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PHYTOCHEMICAL AND ANTHELMINTIC SCREENING OF BUTANOLIC FRACTION OF ETHANOL BARK EXTRACT OF MIMUSOPS ELENGI LINN.

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ABSTRACT

Mimusops elengi (M. elengi) which belongs to the family Sapotaceae. The paper presents the physicochemical and anthelmintic studies of bark of *Mimusops elengi* Linn. The present investigation has been undertaken with an objective to establish physico- chemical parameters standards, and *invitro* anthelmintic activity for *Mimusops elengi* Linn. bark so that authentic plant material could be explored properly for its traditional claims. The present study will provide the information in respect of its identification and its activity on against *Pheretima* posthuma and Ascardia galli.

KEYWORDS: Mimusops elengi Linn. Bark, Physico-chemical, Fluorescence, phytochemical, Pheretima posthuma and Ascardia galli.

INTRODUCTION

Different parts of this plant are used in the indigenous system of medicine for treatment of different ailments.[1] In Ayurveda, the bark, flowers, fruit and seeds are of great value for treating various diseases. The diseases such as cardiotonic, alexipharmic, stomachiac, astringent cooling, anthelmintic, tonic, and febrifuge properties and is also useful in alleviating kapha and pitta doshas is treated by using different parts of *M.* elengi. The bark and fruits of this plant are used in the treatment of diarrhoea and dysentery and decoction of the bark is used as a gargling agent.[2] Rinsing mouth with bark decoction is believed to strengthen the gums, reduce inflammation, prevent bleeding of gums, and to stop bad breath caused by pyorrhea and dental caries.[3] The flowers are used to treat liver complaints, nose related problems, headache. Smoke of flowers is used as good smoke for asthma patients The

flowers are used as fragrances. The bark and root gargling is used to reduce tooth inflammations, strengthens the tooth gums The leaves are well known for analgesic and antipyretic *M. elengi* also used in cleaning dermal wounds, anti-ulcer effects and increase the fertility in women.

MATERIALS AND METHODS

The fresh bark of Mimusops elengi Linn. were collected in the month of September (2018) from salipur, Orissa, India. Collected fresh bark were washed and used to evaluate physiochemical parameters. The powder of dried bark was used for the determination ash values, extractive values and phytochemical investigations. Among the different extracts, ethanolic extract was used to carry out to evaluate its anthelmintic activity. All chemicals and reagents used for testing were analytical grade obtained from SD Fine Chemicals, Mumbai (India).

Extraction

The powdered material was extracted successively with petroleum ether (60-80°), ethyl acetate, chloroform and ethanol by using soxhlet apparatus. The solvent was removed under reduced pressure which gave light yellow, green, deep green, dark brown, colored residue for petroleum ether, ethyl acetate, chloroform, and ethalonic extract respectively. The extracts were concentrated under vacuum at 40-60°C which yields a residue (3.3 w/w, 4.8w/w, 11.24%w/w 17.78w/w,) which were stored in a desiccators at room temperature. The ethanol extract was further fractionated with pet ether, chloroform ethyl acetate, butanol.[4]

Physico-chemical parameters

Percentage of total ash, acid-insoluble ash, water soluble ash and sulphated ash were calculated as per the Indian Pharmacopoeia.[5] The total ash of the powdered bark was tested for different inorganic elements. Different extracts of the bark were prepared for the study of extractive values.[6] Fluorescence analysis of powdered bark was carried out by standard methods.[7,8]

Preliminary phytochemical analysis

For the preliminary phytochemical analysis, 5 g powdered drug was extracted with petroleum ether (60-80), ethyl acetate, chloroform and ethanol successively. The extracts fractions were dried and weighed. The presence or absence ofdifferent phytoconstituents viz. triterpenoids,

steroids, alkaloids, sugars, tannins, glycosides and flavonoids, etc. were detected by usual prescribed methods.[9]

In vitro Anthelmintic activity

Butanol fraction of ethanolic extract from the bark of *Mimusops elengi* Linn. was investigated for its anthelmintic activity against *Pheretima posthuma* and *Ascardia galli*. Various concentrations (25, 50 and 100 mg/ml) of ethanolic extract were tested in the bioassay, which involved determination of time of paralysis and time of death of the worms. Piperazine citrate was included as standard reference and distilled water as control. The anthelmintic assay was carried as per the standard method with minor modifications.[10]

In the first set of experiment, three groups of six earthworms i.e. *Pheretima posthuma* were released in to 50 ml of solutions of piperazine citrate, and butanol fraction of ethanolic extract of bark of *Mimusops elengi* linn. (25, 50 and 100 mg/ml each) in distilled water. Observations were made for the time taken to paralysis and death of individual worms. Time for paralysis was noted when no movement of any sort could be observed except when the worms were shaken vigorously. Death was concluded when the worms lost their motility followed with fading away of their body colors. Same experiment was done for *Ascardia galli* worms only the difference was solutions were prepared in normal saline solutions.

RESULTS

Behavior of bark powder of *Mimusops elengi* Linn. with different chemical reagents were performed to detect the occurrence of phytoconstituents along with color changes under ordinary daylight by standard method which is tabulated in Table 1.

Physico-chemical study

The percentage of total ash, acid-insoluble ash, water-soluble ash, sulphated ash and different extractives are tabulated in Table 2 and 3. The qualitative analysis of ash indicated presence of calcium, aluminum, potassium, chlorides and sulphates.

Fluorescence characteristics

When physical and chemical parameters are inadequate as it often happens with the powdered drugs, the plant material may be identified from their adulterants on basis of fluorescence study of different extract and powdered drug which is tabulated in Tables 4 and 5.

Preliminary phytochemical analysis

The preliminary phytochemical analysis of bark extracts of petroleum ether (60-80°C), ethyl acetate, chloroform and ethanol are tabulated in Table 6.

In vitro Anthelmintic activity

Preliminary phytochemical screening of butanol fraction of ethanolic extract revealed the presence of anthraquinone glycosides, phenolic compounds and steroids. From the results shown in Table 7, the predominant effect of piperazine citrate on the worm is to cause a flaccid paralysis that result in expulsion of the worm by peristalsis. The butanol fraction of ethanolic extract of *Mimusops elengi* Linn. demonstrated paralysis as well as death of worms in a comparable time as compared to piperazine citrate especially at higher concentration of 100 mg/ml.

DISCUSSION

The water soluble ash is almost half of total ash and twice of acid insoluble ash. The alcohol soluble extractive value is more than any other extractive value indicating the solubility of phytoconstituents in alcohol. The fluorescence analysis of powder and extract indicate the any fluorescent phytoconstituent present or adulterants. Preliminary phytochemical analysis indicates the nature ofphytoconstituents present in different solvent extract. This also indicates that the butanol fraction of ethanol extract have more number of phytoconstituent than any other fractions i.e. Carbohydrates, alkaloids, glycosides, phytosterol, saponin flavonoids and phenolics. These are few of the important physico-chemical characters of the bark. Piperazine citrate by increasing chloride ion conductance of worm muscle membrane produces hyperpolarisation and reduced excitability that leads to muscle relaxation and flaccid paralysis. Phytochemical analysis of the butanol fraction of ethanol extract revealed presence of flavonoids as one of the chemical constituent. Polyphenolic compounds show anthelmintic activity.[11] It is possible that phenolic content in the extracts of *Mimusops elengi* Linn. might have interfere with energy generation in helminth parasites by uncoupling oxidative phosphorylation which might haveparalyzed and eventually resulted the death of both species of the worm.[12]

CONCLUSION

It can be concluded from this study that butanol bark extracts fraction of *Mimusops elengi* Linn. possess significant anthelmintic activity. In the current research anthelmintic activity of the bark of the plant was explored next to its traditional claims.

Table 1: Behavior of bark powder with different chemical reagents.

Chemical reagents Sl No.	Acid/ Reagent	Observation
1	powder as such	light brown
2	Powder + picric acid	yellow
3	Powder + conc. Nitric acid	red
4	Powder + con. Hydrochloric acid	light green
4	Powder + conc. Sulphuric acid	brown
6	Powder + Glacial acetic acid	colorless
7	Powder + 5% Ferric chloride solution (aqueous)	green
8	Powder + Sodium hydroxide(5N)	yellow
9	Powder + Potassium hydroxide (5%)	light yellow
10	Powder + Iodine/20	red

Table 2: Ash Value *Mimusops elengi* Linn.bark %w/w (Mean±SEM).

Total ash	**7.55±0.167**
Acid insoluble ash	1.76±0.062
water soluble ash	4.41±0.071
Sulphated ash	6.45±0.256

Table 3: Extractive values of *Mimusops elengi* Linn. bark with different solvents.

Types of solvent	% Extractability (Mean±SEM)
Petroleum ether	0.25±0.05
Benzene	0.61±0.02
Ethyl acetate	1.21±0.07
Chloroform	1.61±0.04
Alcohol	6.24±0.07
Water	2.52±0.05

Table 4: Fluorescence analysis of different fraction of ethanol extract of *Mimusops elengi* Linn. bark under UV and visible light.

Extract	Visible light	Ultra Violet	
		Short wave	Long wave
Petroleum ether fraction (60-80°C)	light yellow	yellowish green	blackish green
Chloroform fraction	yellowish green	green	brownish green
Ethyl acetate fraction	light brown	light green	black
Butanol fraction	light brown	green	dark brown
Reagent	Color in day light	Short wave UV	Long wave UV
Powder as such	Light brown	Light brown	Dark brown
Powder+1 N NaOH in Methanol	Light green	Green	Yellowish green
Powder+1 N NaOH	Yellowish green	Green	Brown
Powder+ Ethanol	Colorless	Colorless	Colorless
Powder+ HNO3+NH3 sol.	Light green	Light green	Pale Brown
Powder+50% HNO3	Light green	Green	Brown
Powder + 1 N HCl	Colorless	Colorless	Light brown

Powder+HCl	Light green	Light green	Brown
Powder+H2SO4	Deep brown	Black	Black
Powder+50% H2SO4	Light green	Green	Brown
Powder+glacial acid	Colorless	Colorless	Light brown
Powder+HNO3	Light yellow	Light green	blue

Table 5: Fluorescence analysis of bark powder of *Mimusops elengi* Linn. with different chemical reagents.

Reagent	Color in day light	Short wave UV	Long wave UV
Powder as such	Light brown	Light brown	brown
Powder+1 N NaOH in Methanol	Light green	Green	Yellowish green
Powder+1 N NaOH	Yellowish green	Green	Brown
Powder+ Ethanol	Colorless	Colorless	Colorless
Powder+ HNO3+NH3 sol.	Light green	Light green	Brown
Powder+50% HNO3	Light green	Green	Brown
Powder + 1 N HCl	Colorless	Colorless	Light brown
Powder+HCl	Light green	Light green	Brown
Powder+H2SO4	Deep brown	Black	Black
Powder+50% H2SO4	Light green	Green	Brown
Powder+glacial acid	Colorless	Colorless	Light brown
Powder+HNO3	Light yellow	Light green	blue

Table 6: Qualitative phytochemical analysis of various fractions of ethanol extract of *Mimusops elengi* Linn. Bark.

Types of constituent	Petroleum ether fraction	Ethyl acetate fraction	Chloroform fraction	Butanol fraction
Alkaloid	-	-	-	+
Carbohydrate and glycoside	-	-	+	+
Saponin	-	+	-	+
Protein	-	-	-	-
Sterol	+	+	+	+
Fixed oils and fats	+	-	+	-
Phenolics and flavonoids	-	-	-	+
Gums and mucilage	-	-	-	-

Table 7: Athelmintic activity of butanol fraction of ethanolic extract of *Mimusops elengi* Linn.

Treatment	concentration mg/ml	*Pheretima posthuma*		*Ascardia galli*	
Butanol fraction of Ethanol extract		P	D	P	D
	25	63.70±0.85	71.2±0.45	61.04±0.95	77.5±0.45
	52	43±0.22	64±0.12	45.5±0.15	67.2±0.1

	100	24±0.96	34±0.47	32.2±0.6	45.7±0.23
Piperazine citrate	25	1.7±0.82	53±0.4	41.5±0.15	54.5±0.45
	52	0.95±0.11	28.5±0.12	28±0.5	30.4±0.1
	100	0.55±0.17	20.5±0.80	21.5±0.3	23±0.85
Distill water		-	-	-	-

Where, P: Time taken for Paralysis of worms (min) D: Time taken for Death of worms (min)

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