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Pharmacognostical and anthelmintic studies on leaf of *Mimusops elengi* linn

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Abstract
Objective: The plant *Mimusops elengi* is an annual or perennial ayurvedic plant, is widely distributed in India. It is used in traditional medicine, especially for skin disease, disease of the gum and teeth, astringent, diuretic, etc.
Methods: The present paper report the macroscopically and microscopically studies of leaf of *Mimusops elengi* linn. Some distinct and different characters were observed with section of fresh leaf. Physiochemical parameter and preliminary phytochemical studies of the leaf powder were also carried out.
Results: Anthelmintic activity of different extracts of leaves of *Mimusops elengi* Linn were investigated against *Pheretima posthuma* at various concentrations (10, 25, 50 mg/ml) of each extract were tested in the bioassay, which involved determination of time of paralysis and time of death of worms. Albendazole was included as standard reference and distilled water as control. The methanolic and ethyl acetate shows more potent anthelmintic activity.
Conclusion: The present study on Pharmacognostical investigation of *Mimusops elengi* Linn. leaves might be useful to supplement information in regard to its identification parameters assumed significantly in the way of acceptability of herbal drugs in present scenario lacking regulatory laws to control quality of herbal drugs.

Keywords: pharmacognostical, anthelmintic, *Mimusops elengi* linn, leaves

Introduction

Anthelminticsare drugs that are used in the treatment of helminthiasis (vomiting of worms) or to treat infections with parasitic worms. The different type of worms.
Round worms: they are of two types, Adult intestinal Nematodes and Larval Tissue Nematodes.
Tape worms: They are found in the intestine or Larva in the tissue. *Flukes:* These are hermaphrodites with an exception of blood flukes. They are found in blood vessels, the intestine, biliary tract, lungs.
Symptoms of Helminthiasis are as follows; Abdominal pain, Diarrhea, Fever, Fatigue, Enlarged liver, gastrointestinal inflammation, Eosinophilia and Dehydration.
Mode of transmission: The disease is transmitted through

1. Fecal- oral route for ascaris, trichuris, and hookworm.
2. Skin penetration for hookworms.

Prevention and control: Following measure can be taken for prevention control of the disease.
Personal hygiene, environmental sanitation, cleans food and drinking water and use of slippers and shoes [1].
They are of huge importance for human tropical medicine and for veterinary medicine. The World Health Organization estimates that a staggering 2 billion people harbor parasitic worm infections (http://www.who.int/wormcontrol/statistics/). Parasitic worms also infect livestock and crops, affecting food production with a resultant economic impact. Also of importance is the infection of domestic pets. Indeed, the companion animal market is a major economic consideration for animal health companies undertaking drug discovery programmes.
Intestinal helminthes infections, such as ascariasis, trichuriasis, hookworm and tapeworm infections, continue to be a cause of major concern to human health in several parts of the world, particularly in the developing nations, causing malabsorption, diarrhea, anemia and other states of poor health. Globally, over 3.5 billion people are infected with intestinal worms, of which children between 5– 15 years account for the highest infection rate of about 400 million cases of worm burden that are mainly attributed to poor sanitation and hygiene. In India, infections with these parasites are regarded as amongst the most common public health problems, particularly in rural areas and urban slums [2].
The genus Mimusops elengi belongs to the family Sapotaceae and comprises of thirty species which are distributed in the tropical parts of hemispheres of these *Mimusops elengi*, commonly known as mulsari or bakul cultivated in gardens due to its scented lowers is indigenous to the subcontinent. The plant has been studied through many years phytochemically.
The seed Kernels from *Mimusops elengi* have been investigated previously by Boorsma in 1902 who found 21% fatty oil and 2% saponin [3, 4, 5, 6, 7].
The bark mainly contains saponin and tannins [8, 9, 10, 11, 12].
The leaves contain steroids. The pulp of the fruit contains mainly sugars and saponin. While the lowers contain volatile oil. The parts of its mostly used in medicines [13, 14, 15, 16, 17, 18, 19, 20]. Bark is tonic and febrifuge. Unripe fruit is a useful masticator and therefore recommended to be chewed for fixing loose teeth. Pulp of ripe fruit is eaten as diet in diarrhea and is used in snake bite. Fruits and lowers are used to prepare a lotion for wounds and ulcers. The bark and

unripe fruit is used by dyers to ix colors. Bark increases fertility in women [21, 22].

Material and Methods

Plant material

The plant specimens for the proposed study were collected from the leaves of *Mimusops elengi* was collected from local area of Dhule district, Maharashtra, India, in July 2012, care was taken to selecthealthy plants and for normal organs. The plant was authenticatedby Dr. J- Jayanthi scientist ' C' H.O.D Deputy Director Botanical Survey of India, Koregaon Road, Pune, by comparing morphological features and a sample voucher specimen of plant was deposited for future reference (Voucher specimen number ANSMIE2). (Annexure 1)**.** The required samples

Of different organs were cut and removed from the plant and fixed in FAA (Formalin – 5 ml + acetic acid – 5ml + 70% Ethyl alcohol – 90ml). After 24 hrs of fixing, the specimens were dehydrated with graded series of tertiary – butyl alcohol as per method11. Infiltration of the

specimens were carried out by gradual addition of paraffin wax (melting point 58 – 600C) until TBA solution attained super saturationThe specimens were casted into paraffin blocks.

Sectioning

The leaves were thoroughly washed with water to remove the debris. The sections were taken by placing the leaf portion cut along with the midrib in between the two flat surfaces of pith. transferred the sections into watch glass containing water, filtered and the sections were stained with Phloroglucinol and conc. hydrochloric acid; and then mounted in glycerin and observed under low power 10X and 45X. The transverse sections were studied**.** Digital images captured using a Motic Digital microscope fitted with DCM(USB 2.0) resolution 350k pixels camera imaging accessory and using Motic analysis software). For studying the venation pattern and

Trichomedistribution, paradermal sections (sections taken parallel to the surface of leaf) as well as clearing of leaf with 5% sodium hydroxide or epidermal peeling by partial maceration employing Jefferey' s maceration fluid was prepared. Glycerin mounted temporary preparations

Were made for macerated/ cleared materials. Powdered materials is boiled with chloral hydrate for 5-10 minutes, and then stained with phloroglucinol and conc. hydrochloric acid, dil. iodine, dil. sulphuric acid, & dil. acetic acid. And observed for the microscopic features under high power. Different cell component were studiedand measured [23, 24].

Photomicrographs

Microscopic descriptions of tissues are supplemented with micrographs wherever necessary. Photographs of different magnifications were taken with Motic Digital microscope fitted with DCM (USB 2.0) resolution 350k pixels camera imaging accessory and using Motic analysis software).

Qualitative and quantitative investigation

Qualitative & quantitative investigation of leaf of *mimusops elengi* Linn. were determined such as foreign organic matter, Total ash, Acid insoluble ash, Water soluble ash, moisture content(loss on drying), alcohol soluble extractive and water soluble extractive were determined [23, 24, 25].

Extraction methodology

The leaves of *Mimusops elengi* was collected and dried in the shade and then pulverized in a grinder. The powdered leaves were utilized for extraction. Material was passed through 120 meshes to remove fine powders and coarse powder was used for extraction. ASohxlet extraction method described in Mukherjee (2002) was used for extraction of powdered plant.

The extraction was carried out by using different solvents in increasing order of polarity, like Petroleum ether (60-80), Chloroform, methanol and water [26, 27].

Preliminary phytochemical screening for various extracts

Conventional standard protocols for detecting the presence of different chemical constituents in the plant extract were employed. The tests for the primary & secondary metabolites viz. carbohydrates, proteins, amino acid, steroids, glycosides, alkaloids, tannins, phenolic compounds and flavonoids were carried out with the different extracts of leaves of *mimusops elengi linn*. Using preliminary phytochemical screening [23].

Anthelmintic activity [28, 29, 30].

Worms Collection

Indian earthworm *Pheritima posthuma* (Annelida) were collected from the water logged areas of soils from local area of Dhule district.

Standard Drug

For the present study Albendazole taken as Standard drug. The concentrations of 10mg/ml were prepared in distilled water.

Extract Preparation

The various concentrations (10, 25, and 50 mg/ml) of each extracts were prepared in Distilled water.

Methodology

The Anthelmintic assay was carried as per the method of Ajaiyeoba *et al*. with necessary modifications. The assay was performed on adult Indian earthworm *Pheritima posthuma*, due to its anatomical and physiological resemblance with the intestinal round worm parasite of human being. Because of easy availability, earth worms have been used widely for initial evaluation of anthelmintic compounds *in* vitro, 50 ml of formulation containing different concentration of crude aqueous & solvent extract (10, 25, and 50 mg/ml in distilled water) were prepared and 6 worms of same type were placed in it. Time for paralysis was noted when no movement of any sort could be observed except when the worms were shaken vigorously. Time for death of worms were recorded after ascertaining that worms neither moved when shaken vigorously nor when dipped in warm water (500C). Albendazole (10 mg/ml) was used as reference standard while distilled water as control.

Statistical Analysis

The data presented as Mean ± SEM. The activities of all extracts were compared with the control. All the extracts showed significantly activity with higher duration of paralysis and death. Values of $P<0.001$ were considered statistically significant.

Result and Discussion

Pharmacognostic studies

In Pharmacognostic study of leaves of *Mimusops elengi* (Linn) macroscopy, microscopy, powder characteristic, physical parameters, and extractive values were studied.

Fig 1: Macroscopy of *Mimusops elengi* leaf part

Table 1: Macroscopical characters of leaf of *Mimusops elengi*

Sr. No.	Macroscopical characters	Observation
1.	Color	Glossy dark green
2.	Odor	Not characteristics
3.	Taste	Astringent
4.	Size	Varying in size
5.	Shape	Oval or Elliptical
6.	Apex	Monocronate
7.	Venation	Dicotyledonous
8.	Margin	Entire
9.	Lamina	Thick
10.	Petiole	Pulvinus

Microscopic characters of leaf of Mimusops elengi

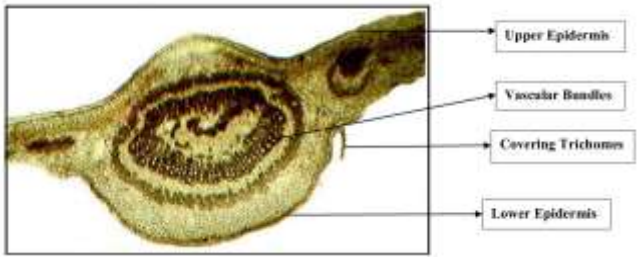

Fig 2: T. S. of Leaf of *Mimusops elengi* stained with phloroglucinol + conc. HCL (Under 40x)

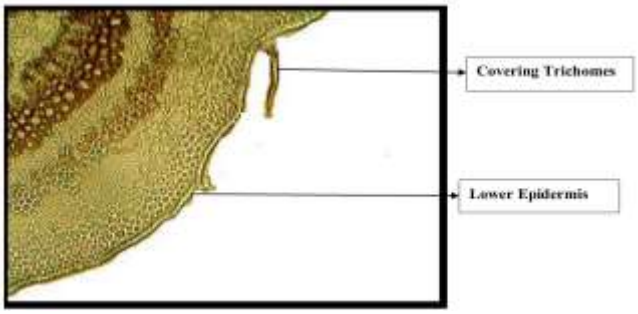

Fig 3: T.S. of Leaf of *Mimusops elengi* (Under 10X) showing various characters stained with phloroglucinol + conc. HCL (1:1)

Powder microscopy of Leaf of Mimusops elengi

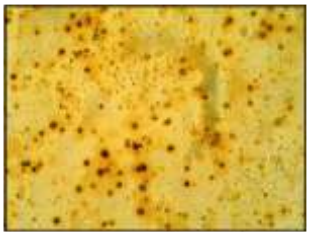

Fig 4: Starch grains stained with dil. iodine solution

Fig 5: Fibre stained with Phloroglucinol + conc. HCL. (1:1)

Fig 6: Calcium oxalate crystals stained with dil. Acetic acid

Fig 7: Calcium oxalate crystals stained with dil. Acetic acid

Fig 8: Calcium oxalate crystals stained with dil. Sulphuric acid

Description of Microscccopy of leaf of Mimusops elengi

The detailed transverse section shows epidermis (both upper and lower) cover the section both in lamina and midrib portion. The vascular bundle are well developed and exposed in the midrib portion. Covering trichomes found on lower surfaces. The epidermis (both upper and lower) followed by hypodermis composed of collenchymatous cells in midrib region. In lamina portion the pallisade cells are in continuation with upper epidermis from above to downward while spongy parenchyma cells followed by lower epidermis from downward to upward. In vascular bundle xylem elements are followed by phloem elements.

Lamina

Upper epidermis - single layered, rectangular epidermal cells with distinct cuticle,

Spongy Parenchyma - Consisted of loosely arranged parenchymatous cells of 4-5 layers with calcium oxalate crystals.

Lower epidermis - was identical to that of upper epidermis.

Powder of leaf of Mimusops elengi

The dried fine powder was stained with Phloroglucinol and Conc. Hcl. Lignified fibers were observed. When stained with dilute iodine solution starch grains were observed. When stained with Dil. H2SO4, calcium oxalate crystals were observed. And when stained with Acetic acid calcium oxalate crystals were observed.

Determination of leaf constant of Mimusops elengi

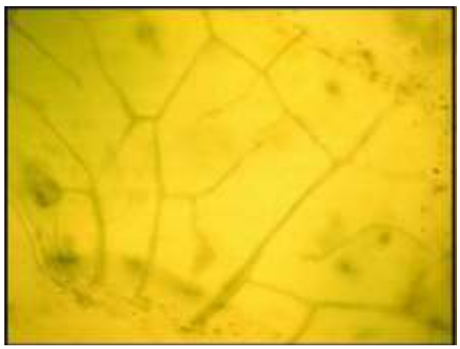

Fig 9: Leaf vein islet number and vein termination number

Table 2: Determination of leaf constant of M*imusops elengi.*

Sr. No.	Particulars	Value
1.	Vein islet number	3-4.26
2.	Vein termination number	2.8-5.42

Qualitative and Quantitative investigation

Table 3: Determination of Leaf constant of *mimusops elengi.*

Sr. No.	Parameters	Values(%w/w)
1.	Foreign organic matter	0.1 %
2.	Loss on drying	11 %
3.	Total Ash value	9.0 %
4.	Water soluble ash value	4.0 %
5.	Acid insoluble ash	2.0 %
6.	Alcoholsoluble extractive	26.4 %
7.	Water soluble extractive	24 %

Table 4: Yield of various extracts obtained from the leaves of *Mimusops elengi* after extraction.

Sr. No.	Extract	Yield %w/w
1.	Petroleum ether	2.99
2.	Chloroform	2.11
3.	Methanol	10.32
4.	Aqueous	16.45

Table 5: Preliminary phytochemical screening of different extract

Sr. No.	Plant constituents	Pet. etherextract	Chloroform extract	Methanol extract	Aqueous extract
1.	Carbohydrates	-	+	+	+
2.	Proteins	-	-	-	-
3.	Amino acids	-	-	+	+
4.	Steroids	+	+	+	+
5.	Glycosides	+	-	+	+
6.	Alkaloids	-	-	+	+
7.	Flavonoids	-	+	+	+
8.	Tannins	-	+	+	+
9.	Triterpenoids	-	-	-	+
10.	Saponins	-	-	+	+

Anthelmintic activity

Table 6: Effect of extract of various concentration of methanolic, aqueous, petroleum ether, chloroform and ethyl acetate extracts of *Mimusops elengi*leaves on paralysis and death time in min. of *Pheretima posthuma* earthworm for studying in vitro anthelmintic activity

Test Substance	Concentration (mg/ml)	Time taken by *Pheretima posthuma* for Paralysis (P) and death (D) of worms in min	
		P	D
Methanolic extract	10	46.08 ± 3.50	62.07 ± 3.79
	25	27.77 ± 2.30	48.06 ± 3.80
	50	18.07 ± 1.89	34.40 ± 2.89
Chloroform extract	10	61.26 ± 3.49	89.50 ± 3.94
	25	45.05 ± 2.99	69.12 ± 3.43
	50	32.06 ± 2.52	55.09 ± 2.99
Pet. extract	10	69.18 ± 3.63	105.37 ±4.13
	25	56.43 ± 3.16	84.20 ± 3.57
	50	36.29 ± 2.68	68.37 ± 3.13
Aq. extract	10	56.04 ± 1.76	78.40 ± 3.0
	25	40.13 ± 2.30	63.15 ± 2.80
	50	27.22 ± 3.10	50.25 ± 3.76
E. A. fraction	10	49.18 ± 3.13	70.53 ± 4.0
	25	30.24 ± 2.44	52.45 ± 3.43
	50	20.23 ± 2.0	40.22 ± 3.03
Albendazole	20	14.06 ± 1.67	33.36 ± 2.56
Control	-	-	-

Graph 1: Time of paralysis and Death of all extract and STD drug

Values are expressed as MEAN ± SEM, one way ANNOVA followed by Dunne' s test.

Note: - n=5 in each group. *P<0.05, **P<0.01, ***P<0.001
Where, ME: Methanolicextract
Aq: Aqueous extract
Pet: Petroleum ether extract
Chl: Chloroform extract
E.A: Ethyl acetate soluble fraction of methanolic extract
STD: Standard Albendazole

Discussion

The pharmacognostic, phytochemical and antioxidant, antimicrobial, anthelmintic potential of leaves of *Mimusops elengi* was evaluated.In pharmacognostic study the morphology of leaves of plant were shows presence of color-glossy dark green, odor-not charecterstics, Taste-astringent, Size-varying in size, Shape-Oval or elliptical, Apex-monocronate, Venation-dicotyledonous, Margine-entire, Lamina-thick, Petiole-pulvinus. In case of microscopy of leaves were studies &shoes presence of epidermis, vascular bundles, covering trichomes, collenchymas& parenchyma etc. In case of microscopic powder characteristics were shows presence of starch grains when stained with dilute iodine solution, calcium oxalate crystals when stained with dilute acetic acid and dilute sulphuric acid, Fibres when stained with phloroglucinol &conc. HCL (1:1).

The physicochemical properties of powder of leaves were examined like as total ash, water soluble ash, acid insoluble ash, extractive values (water soluble & alcohol soluble extractives), loss on drying etc. Determination of leaf constant of mimusops elengi such as vein islet number, vein termination number were examined. Proximate values for the leaf of Mimusops elengi L are as follows. Foreign organic matter (0.1%), Total ash value (9.05%), acid insoluble ash value (2.0%), water soluble ash value (4.0%), alcohol soluble extractives (26.4%), water soluble extractives (24.0%), and, loss on drying (11.0%). These values are criterion to put the guidelines of identity or purity of drugs.Preliminary phytochemical investigation of pet. Ether, chloroform, methanol and aqueous extract were revealed that presence of tannins, flavonoids, alkaloids, steroids, saponins, triterpinoids, glycosides, where in, the steroids, glycosides may present in petroleum ether extract, the Methanolic extract may contains steroids, flavonoids, alkaloids, tannins & phenolic compounds, the Aqueous extract may contains Alkaloids, flavonoids, tannins, saponins & phenolic compounds and the chloroform extract contains steroids, tannins, flavonoids.

The present study revealed that the aqueous, methanol, pet.ether, chloroform and ethyl acetate soluble fraction of methanol extracts of leaf of *Mimusops elengi linn* possess potent anthelmintic property in a dose dependent manner for the parameters studied *viz.* paralysis and death which is quite comparable with standard Anthelmentic drug Albendazole in organisms *Pheritima postuma.* It is due to the presence of active principles in the plant extracts. It acts as potent anthelmintic, because the extracts of the plant contains flavonoids, triterpenoids, alkaloids, steroids, phenolic compounds and tannins. Specifically, tannins present in the extract may be attributed to the anthelmintic activity. Aqueous, methanolic, petroleum ether, chloroform, ethyl acetate soluble fraction of methanol extract of Leaves of *mimusops elengi* exhibited anthelmintic activity using *Pheretima posthuma and* worms in dose dependent manner giving shortest time of paralysis (P) and death (D) with 50mg/ml concentration. The methanolic extract caused paralysis at 18.07±1.89 min. and time of death at 34.40±2.89 min. while aqueous extract revealed paralysis at 27.22±3.10 and time of death 50.25±3.76 min. The ethyl acetate soluble fraction revealed paralysis at 20.23±2.0 and time of death at 40.22±3.03, the chloroform extract revealed paralysis at 32.06±2.52 and time of death at 55.09±2.99 and the petroleum ether extract revealed paralysis at 36.29.23±2.68 and time of death at 68.37±3.13 respectively against the earthworm *Pheretima posthuma*. The standard drug Albendazole showed paralysis at 14.06±1.67 min. and the time of death at 33.36±2.56 minutes. Albendazole by increasing chloride ion conductance of worm muscle membrane produced hyper polarization and reduced excitability that lead to muscle relaxation and flaccid paralysis. The extract of *Mimusops elengi* not only demonstrated paralysis but also caused death of worms especially at higher concentration of 50mg/ml, but not in shorter time as compared to standard drug Albendazole. Phytochemical analysis of crude extract revealed the presence of tannins among other chemical constituents contained within them. Tannins were shown to produce anthelmintic activities. Chemically tannins are polyphenolic compounds. Reported anthelmintic effect of tannins is that they can bind to free proteins in the gastrointestinal tract of host animal or glycoprotein on the cuticle of parasite and may cause death.

Conclusion

It can be concluded that the complete and accurate physicochemical values of the present study will be beneficial for identification and authentification of *Mimusops elengi* leaf powder. Preliminary phytochemical investigation of petroleum ether, chloroform, methanol and aqueous extracts have revealed the presence of tannins, flavonoids, triterpenoids, steroids, saponins,glycosides, alkaloids and phenolic compound.

The study has also shown that methanolic, petroleum ether, chloroform, aqueous extract and ethyl acetate soluble fraction of leaf of *Mimusops elengi* have significantly determined anthelmintic activity. But methanolic extract and ethyl acetate soluble fraction of methanol extract of *Mimusops elengi* showed most significant anthelmintic activity as compare to the other extracts.

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