development of new generation standardization of botanical medicines.

Seeds and bark are two most useful parts of *M. elengi*. Seeds of *M. elengi* are astringent to bowels, and bruised seed kernels are applied locally within the anus of children in case of constipation[4]. Hot water extract of dried seeds is used to fix loose teeth[5]. Bark is used as an astringent and applied externally too. Bark extract is also given orally to cure diseases of gums and teeth, biliousness as an anthelmintic, stomachic and cardiotonic[5]. Bark extract of *M.elengi* showed moderate inhibitory activity against HIV type 1 protease[6] and non-significant activity against Herpes simplex virus type -1[7]. Bark extracts is used as a gargle for odontopathy[8]. Increasing demand of bark and seeds in several herbal formulations leads adulteration with several unknown herbal plants. Thus market demand is responsible to evaluate this important medicinal plant. In the present investigation we reported pharmacognostic evaluation of seed and bark of *M. elengi*.

MATERIALS AND METHOD

Dried parts of *M. elengi* (bark, seed) were used for pharmacognostic evaluation. Plant parts were collected from the medicinal plant garden of Dharmaj –Gujarat. Plant was identified and confirmed with the help of "Flora of Gujarat"[9]. Dust and debris were removed from the plant parts and dried in hot air oven (Cintex –India) at 50°C for complete drying. Powder of dried part was made by a domestic grinder.

Extractive value determination

Coarsely powdered air-dried material 4 g was placed in a glass stoppered conical flask and macerated with 100 ml of solvents (water, methanol, and chloroform) shaking frequently, and then allowing it to stand for 18 hours. Filter it rapidly through whatman No. 1 filter paper, taking care not to lose any solvent. Transfer 25 ml filtrate to flat- bottom dish and evaporate solvent on a water bath. Dry at 105 °C for 6 hours, cool in a desiccator for 30 minutes and weigh it immediately. Calculate the content of extractable matter in % of air-dried material[10].

Ash values

Ash values such as total ash, acid insoluble ash, water-soluble ash, and sulfated ash were determined according to Indian pharmacopoeia. For determination of ash values, powder was prepared of bark and seed and sifted through sieve no. 20 and following tests were performed.

Total ash

About 3 g each of powdered parts were accurately weighed and taken separately in silica crucible, which was previously ignited and weighed. The powder was spread as a fine layer on the bottom of crucible. The powder was incinerated gradually by increasing temperature to make it dull red hot until free from carbon. The crucible was cooled and weighed. The procedure was repeated to get constant weight. The percentage of total ash was calculated with reference to the air-dried powder[11].

Acid insoluble ash

The ash obtained as described above was boiled with 25 ml of 2N HCl for 5 minutes. The insoluble ash was collected on an ash less filter paper and washed with hot water. The insoluble ash was transferred into a crucible, ignited and weighed. The procedure was repeated to get a constant weight. The percentage of acid insoluble ash was calculated with reference to the air-dried drug[11].

Water soluble ash

The ash obtained as described for the total ash, was boiled for 5 minutes with 25 ml of water. The insoluble matter was collected on ash less filter paper and washed with hot water. The insoluble ash was transferred into silica crucible, ignited for 15 min. and weighed. The procedure was repeated to get a constant weight. The weight of insoluble matter was subtracted form the weight of total ash. The difference of weight was considered as water-soluble ash. The

percentage of water-soluble ash was calculated with reference to air-dried parts respectively[11].

Sulfated ash

A silica crucible was heated to red for 10 min. and was allowed to cool in a desiccator and weighed. A gram of substance was accurately weighed and transferred to the crucible. It was ignited gently at first, until the substance was thoroughly charred. Then the residue was cooled and moistened with 1 ml of concentrated sulfuric acid, heated gently until white fumes are no longer evolved and ignited at 800 °C ± 25 °C until all black particles have disappeared. The ignition was conducted in a place protected from air currents. The crucible was allowed to cool. A few drops of concentrated sulfuric acid were added and heated. Ignited as before and was allowed to cool and weighed. The operation was repeated until two successive weighing do not differ by more than 0.5 mg [11].

Fluorescent analysis

The fluorescent analysis of various extracts of aerial parts of *M. elengi*(seeds and bark) was studied under UV light and daylight[12, 13]. Powder was extracted with chemicals like, 1N NaOH, Acetic acid, 1N HCl, 1N HNO_3, 5% Iodine, 5 % $FeCl_3$, 1 N NaOH in methanol, and methanol.

Micro chemical tests

Powder of aerial parts of *M. elengi* was treated with chemicals to observe the colour change under ordinary light [14]. Fine powder 1 g was treated with 5ml of chemicals like, HCl, H_2SO_4, HNO_3, Acetic acid, Ammonia liq., Ferric chloride, Iodine solution and 10% NaOH.

Phytochemical investigation

Qualitative Phytochemical analysis was done by Harbone method [15].

XRF analysis

Powder of dried bark and seed was made in a domestic grinder. Powder was used for the element analysis by the automatic X-ray refraction spectrophotometer (Shimadzu, Japan).

RESULTS

Results for extractive value, filtrate colour, and residual nature of bark and seeds were recorded in Table 1. Extractive value in methanol was not much different than the extractive value in water, but nature of residual and filtrate colour was quite different than water as extractive solvent. Among selected solvents, chloroform extracted less matter from the plant powder, and filtrate colour was also different as compared to water and methanol. Florescence analysis of bark and seed powder of *M. elengi* was performed after treating with several chemicals and results were recorded as mention in Table 2. Four types of ash were determined of the bark and seed of the *M. elengi*, and their percentage values were recorded in Table 3. Sulfated ash was higher than the total ash, acid insoluble ash, and water-soluble ash in parts of *M. elengi* (seeds). Powders of bark and seed were treated with concentrated acids, ammonia solution, ferric chloride solution, iodine solution and base and colour changes in the powder were recorded in Table 4, Extract of bark and seeds (water, methanol, and chloroform) were qualitatively analyzed for the major chemical groups (carbohydrate, protein, alkaloid, steroids, flavanoid, terpenoids, glycoside, saponin, tannin and fixed oil) and results are recorded in Table 5. Results of elemental analysis of bark and seed of *M. elengi* by XRF are recorded in Table 6. Elements like Ca>K>S>Fe>Sr>Ti and Ca>K>S>Mn>Fe>Cu>Sr were found in bark and seed respectively at concentration in decreasing order as mention here.

Table 1: Extractive value of bark and seeds of *M. elengi* in different solvent

Treatment	Parts	Percentage of extraction	Filtrate colour	Residual nature & colour
Water	Bark	71.73±1.51	Reddish brown	Crystalline Reddish brown
	Seed	36.86±0.70	Off white	Crystalline white
Methanol	Bark	66.73±0.46	Reddish brown dark	Crystalline reddish brown
	Seed	34.08±0.52	Off white	Solid off white
Chloroform	Bark	51.10±1.64	Reddish brown	Solid light orange
	Seed	19.76±0.49	Light yellow	Sticky yellowish white

Table 2: Fluorescence analysis of bark and seeds powder of *M. elengi* under visible and UV light after treated with different chemical

Treatment	Visible Light		UV light (366nm)	
	Bark	Seed	Bark	Seed
Powder +1 N NaOH	Greenish black	White	Yellowish green	No florescence
Powder +Acetic acid	Creamy white	White	No florescence	No florescence
Powder + 1N HCl	Dull white	White	No florescence	No florescence
Powder +1 N HNO_3	Dull white	White	No florescence	No florescence
Powder +HNO_3 + ammonia	Orange	White	No florescence	No florescence
Powder +5 % $FeCl_3$	Light brown	Yellow	Fluorescent green	Fluorescent green
Powder +5 % Iodine	Orange red	Yellow	Yellow	Fluorescent green
Powder +1 N NaOH in methanol	Yellowish brown	White	Yellowish green	No florescence
Powder +Methanol	Reddish brown dark	Off white	Violet	No florescence

Table 3: Ash analysis of bark and seeds of *M. elengi*

Plant part	Ash (%)			
	Total ash	Acid insoluble ash	Water soluble ash	Sulfated ash
Bark	12.5	7.0	2.0	9.0
Seed	6.0	3.5	2.2	10.3

Table 4: Colour analysis of bark and seeds powder of *M. elengi* after treated with different chemical.

Treatment	Bark	Seed
HCl	Reddish brown	Light pink
H_2SO_4	Reddish brown	Dark reddish orange
HNO_3	Yellowish red	Yellow
Acetic acid	Reddish brown	White
Ammonia liq.	Brown	Creamy white
Ferric chloride	Black	Black
Iodine sol.	Orange red	Yellow
10% NaOH	Brownish red	Creamy white

Table 5: Preliminary phytochemical analysis of bark and seeds of *M. elengi* in water, chloroform and methanol extract.

Phytochemical group	Bark			Seed		
	D/W	CH_3OH	$CHCl_3$	D/W	CH_3OH	$CHCl_3$
Carbohydrate	+	+	-	+	+	-
Protein	+	+	-	+	+	-
Alkaloid	-	-	-	-	-	-
Steroids	+	+	+	+	+	+
Flavanoid	+	+	+	-	-	-
Terpenoids	+	+	+	-	-	-
Glycoside	+	+	+	+	+	-
Saponin	+	+	-	+	+	-
Tannin	+	+	-	-	-	-
Fixed oil	-	-	-	-	-	+

+ = Positive, - = Negative, D/W = Distilled water, CH_3OH = Methanol, $CHCl_3$= Chloroform

Table 6: Element analysis of bark and seeds of *M. elengi,* values are mg/100g.

Elements	Bark	Seed
Calcium	78.152	58.642
Potasium	10.462	34.070
Sulphur	2.890	2.989
Manganese	0.0	1.663
Iron	6.391	1.495
Copper	0.0	0.679
Strontium	1.295	0.462
Titanium	0.804	0.0

DISCUSSION

Extractive value, ash value, colours under ordinary and UV light, microchemical tests, and the qualitative evaluation of extract for the phytochemical groups, were parameters used for the characterization of botanical drug, and these are the preliminary steps of the quality control for herbal drugs. Biological activity of crude drug is mainly due to the active chemical constituents, and the constituent may be soluble in different polar, semi polar and non-polar solvents[16]. Methanol and water showed highest extractive values, and both are able to extract most of phytoconstituents from both seed cotyledons and bark. As the water decoction is the usual method for most of the medicinal plant based preparations of drugs[17]. In present study cold maceration was used to extract secondary metabolite, are probably the reason for low yield of extracts, as maceration[18] and cold extraction[19, 20] generally been reported to give lower yield of plant extracts compared to hot and soxhlet extraction.

Ash value of medicinal plants reflects the carbonate, phosphate, oxides, silicate, and silica. Moreover the total ash of a crude drug also reflects the care taken in drug preservation, and the purity of crude and the prepared drug[16]. Acid insoluble ash reflects the calcium oxalate content of the drug. In the present investigation considerable amount of total ash was noticed in bark and seed, findings can be employed as quality parameter to evaluate *M.elengi* biomass for any adulteration. Fluorescence analysis [12,13] and micro chemical tests[14] are used to characterize the crude drug. Among employed chemical test, seeds of *M. elengi* produced noticeable colour with concentrated acids, it can be an important character to ascertain genuineness of the powdered drug.

Phytochemical profiling of methanol and water extract of bark and seed for carbohydrate, protein, alkaloids, flavanoid, terpenoids, glycoside, saponin and tannin emerged with noticeable results. Like alkaloids were absent in bark and seed, same results were reported by Misra and Mitra[21] and Gunatilaka[22], while presence of tannin was only found in bark. Such outstanding phytochemical screening results can be good tool for identification of *M.elengi* biomass particularly when grinded to fine powder.

Calcium, which is essential element for several life processes, was found to be highest in seeds of *M. elengi*, and results corroborated with Gopalan *et al.*[23]. The mineral content are essential part of plant biomass like; iron, copper and sulphur were detected in different crops[24,25,26,27]. Several (*Nelumbo nucifera*, *Embelia ribes*, and *Eugenia Jambolana)* plant seeds contain elements like potassium, manganese, iron and copper with some medicinal activity[28]. Same way seeds of *M.elengi* posses' greater percentage of such element which may have contribution in medicinal value of seeds.

Other element like strontium was also reported in *M. elengi* seeds and bark strontium commonly occurs in nature, and it is the 15th most abundant element on earth, chiefly found in the form of $SrSO_4$ and $SrCO_3$[29]. Its occurrence in plants is a due to many factors, type and chemical composition of soil[30,31], rainfall[32], agricultural practice[33] and kind of plant [34]. Several salts of strontium such as strontium carbonate or strontium citrate are often presented as natural therapies and sold at a dose that is several hundred times higher than the usual Strontium intake. And such salts can still be sold in United States under the Dietary Supplements Health and Education Act of 1994[35]. So occurrence of strontium in bark and seeds of *M. elengi* will not affect any way to the drug quality. Precise

estimation and documentation would also be the steps for quality parameter for *M. elengi*.

CONCLUSION

Pharmacognostic values for seeds and bark for parameters like extractive value, determination of ash, florescence analysis of extract, treatment of powder with acid & alkali, phytochemical analysis of different extract and elemental analysis with significant results can be employed as evaluating parameter. These parameters, which are being reported for the first time in this way for *M.elengi* bark and seed, could be useful as quality control parameter. Any crude drug which is claimed to be *M. elengi* but whose characters significantly deviate from the character above would then be rejected as contaminated, adulterated or downright fake.

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