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Anthelmintic Activity of *Manilkara hexandra* (Roxb) Dubard Leaves Extract on Indian Earthworm (*Pheretima posthuma*)

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Research Article

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ABSTRACT

The aim of present study was to evaluate the anthelmintic activity of aqueous extract of *Manilkara hexandra* (Roxb) dubard, is a tree species in the tribe Sapoteae, in the family Sapotaceae has been used in different system of traditional medication for the treatment. The therapeutic properties of the plant's leaves, fruits, roots, and bark are well-known. Anthelmintic activity can be determined by time of paralysis and time of death of the worms. Piperazine citrate is used as standard refrance drug. *Manilkara hexandra* (Roxb) is a native of India, primarily found as a wild tree in the southern and northern parts of the country. It is widely spread in Gujarat, Rajasthan, Madhya Pradesh, Andhra Pradesh, Kerala and Maharashtra. Our goal is to gather useful information about the plant's morphology, microscopy, phytoconstituents and pharmacological aspects.

INTRODUCTION

Manilkara hexandra (Roxb) dubard (family-Sapotaceae) is a small to medium sized evergreen tree. It is widely prevalent to South Asia and tropical countries, and it can be found in large numbers in South, North, and Central India particularly in Rajasthan, Gujarat, Madhya Pradesh, and Maharashtra. *Manilkara hexandra* is a huge slow-growing evergreen plant. It can be found in both tropical and temperate rainforests. The tree can grow to be 12 to

25 m tall with a trunk Circumference of 1 to 3 m. The bark is tough and grey in colour. The wood is robust, long-lasting and strong with a density ranging from 0.83 to 1.08 t/m^3 depending on the degree of drying. Despite the difficulty of working with such solid wood it is utilized for heavy structural work, gate posts, and large beams, as well as turning and carpentry. It serves as a rootstock for *Manilkara zapota* and its own fruits are edible [1,2].

The tree is long lived, small to medium size, with a spreading crown, straight growing and massive hole. Flowering occurs in the month of October-November-December. And fruit ripens during May-June. Ripe fruits are eaten fresh or after dehydration, they are sweet but astringent. The seed contains 24.6% of edible oil. Although the fruits and plants have immense importance and potential the commercial cultivation of khirni is restricted due to lack of healthy planting material. Because work on vegetative methods of propagating khirni is limited, new plants are only prepared by raising seedlings. The recalcitrant nature of khirni seeds and their hard seed coat mess dormancy or "hardseededness." The tough seed coat prevents water absorption and limits gaseous exchange. The short viability of seeds prevents long-term storage, reducing bulk availability of planting material even further. Furthermore, the slow growth rate of khirni Seedlings is a disadvantage in terms of instant and mass multiplication [3,4].

An examination of the literature reveals extracts and metabolites from this plant possess pharmacological properties such as anti-inflammatory, antiulcer, aphrodisiac, alexipharmic, anthelminthic, antibacterial, and free radical scavenging activity. Beside medicinal uses plant has high economic value due to its edible and nutritive fruit, useful wood, latex and bark and provides substantial livelihood support to local inhabitants. A wide range of chemical Compounds including sterols, starch, terpenoids, anthraquinone glycoside, cardiac glycoside, saponinand tannins etc. have been isolated from this species. The presented research summarizes the information concerning the traditional uses, phytochemistry and biological activity of *Manilkara hexandra* (Figure 1).

Figure 1: Leaves and fruit of *Manilkara hexandra*.



Vernacular names

- English: Obtuse leaved mimusops
- Hindi: Khirni, khirani
- Marathi: Khirni
- Sanskrit: Raajaadan, Phalaadhyakhsa
- Tamilnadu: Ulakkaippa-lai Palai
- Telugu: Patla, Pola, Kirni
- Ayurvedic: Khirni
- Siddha: Palai
- Malayalam: Krini and Palamunpala
- Kannada: Hale and Hannu

Botanical classification

- Kingdom: Plantae
- Phylum: Tracheophyta

- Sub-phylum: Euphyllophytina
- Class: Magnoliopsida
- Sub-class: Magnoliidae
- Order: Ericales
- Family: *Sapotaceae*
- Genus: *Manilkara*
- Species: *Manilkara hexandra*

Traditional uses

It is commonly used in remedial herbal medicine to treat conditions such as jaundice, ulitis, odontopathy, fever, colic dyspepsia, helminthiasis, hyper dyspepsia, and burning Sensation. It helps with swelling, abdominal colic, gout, rheumatism, and toxicosis by purifying the blood. It is made up of a combination of constituents that have anti–inflammatory, diuretic, antiurolithiatic, analgesic, antipyretic, and antimicrobial properties. The oil extracted from the seeds is used as a cooking oil by the indigenous people. The Koli tribe uses the bark decoction to treat diarrhoea in children. The stem bark is also used to treat fever, jaundice, helminthiasis, flatulence, stomach disorders, and other ailments. The seeds are being studied for their pharmacology but there have been no reports on the plants stem bark. The seeds are used as a cardiac tonic and to treat anorexia. It also causes muscle weight gain and obesity. According to a survey conducted in the Jalgaon district of North Maharashtra, its fruits are used to treat digestive disorders. *Manilkara hexandra* leaf extract is used to treat asthma in the Rayalaseema region of Andhra Pradesh by tribal people [5,6].

Introduction to helminthis

Helminth is a term that refers to any worm. Invertebrates with elongated, flat, or circular bodies are known as helminths. Flukes and tapeworms are flatworms or platyhelminths (platy from the Greek root meaning “flat” in medically focused schemes. Roundworms are a type of worm. Nematodes (from the Greek word nematos, which means “thread”). These divisions are made for lung flukes for example are convenient according to the host organ in where they dwelt. Tapeworms and roundworms in the intestine. There are three types of helminthes are Flukes (Trematodes), Tapeworms (Cestodes), Roundworms (Nematodes) [7,8].

Pheretima posthuma

Pheretima posthuma is a species of earthworm found in Asia. It belongs to the *Megascolecidae* family's of earthworms, which are found primarily in portions of Southeast Asia, such as India, As well as parts of oceania and North America (Figure 2).

Figure 2. Indian earthworm (*Pheretima posthuma*).

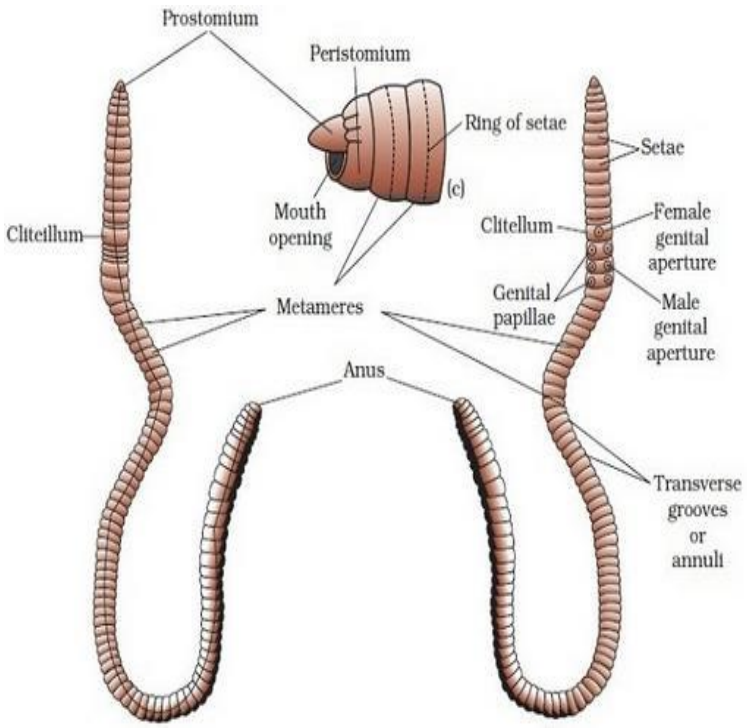
Scientific classification

- Kingdom: Animalia
- Phylum: Annelida
- Class: Clitellata
- Order: Opisthopora
- Suborder: Lumbricina
- Family: *Megascolecidae*
- Genus: *Pheretima*

Structure of *Pheretima*

The body of earthworm is elongated, narrow and cylindrical measuring about 20 cm in length and 3 to 5 mm in width. The anterior end is more pointed than the posterior end. The dorsal side of the body is brown in colour and can be distinguished from the ventral side which is lighter in colour. The body of earthworm consists of about 100-120 small ring-like segments somites or metameres. It exhibits true segmentation i.e. the external segmentation corresponds with internal segmentation (Figure 3) [9,10].

Figure 3. Structure of *Pheretima posthuma*.



MATERIALS AND METHODS

Collection of plant material

Fresh plant leaves of *Manilkara hexandra* (*Sapotaceae*) were collected from the local area at lonkheda and authentified by Dr. Santosh K Tayade. Head, Dept. of Botany, Arts science and Commerce College, lonkheda, Shahada, Dist- Nand urbar. After authentification fresh leaves of plant were collected wash under running tap water dried under shade for period of 7 days and then pulverized in mechanical grinder to obtain coarse powder. The dried powder was store in airtight bottles [11].

Animals

Indian adult earthworms (*Pheretima posthuma*) were used to study anthelmintic activity. The earthworms were collected from moist soil at local area of lonkheda and authentified by Dr. Head R.M. Chaudhari Dept. of zoology arts science and commerce college lonkheda, shahada dist.nandurbar (Figure 4).

Figure 4. Earthworm (*Pheretima posthuma*).

Prepration of extract

Aqueous extract: The course powered material (50 gm) was soaked in distilled water (250 ml) by simple cold maceration method for continuous 7 days and then concentration was evaporated until concentrate is left and then dry.

Ethanolic extract: The coarse powder material each (50 gm) was soaked in ethanol solution (250 ml) by simple maceration method for continuous 7 days and strained and evaporated until concentration is left and then dry.

Petroleum ether extract: The coarse powdered material (50 gm) was soaked in Petroleum ether solution (250 ml) by maceration method for continuous 7 days and strained and evaporated until concentrate is left and then dry.

Chemicals: Saline solution, Piperazine citrate, Tween 80 [12].

Anthelmintic activity

The anthelmintic assay was carried out as per the method of Ajaiyeoba et al. with necessary modification. The assay was performed on adult Indian earthworms *Pheretima posthuma* due to its anatomical and physiological resemble with the intestinal round warm parasite of human being because easy of availability. Earthworms have been used widely for initial evaluation of anthelmintic compound. 20 ml of formulation containing different concentration of crude aqueous extract (25, 50, 75 mg/ml) were prepared and 3 worms of same type were placed in Petri plate and observe them same concentration for ethanolic extract (25,50,75 mg/ml) were prepared [13]. Concentration of crude petroleum ether extract (5, 10, 15 mg/ml) were prepared to and 3 worms of same type were placed in it and observe them. Piperazine citrate (10 mg /ml) was used as reference standard and saline solution as control observation were made for the time take for paralysis was noted. When no movement of any sord could be observed except when the worms were shaken vigorously. Time for death of worm were noted after ascertaining that worms neither moved when shaken vigorously nor when dipped in saline solution as shown in Figures 5-8 (Table 1 and Figure 9).

Figure 5. Aqueous extract 75 mg/ml.

Figure 6. Ethanolic extract 75 mg/ml.

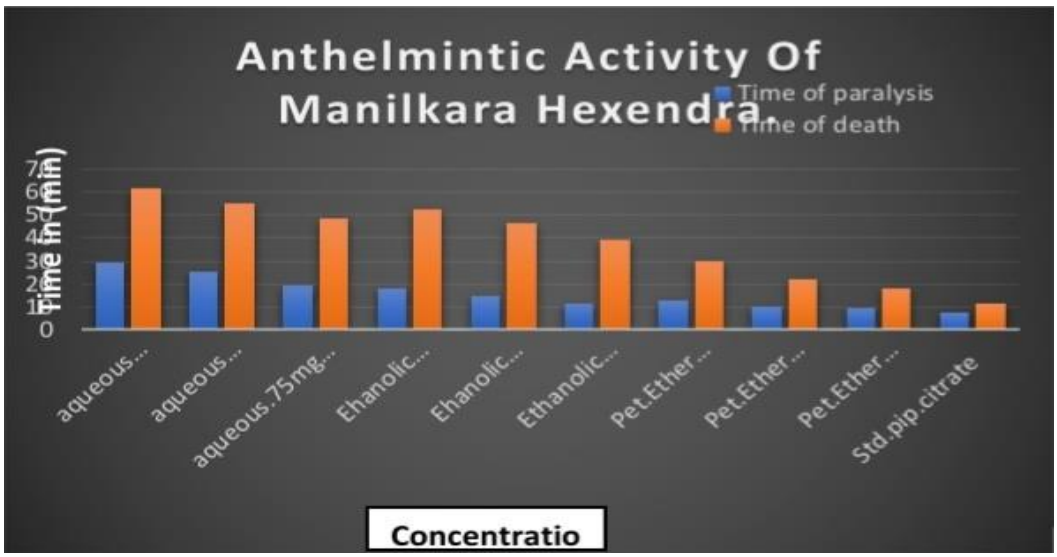
Figure 7. Petroleum ether extracts 15 mg/ml.

Figure 8. Standard drug (Piperazine Citrate) 10 g/ml.

Table 1. Obesevation table of *Manilkara hexendra* leaves extract.

Sr.no.	Extract	Concentration in mg/ml	Indian earthworm (Pheretima posthuma)	
1	Aqueous extract	25 mg/ml	29:36 min	62:00 min
2		50 mg/ml	12:28 min	55:24 min
3		75 mg/ml	19:42 min	48:19 min
1	Ethanolic extract	25 mg/ml	18:37 min	52:30 min
2		50 mg/ml	15:05 min	46:24 min
3		75 mg/ml	11:31 min	39:17 min
1	Petroleum ether extract	5 mg/ml	13:15 min	30:14 min
2		10 mg/ml	10:08 min	22:08 min
3		15 mg/ml	9:44 min	18:12 min
1	Piperazine citrate	10 mg/ml	7:46 min	11:57 min
2	Control normal (Saline solution)	-	-	-

Figure 9. Anthelmintic activity of *Manilkara hexandra* (roxb) dourb leaves extract on indian earthworms (*Pheretima posthuma*). **Note:** (▮) Time of death; (▮) Time of paralysis.



PHYTOCHEMICAL TEST

Test for alkaloids

Mayer's test: The test was done by adding 1-2 ml of the mayer reagent to 5 ml of plant extract. The formation of white creamy precipitate wound show positive result for the presence of alkaloids.

Wagner's test: Wagner's reagents add into the plant extracts presence of brownish to yellowish precipitate shows presence of alkaloids.

Hager's test: To the extract 2 ml of Hager's reagent was added the formation of yellow precipitate confirmed the presence of alkaloids.

Dragendorff's test: In a test tube containing 1 ml of extract few drops of Dragendorff's reagent was added and the colour developed was noticed. Appearance of orange colour indicates the presence of alkaloids (Table 2) [14].

Table 2. Test for alkaloids present in *Manilkara hexendra* leaves extract.

Sr.no.	Test name	Procedure	Observation
1	Mayer's test	2-3 ml filtrate add few drops of Mayer's reagent	Positive
2	Hagers test	2-3 ml filtrate add few drops of Hagers reagent	Positive
3	Wagers test	2-3 ml filtrate add few drops of Wagner reagent	Positive
4	Dragendroffs test	2-3ml filtrate add few drops of Dragendroffs reagent	Positive

Test for flavonoids

Shinoda test: To the extract, a few magnesium turnings and 2 drops of concentrated hydrochloric acid were added, formation of red color showed the presence of flavones.

Zinc HCl test: To test the sample solution for the flavonoids added a mixture of zinc dust and concentrated hydrochloric acid results in red color (Table 3).

Table 3. Test for flavonoids present in *Manilkara hexendra* leaves extract.

Sr.no	Test name	Procedure	Observation
1	Shinoda test	Extract+5ml of 95%of ethanol few drop of conn.HCl+0.5 g of magnesium turning	Positive
2	Zinc HCl test	Drug sol+Zinc dust +HCl	Positive

Test for Carbohydrate

Molisch's test: To the extract, 1 ml of alpha-naphthol solution, and concentrated sulphuric acid through the sides of test tube were added. Purple or reddish violet colour at the junction of the two liquids revealed the presence of carbohydrates.

Fehling's test: To the extract equal quantities of Fehling's solution A and B were added and on heating, formation of a brick red precipitate indicates the presence of carbohydrates [15,16].

Benedict's test: To 5 ml of Benedict's reagent, extract was added and boiled for two minutes and cooled. Formation of red precipitate showed the presence of carbohydrates (Table 4).

Table 4. Test for carbohydrates present in *Manilkara hexendra* leaves extract.

Sr.no	Test name	Procedure	Observation
1	Molish test	Extract+Alpha napthol in alcohol+conn.H_2SO_4	Positive
2	Fehlling test	Extract+Fehlling A+Fehlling B boil on water bath for 5min	Positive
3	Benedic test	Extract+Benedic reagent	Positive
4	Barford test	Equal volume of Barford reagent and test solution heat for 2-3min	Positive
5	Iodine test	Extract+few drop of dil.iodin solution	Positive

Test for tannin

Lead acetate test: The extract was mixed with basic lead acetate solution formation of white precipitate indicated the presence of tannins.

Bromine water test: To the extract 3-4 drop of bromine water appearance of buff colure indicates the presence of tannin.

Iodine test: Extract treated with few drop of diluted iodine solution appearance of transient red color indicated the presence of tannins [17].

Potassium dichromate test: The extract was dissolved in water and then added potassium dichromate solution; to give a yellow color precipitate indicates the presence of tannins (Table 5).

Table 5. Test for tannin present in *Manilkara hexendra* leaves extract.

Sr.no	Test name	Procedure	Observation
1	$KMnO_4$ test	Extract+dil $KMnO_4$	Positive
2	Bromine test	Extract+bromine water	Positive
3	Nitric acid test	Extract+Dil.HNO_3	Positive
4	Lead acetate test	Extract+lead acetate solution	Positive
5	Iodine test	Extract+Dil.Iodine	Positive

RESULTS AND DISCUSSION

The preliminary investigation of all the extract of *Manilkara hexandra* shows presence of tannin, alkaloids, flavonoids, carbohydrates. Some of these phytoconstituents may be responsible to show a potent anthelmintic activity from the observation made higher Concentration of extract produced paralytic effect much earlier and the time of death was shorter for all worms. All the extract show anthelmintic activity but Petroleum ether extract show anthelmintic activity in dose dependent manner giving shorter time of Paralysis (P) and Death (D) with 5, 10, 15 mg/ml concentration for worms. The petroleum ether extract of *Manilkara hexandra* caused paralysis time for 5 mg/ml is 13:15 min and death time 30:14 min. 10 mg/ml time for Paralysis 10:08 min and death time 22:08 min. 15 mg/ml time for paralysis 9:44 min and death Time 18:13 min respectively against worms. The reference drug piperazine citrate (10mg/ml) showed the paralysis at 7:46 min and death time 11:57 min respectively. The experimental evidence obtained in the laboratory model could provide a rational for the traditional used for this plant as antihelminetic. The plant may be further explore for its phytochemical profile to recognize the active constitute accountable for anthelmintic activity [18].

CONCLUSION

In conclusion from the above results it is conclude that the petroleum ether extract of plant *Manilkara hexandra* show potent anthelmintic activity to standard anthelmintic drug. Further studies using *in vivo* model are required to carry out and established the effectiveness and pharmacological rational for the use of *Manilkara hexandra* as an anthelmintic drug. The drug can be further explored for the isolation and characterization of the active constitute responsible for anthelmintic activity. The medicinal value of the plant's leaves, fruit and vegetables, roots, and bark is well known. Anthelmintic activity is determined by the time of paralysis and dying of the worms. Our goal is to gather useful information on plant morphology, microscopy, phytoconstituents, and therapeutical aspects.

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CONFLICT OF INTEREST

The author declared that they have no competing interests

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