

Pharmacognostical, Phytochemical and Pharmacological Evaluation of *Mallotus philippensis*

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Abstract

The present study was aimed at Pharmacognostical, Phytochemical and Pharmacological evaluation of the plant *Mallotus Philippinensis* family- *Euphorbiaceae*. Pharmacognostical investigation were carried out by performing organoleptic, microscopical and physicochemical evaluation i.e. ash values, extractive values, moisture content, swelling index, foaming index and foreign matter. The obtained results showed that the moisture content was found to be 1.74%. Similarly swelling index was recorded to be (0.8cm). Foaming index (less than 100). Phytochemical investigation included successive soxhlet extraction, the obtained extractive values were 4.9%, 5.45%, 9.77%, 8.75%, and 4.4% for petroleum ether, chloroform, ethyl acetate, ethanol and distilled water respectively. The preliminary qualitative phytochemical screening revealed the presence of carbohydrates, phenolic compounds, alkaloids, glycosides, flavonoids, steroids, fixed oils and fats. Pharmacological investigation included the Anthelmintic activity by using the *Mallotus Philippinensis* plant ethyl acetate extract. The 200 mg/kg dose to be found positively in Pharmacological responses.

Keywords: *Mallotus Philippinensis*, Anthelmintic activity, Phytochemical investigation, Pharmacological investigation, Pharmacognostical investigation

INTRODUCTION

The plants employed for medicinal purpose are considered to include all plant material such as flower, fruit, root, foliage and seed which may be useful as such or in the form of extracts and chemical compounds isolated from them to produce drugs for human and veterinary medicine. These plants are closely related to those that produce stimulants, condiments, spices, essential oils, and such other higher forms of plants life that produce specific influence on cell metabolism. Among the kingdom of crude drugs (plants, animals and minerals), medicinal plants were first to be used by men. Medicinal plants account for 20% of all medical prescriptions in industrialized countries and for 80% in developing countries¹. The World Health Organization (WHO) has compiled a list of over 20,000 common medicinal plants used in different parts of the globe and many of them are known for their efficacy against different human ailments. India has been endowed with a very rich flora due to the extreme variations in geographical and climatic conditions. These plants have been used since ancient times for the treatment of human diseases². The traditional system of medicines (Ayurveda, Siddha and Unani-Tibb) together with folklore medicine still continues to serve, in spite of the advent of modern medicine, to large

portions of the population, particularly in the rural areas. India is one of the world's twelve leading biodiversity centres with the presence of over 45,000 different plant species, of which about 15,000-20,000 plants have got medicinal values. However, only about 7,000-7,500 is used for their medicinal values by traditional communities. The medicinal potential of plant drugs is well recognized now, as for instance, the consumption of medicinal plants has doubled in last ten years in Western Europe³. It has been estimated that up to 50% of the prescriptions presently dispensed in USA may contain one or more natural product drugs. It seems certain that the continued scientific study of medicinal plant will afford a plethora of novel, structurally diverse bioactive compounds. The WHO has emphasized the utilization of indigenous system of medicine based on the ideally available raw materials i.e. medicinal plant⁴.

Plant Description:

A bush to small or medium-sized tree, up to 25 metres tall and a trunk diameter of 40 cm. The trunk is fluted and irregular at the base¹⁰. Leaves are opposite on the stem, ovate to oblong in shape. 4 to 12 cm long and 2 to 7 cm wide with a long pointed tip. The upper surface is green without hairs, the underside

pale grey in colour. Leaf stems 2 to 5 cm long, somewhat thickened at both ends. The first leaf vein on either side of the mid rib extends from the leaf base, to over half the length of the leaf¹¹

Plant Description:

- **Bark:** Slender branch bark is pale, and the younger branch is covered in rust-red matted hairs.
- **Leaves:** Alternate, ovate-lanceolate, 8-22 x 3-8 cm, 3-nerved at base, glabrous above, pubescent and with numerous red glands beneath.
- **Flowers:** Small; dioecious, males in erect terminal spikes forming elongated panicle racemes; females solitary in short spikes, ovary covered with red glands. Flowers are covered by rust red matted hairs.
- **Fruits:** Globose, 3-lobed, 8-10 mm in diameter, covered with bright red powder.
- **Seeds:** Subglobose, black, 3-4 mm across.
- **Plant type / Growth Habit:** Tree
- **Duration:** Perennial
- **Distribution:** Found throughout India, occasionally ascending to 1500 m in the outer Himalayas; also found in Sri Lanka, Southern China, Myanmar, Thailand, and throughout Malaysia to Australia.
- **Habitat:** Subtropical and tropical areas.
- **Vernacular names / Synonyms:**

Ayurvedic: Kampillaka, Kampilla, Kapila, Karkasha, Raktanga, Rechi, Kampilla.

Bengali: Kamala, Kamalagundi

English: Kamala tree, Monkey Face Tree, Dyers rotlera, Kamala dye tree, Monkey face tree, Orange kamala, red kamala, scarlet croton

Gujrat: Kabilo

Hindi: Kamala, Sindur, Rohini, Kambhal

Kannada: Kampillaka, Kunkumadamara

Punjabi: Kumila, Kamal, Kambal, Kamela

Siddha: Kamela

Unani: Kamila

METHODOLOGY

1. Aims and Objectives

It comprised of consecutive three steps:

Part A: Pharmacognostical Studies

Part B: Phytochemical Studies

Part C: Pharmacological Studies

Part A: Pharmacognostical Investigation

It included collection, identification and authentication of plant material, drying and size reduction, organoleptic evaluation, microscopic evaluation (transverse section of leaf, stem and root), powder microscopy and determination of leaf constant⁹.

Physico-chemical investigation included determination of foreign organic matter, ash value (total ash, acid insoluble ash and water soluble ash), extractive value, moisture content (loss on drying), swelling index and foaming index.⁵

Part B: Phytochemical investigations

It included extraction (successive soxhlet extraction with increasing polarity of various solvents- petroleum ether, chloroform, ethyl acetate, ethanol and water), phytochemical screening (chemical tests of various extracts) and fluorescence analysis⁶.

Part C: Pharmacological Study

Evaluation of *in vitro* Anthelmintic activity:

All the experiments were carried out in Indian adult earthworms (*Mallotus philippensis*) due to its anatomical resemblance with the intestinal roundworm parasites of human beings. They were collected from moist soil and washed with water to remove all fecal matters.⁷

Experimental Design:

The Anthelmintic activity was performed according to the Ghosh *et al.*, method⁸. On adult Indian earth worm *Mallotus philippensis* as it has anatomical and physiological resemblance with the intestinal round worm parasites of human beings. *Mallotus philippensis* was placed in petridish containing four different concentrations (25, 50, 100 and 200mg) of methanolic & aqueous extract of *Mallotus philippensis*. Each petridish was placed with 4 worms and observed for paralysis or death. Mean time for paralysis was noted when no movement of any sort could be observed, except when the worm was shaken vigorously; the time death of worm (min) was recorded after ascertaining that worms neither moved when shaken nor when given external stimuli. The test results were compared with reference compound albendazole (15 mg/ml) treated samples.

RESULT:

1. Pharmacognostical Investigation:

A. Organoleptic Evaluation:

Table 1: Organoleptic evaluation of the plant *Mallotus philippensis*

Aerial part	
Characters	Observation
Colour	Reddish brown
Texture	Coarse
Taste	Bitter
Odour	Odourless

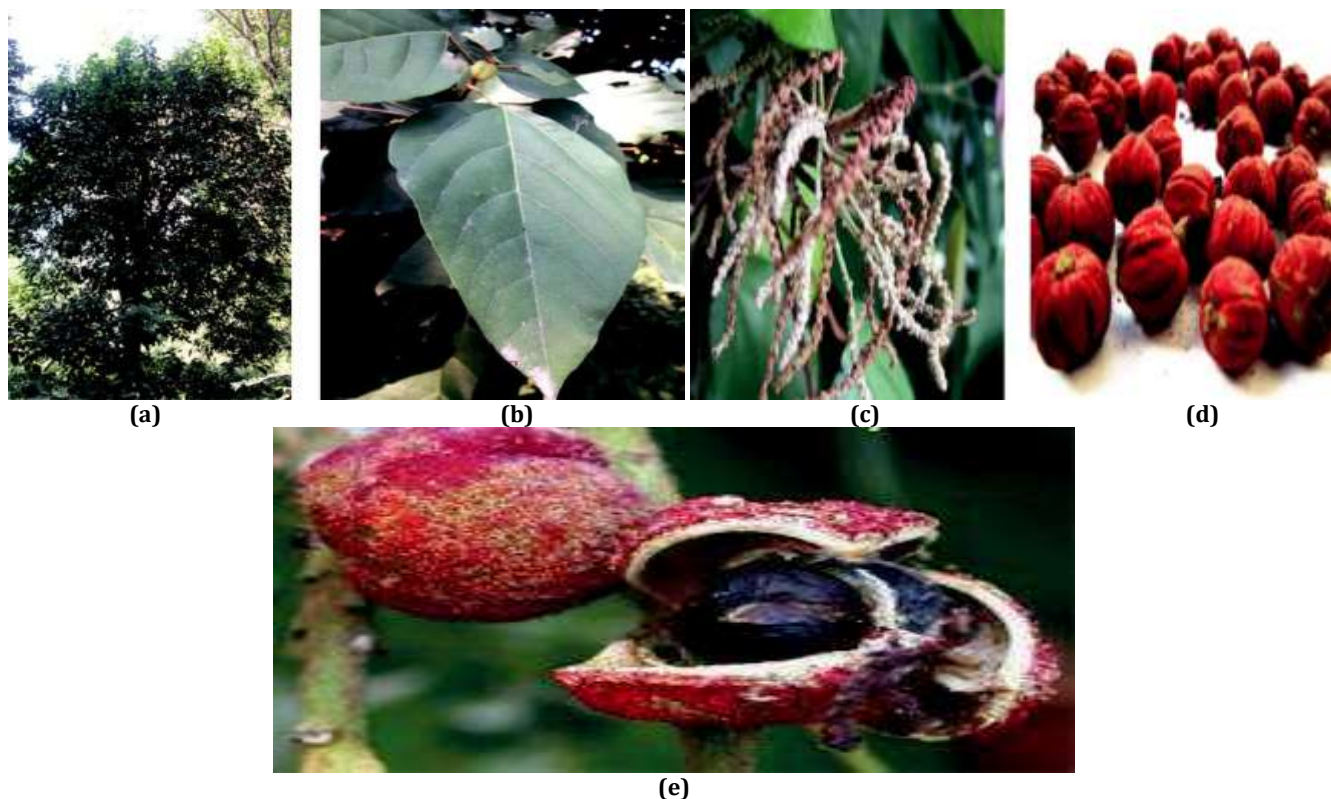


Figure 1: *Mallotus philippinensis*. (a) Mature plant; (b) leaf; (c) initial inflorescence of seed setting; (d) mature fruits twig; (e) mature fruit with seed

B. Powder microscopy:

Table 2: Powder microscopy of the aerial part of the plant. *Mallotus philippinensis*

SI.NO.	Part of the plant	Characters
1.	Dry powder of the aerial part of <i>Mallotus philippinensis</i>	Uniseriate multicellular trichomes Anisocytic stomata cruciferous Sclerenchymatus fibers Starch grains Bundle of acicular Cluster crystals

C. Stomata number:

Table 3: Stomatal number of the plant *Mallotus philippinensis*

SI NO.	Parameters	Value (1mm ²)
1	Vein islet number (1 mm ² leaf surface)	22
2	Vein termination number	15
3	Stomatal index (per sq.mm)	Upper surface-0.22 Lower surface- 035
4	Stomatal number (per sq.mm)	Upper surface-15.94 Lower surface-27.09

D. Transverse section examination of stem and flower:

It was carried out by using the fresh *Mallotus philippinensis* plant parts for section cutting. Stems and leaf were soaked in chloral hydrate for few minutes in order to make them soft and

then the cross sections were prepared by taking free hand section. The finally prepared slides were then captured through compound microscope and labeled .The labeled characters was as shown in figure.

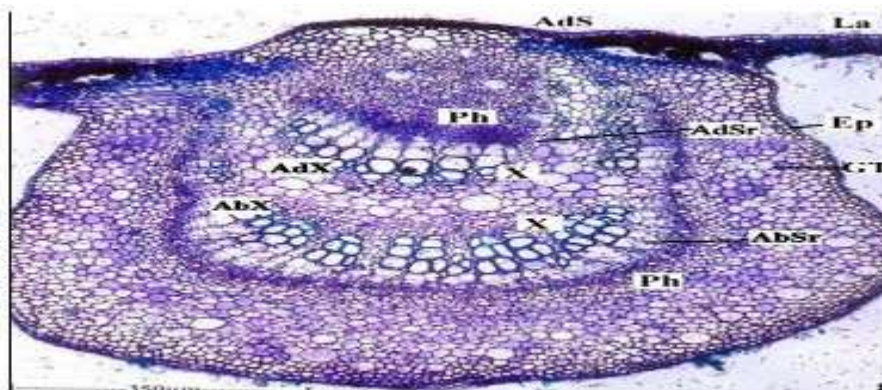


Figure 2: Transverse Section of leaf through Midrib (10X)

(AdS - Adaxial side, AbSr - Abaxial strand, AdSr - Adaxial strand, AbX - Abaxial xylem, AdX - Adaxial xylem, Ep - Epidermis, GT - Ground Tissue, La - Lamina, Ph - Phloem, X - Xylem)

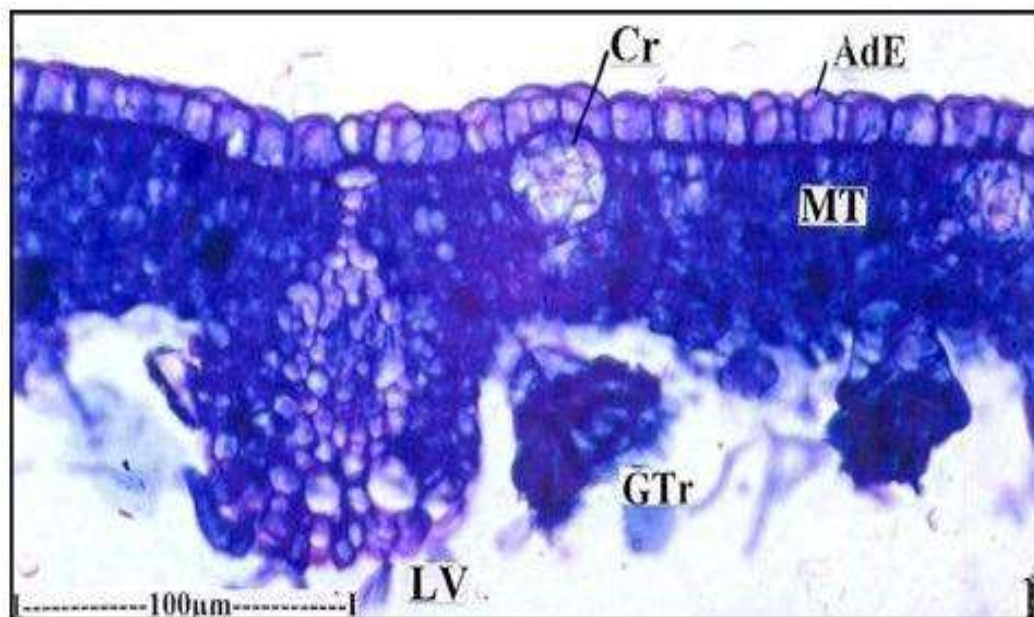


Figure 3: Transverse section of lamina showing crystals, glandular and non glandular trichomes (40X)
(AdE - Adaxial epidermis; Cr - Crystal; GTr. - glandular trichomes; LV - Lateral vein; MT - Mesophyll tissue)

2. Physico-Chemical Investigation

A. Foreign organic matter:

Table 4: foreign organic matter of the plant *Mallotus philippensis*

SI.NO.	Parameter	% yield (w/w)
1	Foreign Organic matter	0.18

B. Ash value:

Table 5 Ash value of the plant *Mallotus philippensis*

SI.NO.	Parameters	% Values (w/w)
1	Total ash	27-37%
2	Acid insoluble ash	37%
3	Water soluble ash	11-16%

C. Moisture content (Loss on drying)

Table 6 Moisture content of the plant *Mallotus philippensis*

SI.NO.	Parameter	%Value (w/w)
1	Moisture content	1.74

D. Swelling index

Table no: 7 swelling index of the plant *Mallotus philippensis*

SI.NO.	Parameter	Value (cm.)
1	Swelling index	0.8

E. Foaming index

Table no: 8 foaming index of the plant *Mallotus philippensis*

SI.NO.	Parameter	Value
1	Foaming index	Less than 100

3. Phytochemical Investigations

A. Extractive Yield of Different Extracts

Table no: 9 Extractive values of the *Mallotus philippensis*

Extracts	% Yield	Color
Petroleum Ether	4.9%	Reddish brown
Chloroform	5.45%	Reddish brown
Ethyl acetate	9.77%	Reddish brown
Ethanol Extract	8.75%	Reddish brown
distilled water	4.4%	Reddish brown

B. The phytoconstituent of the plant *Mallotus philippensis***Table 10** The phytoconstituent of the plant *Mallotus philippensis*

Sl. No.	Test/ reagent used	Extracts		
		Pet. ether extract	Chloroform extract	Ethanol Extract
1	Alkaloids			+
	Mayer's Reagent	-	-	+
	Dragendroff's Reagent	-	-	+
	Wagner's Reagent	-	-	+
	Hager's Reagent	-	-	+
2	Carbohydrates:-			
	Molisch's Test	-	-	+
	Fehling's Test	-	-	+
	Benedict's Reagent	-	-	+
	Barfoid's Test	-	-	+
	Iodine Test	-	-	+
3	Glycosides:-			
	Keller-Killiani Test	+	-	-
	Legal Test	+	-	-
	Modified Borntrager's Test	+	-	-
	Borntrager's Test	+	-	-
4	Proteins and Amino acids:-			
	Ninhydrine Test	-	-	+
	Biuret Test	-	-	+
	Millon's Test	-	-	+
	Xanthoproteic Test	-	-	+
5	Tannin:-			
	Ferric chloride solution	-	+	+
	Gelatin solution	-	+	+
	Lead acetate solution	-	+	+
6	Terpenoids	+	+	-
7	Saponin	+	-	-
	Foam Test	+	-	-
	With NaHCO ₃	+	-	-
8	Flavonoids			
	With NaOH	-	-	+
	With H ₂ SO ₄	-	-	+
	With Mg/HCl	-	-	+
9	Steroids:-			
	Liebermann's Test	-	+	+
	Salkowski test	-	+	+

Pharmacological Study

Table 12- indicates the phytochemical constituents of methanolic, benzene and aqueous extract of the *Mallotus philippensis* when subjected to qualitative analysis for carbohydrates, protein, alkaloids, flavonoids, steroids, saponin, glycosides, terpenoids, tannins and phenols. By preliminary phytochemical screening it was found that all the three extract of plant contain carbohydrates, protein, alkaloids, flavonoids, steroids, saponin, glycosides, terpenoids, phlobatannins, tannins and phenols.

Table 13 - shows higher concentration of extract produced paralytic effect much earlier and time taken for death was shorter for worms. Aqueous and methanol extract of *Mallotus philippensis* exhibited anthelmintic activity in dose - dependent manner showing maximum efficacy at 25,

50, 100 and 200, mg/ml concentration for worms than benzene extract of *Mallotus philippensis*

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Figure 13- shows higher concentration of extract time taken for death was shorter for worms. Aqueous and methanol extract of *Mallotus philippensis* exhibited anthelmintic activity in dose- dependent manner showing maximum efficacy at 25, 50, 100 and 200 mg/ml concentration for worms than benzene extract of *Mallotus philippensis*.

Table 11- Phytochemical analysis of different solvent extracts of *Mallotus philippensis*

S.No	Phytochemical Constituents	Methanolic extract	Benzene extract	Aqueous extract
1.	Carbohydrate	+	+	+
2.	Protein and amino acids	+	+	+
3.	Alkaloids	+	+	+
4.	Flavonoids	+	+	+
5.	Steroids	+	+	+
6.	Saponin	+	+	+
7.	Tannins	+	+	+
8.	Phenols	+	+	+
9.	Glycosides	-	+	+

: (+) Present, (-) Absent

Table-12 *In vitro* Anthelmintic activity of various extracts *Mallotus philippensis*

Group	Treatment of extracts	Concentration (mg/ml)	Time taken for paralysis (min)	Time taken for death (min)
1.	Normal control	-	-	-
2.	Experimental control	-	-	-
3.	Albendazole	15	43 ± 1.61	55 ± 1.60
4.	Methanol	25	58 ± 4.16	74 ± 6.35
		50	52 ± 4.54	63 ± 2.94
		100	33 ± 2.38	42 ± 2.64
		200	27 ± 1.63	35 ± 2.00
		Hydroalcoholic	25	77 ± 1.11
	50	64 ± 1.11	81 ± 1.11	
	100	53 ± 1.11	69 ± 1.11	
	200	38 ± 1.11	55 ± 1.11	
6.	Aqueous	25	56 ± 9.91	69 ± 9.91
		50	50 ± 9.72	60 ± 9.72
		100	42 ± 1.29	48 ± 1.41
		200	27 ± 1.29	32 ± 1.41

All values represents mean ± SD; n=4 in each group. Comparisons made between standard / treated groups.

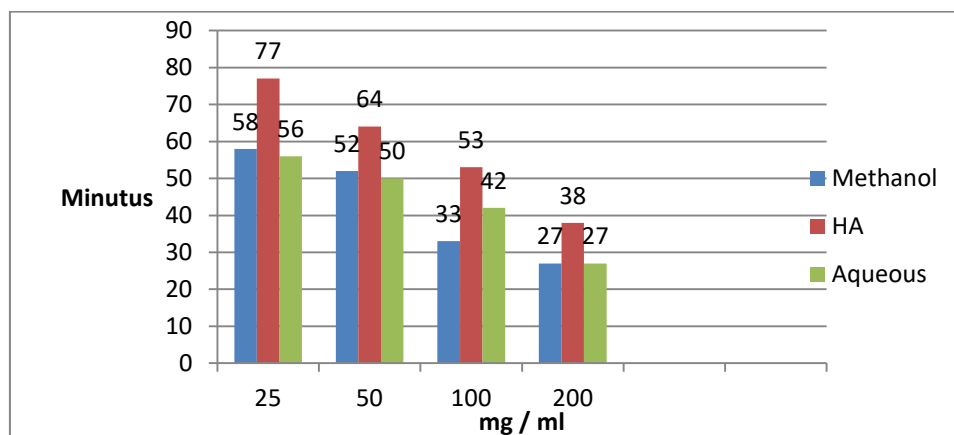


Figure 4: Time taken for paralysis of *Pheretima posthuma* by various solvent extracts of *Mallotus philippensis*

DISCUSSION

Helminthes infections are among the most widespread infections in humans, distressing a huge population of the world. Although the majority of infections due to helminths are generally restricted to tropical regions and cause enormous hazard to health. To evaluate compounds with anthelmintic activity, a number of substances were analyzed using different species of worms, for example, earthworms, *Ascaris*, *Nippostrongylus* and *Heterakis*. From all these species, earthworms have been used extensively for the preliminary evaluation of anthelmintic compounds *in vitro* because they are similar to intestinal "worms" in their reaction to anthelmintics and are easily accessible. It has been verified that all anthelmintics which are toxic to earthworms are creditable to study as an anthelmintic. Earthworms have the ability to move by ciliary movement. The outer layer of the earthworm is a mucilaginous layer and composed of complex polysaccharides. This layer being slimy enables the earthworm to move freely. Any damage to the mucopolysaccharide membrane will expose the outer layer and this restricts its movement and can cause paralysis. This action may lead to the death of the worm by causing damage to the mucopolysaccharide layer. This causes irritation leading to paralysis. Commonly used anthelmintic drugs like piperazine citrate and albendazole by increasing chloride ion conductance of worm muscle membrane produces hyper polarization and reduced excitability that leads to muscle relaxation and flaccid paralysis.

CONCLUSION

The process of new drugs development is enforced by the success of herbal drugs in pharmaceutical market. Another approach to natural product drug discovery is to utilize the information derived from traditional system that have used plant product to control disease and injury. From industrial view point regarding sufficient supply of natural product derived active constituents. As the amount of secondary metabolites are less so the yield is less. One approach that has been successful in development of simpler semi-synthetic or synthetic analogs is designed which also improves its pharmaceutical properties. A great approach to meet the demand of secondary metabolites is the use of tissue culture techniques.

From the experimental work it was clear that the Aqueous shows positive test for terpenoids, Methanolic shows to contain terpenoids and steroids, chloroform extract shows positive test for carbohydrate, glycosides and steroids whereas ethanol extract found to contain carbohydrate,

cardiac glycosides, tannins, flavonoids and saponins. The therapeutic value reported that it has an Anthelmintic effect in ethanolic extract and significant at a high dose of 200mg/ml by is show the good pharmacological activity.

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Conflict index:

No conflict of interest

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