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Anthelmintic Activity of *Limonia Acidissima* leaves Extract on Indian Earthworm (Pheretima Posthuma)

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Abstract

One of the oldest systems of medicine in the world, ayurveda has contributed to the developmentof several substances with medicinal benefits. *Limonia Acidissima* also has several medical andnutritional benefits. The monotypic genus Limonia (also known as Feronia elephantum, Hesperethusa crenulata, Feronia limonia, and Schinus limonia) is a member of the Rutaceae family of citrus plants. In India, *Limonia Acidissima* is also known as kaitha, kavit, elephant apple, curd fruit, monkey fruit, and wood apple. The leaves, bark, roots, and fruits are widely used in medicine. They are also frequently used to treat peptic ulcers, chronic diarrhea, and dysentery,among other conditions.

Anthelmintic activity of different extracts of *Limonia Acidissima* leaves were evaluated against Indian earthworm. Various concentrations of the extracts were bioassay for the determination of time of paralysis (p) and time of death (d) of the worm. Albendazole at a concentration of 20 mg/ml has been used as reference standard. In our present study, the crude pet. ether and ethanol extract demonstrated paralysis and also caused death of worms especially at the higher concentration (50 mg/ml). This study confirmed the use of plant leaves as an anthelmintic agent. Themain focus of the current article is on the morphology, nutritional value, phytochemistry, and pharmacological activities of the many components of *Limonia Acidissima*, all of which have received little attention or use.

Keywords: *Limonia Acidissima*, pharmacological action, Rutaceae, phytochemistry, nutraceuticals.

1. Introduction

The Rutaceae family member *Limonia Acidissima*, also known as Feronia Limonia Swinglel, Feronia elephantum, Schinus Limonia, and more commonly known as wood apple and elephantapple, is native to the dry plains of India and Ceylon, where it grows wild and is frequently cultivated alongside roads, at the edges of fields, and occasionally in orchards. It is also cultivated in Southern America, northern Malaysia, temperate Asia, and tropical Asia. The monotypic genus Limonia is only found in India, Pakistan, Sri Lanka, and Southeast Asia.[1] Together with these names, it is also referred to as wood apple, elephant-apple, monkey fruit, curd fruit, Kath bel, and kaitha. Many ailments are treated using this plant's medication.[2] *Limonia Acidissima* L. is one of several herbs that are utilized for its therapeutic and cosmetic characteristics.[3] L. acidissima is a slow-growing, deciduous tree that is upright with a few upward-reaching branches that curve outward at the summit and are split into thin branchlets with drooping ends.[4] Fruit is widely used in India as a liver and heart tonic, as a way to stop diarrhea and dysentery when unripe, and as an efficient therapy for high cough, sore throat, andgum disease.[5] The fruit includes saponins, which cause foaming and have anti-fungal properties, as well as flavonoids, which have antioxidant properties.[6]

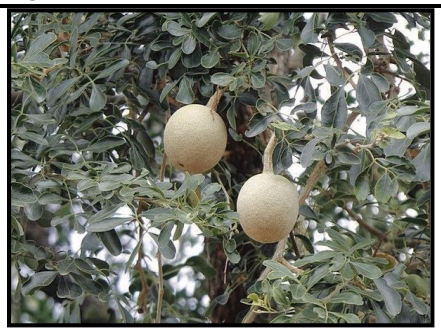

Fig 1: *Limonia Acidissima* Fruits [7]

Fig 2: *Limonia Acidissima* fruit pulp.[8]

1.1 Scientific classification [9]

Kingdom : Plantae
Sub-kingdom : Tracheobionta Superdivision : SpermatophytaDivision : Division
Class : Magnoliospida
Subclass : Rosidae
Order : Sapindales
Family : Rutaceae
Genus : Limonia L.
Species : L. acidissima.

1.2 Synonyms [10]

Feroniaele phantum Correa, Feronia limonia (L.) Swingle,Schinus limonia LL.

1.3 Vernacular names [11]

English : Wood Apple, Elephant Apple, Curd fruit, Monkey fruitHindi : Kaitha, Kath Bel or Kabeet
Oriya : Kaitha
Sanskrit : Kapittha or Dadhistha.
Telgu : Vellaga Pandu
Bengali : Koth Bel
Gujarati : Kothu
Malayalam : Vilam Kai

2. Morphology

Fig 3: *Limonia Acidissima* tree [12]

Prior to the development of processing methods in the middle of the 1950s, the fruit in India was generally considered a "poor man's meal.". Panicules typically appear on fresh shoots. Moreover, the blooms may be a hybrid of staminate and hermaphrodite.[13] The leaves may grow up to 4.7 inches (12 cm) long, are imparipinnate, and have a slightly winged rachis and petiole.The terminal leaflet is obovate, up to 1.6 inches (4 cm) long, dotted with oil glands, and has a subtle perfume when crushed. The opposing leaflets are opposite in 2-3 pairs. White, green, orreddish-purple 5-merous staminate and perfect blooms are typically seen combined in lax, terminal, or axillary inflorescences. Seeds are hairy, 0.2-0.24 in. (5-6 mm) long, and have thick,green cotyledons; epigeal germination.[14] The fruit has a stiff, woody, grayish-white, scurfy skin that is approximately 1/4 in (6 mm) thick, is oblong to spherical in shape, and is 2 to 5 in (5-12.5 cm) broad. The pulp is dotted with countless tiny, white seeds and is dark, mealy, odorous, resinous, astringent, acidic, or sweet-tasting. In Malaya, flowering takes place in February and March, the leaves fall off in January,

and the fruit ripens in October and November. The fruit ripens in India between early October and March. There are two varieties,one with big, sweetish fruits and the other with little, sour ones.[15]

3. Growth & Distribution

This plant may be found all throughout India [16] and is also grown in Bangladesh, Pakistan, andSri Lanka.[17] Both the seed and vegetative methods are used for propagation.[18] Yet, this plant'shigh incidence of seedling death and propensity for out-crossing account for its poor regeneration and subpar germplasm. In order to get around this, axillary bud proliferation in invitro propagation has been devised. [19-20]

3.1 Ethnomedical uses

Ethnomedicine has long employed the fruits, gum, leaves, bark, and pulp of Feronia Limonia tocure a number of diseases and problems. [21]

3.2 Leaves

The leaves are beneficial for anorexia, bronchitis, calculus, cough, diarrhea, gastropathy, and hiccough in addition to being antiemetic, carminative, cardiotonic, and expectorant, as well as purgative. Children with digestive problems are given leaf juice. [16,17, 20, 22]

3.3 Bark

Pitta and several liver illnesses can benefit from the bark.[23]

3.4 Gums

The gum is demulcent and constipating, and it is helpful for diabetes, hemorrhoids, gastropathy,diarrhea, and other conditions. [11, 16, 24, 25]

3.5 Unripe fruits

The unripe fruits are used to treat diarrhea, dysentery, pruritus, and pharyngodynia. They are sour, fragrant, astringent, constipating, and alexipharmic. The unripe fruit is astringent to the bowels and helps with whooping cough as well as body itching. [16, 17, 25, 26, 27]

3.6 Ripe fruits

The juice from Feronia Limonia ripe fruits treats earaches, fatigue, thirst, asthma, tumor's, opthalmia, leucorrhoea, and scurvy. The fruits also treat cough, diarrhea, heart conditions, and vomiting. The fruits, in accordance with Yunani, are cardiotonic, liver tonic, lungs tonic, diuretic, strengthening the gums; the juice is helpful for sore throat and stomtitis; topically, it eases pain brought on by wasp and other insect stings. The fruit pulp is administered topically as a remedy for venous bug bites. In amoebiosis, as a vermifuge, and to treat diabetes, pulp is utilized. [26, 11, 17, 18, 20]

3.7 Ecology

The native and popular wood apple grows on dry plains. It prefers a climate with a monsoon and dry season. The tree in the western Himalayas can reach a height of 450 metres. Generallyspeaking, it can withstand droughts and is best suited to light soils.[28]

3.8 Propagation

Trees that are propagated vegetatively bloom after three years.[13] The wood-apple is typically produced from seeds, although the first fruit will not appear on seedlings until they are at least 15 years old.[15] In a nursery, seeds begin to germinate in around two to three weeks; long-termstorage of seeds may result in 80% germination. It is reported that grafting mature wood buds onto seedlings produces miniature trees with early fruiting.[14]

3.9 Harvesting

The fruit is dropped onto a hard surface from a height of one foot to determine its maturity (30cm). Fruits that are immature bounce, but adult fruits do not. The fruit is left in the sun to fullyripe for two weeks after harvest.[15] Closed, complete Buah Kawista can be preserved in the refrigerator for one to two months or at room temperature for up to ten days. For the optimum quality, the flesh should be eaten right after after opening. Buah Kawista can also be frozen forup to six months in a combination of lemon juice.[29]

4. Medicinal properties

In the indigenous medical system, every component of Limonia is recommended for the treatment of various illnesses. Fruits are cooling, stomachic, stimulating, astringent, aphrodisiac, diuretic, cardiotonic, tonic to liver and lungs, and excellent for asthma, consumption, tumor's, opthalmia, and leucorrhoea. They also heal cough and hiccup.[30] Fruit that is not quite ripe has astringent properties, whilst seeds are utilized to treat cardiac conditions. When treating diarrhea and dysentery, the fruits are used instead of Bael (Eagle marmelos).[31] For vitiated states of vata and pitta, the bark and leaves are employed.[32] The astringent and carminative properties of the leaves help with nausea, indigestion, hiccups, and dysentery. The foliage has hepatoprotective properties.[33] The gum helps with constipation and demulcent symptoms of diarrhea, dysentery, gastropathy, haemorrhoids, and diabetes.[34]

4.1 Other usage

The fruit's rind is so thick and sturdy that it can be carved into a bowl or ashtray-style device. Hard wood from the tree can be utilized for woodworking. The wood is used to build hubs, supports, rollers for mills, hub structures, and agricultural tools. The wood is also used as fuel.To use as feed, the leaves are lopped. Because to its water tolerance, the plant has also been utilized in Thailand as citrus rootstock. An edible gum is also produced from the bark. Unsaturated fatty acids are abundant in the bland, non-bitter oil found in seeds. [35] The wood isthick, hard, and pale or yellow-gray in color. A white, transparent gum that is produced by the boot and branches are used as a filler or adulterant for gum Arabic. Watercolors, ink, dyes, and varnish are also made with the gum. The gum contains traces of rhamnose and glucuronic acid,as well as 35.5% arabinose and xylose, 42.7% d-galactose, and other sugars.[13]

5. Phytochemical constituents

The presence of flavonoids, alkaloids, terpenoids, phenols, lipids, steroids, tannins, glycosides,saponins, gum, mucilage, and settling oils in the main phytochemical analysis of the *Limonia Acidissima* tree. [9,36,37,38] The tree's leaves can hold psoralen, stigmasterol, and orientin. Tannins, saponarin, bergapten, vitedin, and certain essential oils.[39] Marmesin, feronolide, and feronone are all found in bark.[40] Feronia lactone, bargapten, Geranylum belliferone, isopimpinellin, osthol, marmesin, and marmin are the main components of the root, while the seeds are rich incarbohydrates, proteins, fixed oil, and amino acids.[41] The crude powder of *Limonia Acidissima*leaf and stem was subjected to qualitative phytochemical analysis, which revealed that the leaf contained the greatest concentration of tannins and flavonoids while the stem contained just flavonoids. Tripenes and steroids were detected in small amounts in the leaf and stem, but saponins were completely missing.[42] The plant's unripe fruits contain stigmasterol, and the fruitpulp contains a huge amount of citric acid along with fruit acids, minerals, and mucilage. Fruit also contains scoparone, dictamnine, xanthotoxin, umbelliferone, coumarins, fatty acids, sterols, and alkaloids in the pericarp, as well as isopimpinellin, isoimperatorin, and marmin.[43]

Table 1: Phytochemical constituents shown in following table

Sr. No.	Phytochemicals	Fruit pulp	Leaf	Stem
1.	Saponins	-	+	++
2.	Steroids	+	+	-
3.	Triterpenes	-	-	+
4.	Cardiac glycosides	++	+	+
5.	Tannins	+	+	-
6.	Flavonoids	+++	+++	++
7.	Carbohydrates	+++	-	+
8.	Alkaloids	++	-	-
9.	Amino acids	-	+	+

6. Materials and methods

6.1 Collection of plant material

Fresh *Limonia Acidissima* plant leaves that were acquired from the Kudhavad Tal- Shahada neighborhood in the Nandurbar district were authenticated by Dr. Santosh K. Tayade, College of Arts, Science, and Commerce in Lonkheda, Shahada District, Nandurbar. Fresh plant leaves were collected, cleaned using running tap water, and dried for seven days in the shade following verification. and then using a mechanical grinder, made intoa coarse powder. Dry powder was stored in airtight vials.

6.2 Animals

Anthelminthic action was investigated using adult Indian earthworms (Pheretima Posthuma). The earthworms were collected from a local area of Lonkhede's moist soil and authenticated by Dr. R.M. Chaudhari Head of the department of zoology at the college of arts, sciences, and commerce in Lonkhede, Shahada district, Nandurbar.

Fig 4: Indian Earthworm (Pheretima Posthuma)

7. Preparation of extract

7.1 Ethanolic extract

Each 100 g of powdered coarse leaves was steeped in 250 ml of ethanol using the maceration method for a continuous 1 week. The concentrate was then filtered, evaporated ona water bath until no more concentrate remained, and dried.[44]

7.2 Petroleum ether extract

Each 100 g of powdered coarse leaves was steeped in 250 ml of petroleum ether usingthe maceration method for a continuous 1 week. The concentrate was then filtered, evaporatedon a water bath until no more concentrate remained, and dried. [44]

7.3 Chemicals

a. Saline solution
b. Albendazole
c. Tween 80

8. Anthelminthic activity

The anthelmintic activity was conducted using the Ajaiyeoba et al. method with the appropriate modifications.[45] The assay was performed on adult Indian earthworm (Pheretima Posthuma). Earth worms have been employed frequently for preliminary in vitro testing of anthelmintic drugs due to their ease of availability.[46]

6 adult worms of the same kind, each with a different concentration of crude ethanolic and petroleum ether extract (25, 50 mg/ml in saline solution), were added to a 20 ml formulation. When no movement of any kind could be seen until when the worms were violently disturbed,this condition was known as paralysis. The time of death of the worms was recorded. Saline solution served as the control and albendazole (20 mg/ml) as the reference standard.[46]

Fig 5: Ethanol 25 mg/ml

Fig 6: Ethanol 50 mg/ml

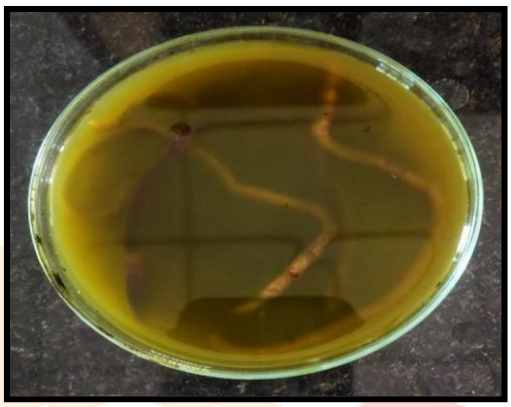

Fig 7: Pet ether 25 mg/ml

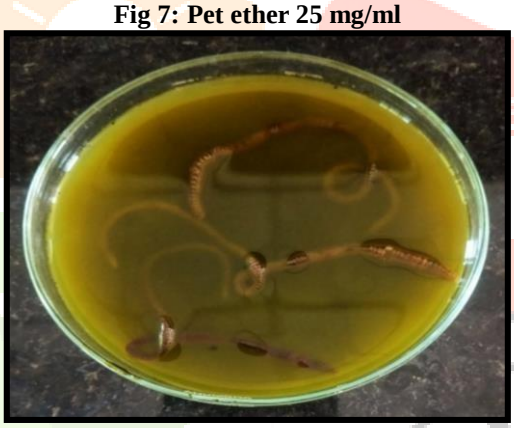

Fig 8: Pet ether 50 mg/ml

Fig 9: Std Albendazole Solution

Table 2: Anthelmintic activity of Petroleum ether and ethanolic extract of *Limonia Acidissima* Leaves

Sr. No.	Extract	Concentration in mg/ml	Indian earthworm (Pheretima Posthuma)	
			Time of paralysis in min (P)	Time of Death in min (D)
1.	Petroleum ether	25	16.50 ± 1.62	66.05 ± 1.0
		50	11.15 ± 1.38	53.44± 2.16
2.	Ethanolic extract	25	22.16 ± 1.26	62.98 ± 1.12
		50	16.13 ± 1.44	44.22± 1.42
3.	Standard Albendazole	20	8.52 ± 0.72	22.36 ± 1.16
4.	Control (Normal saline solution)	-	-	-

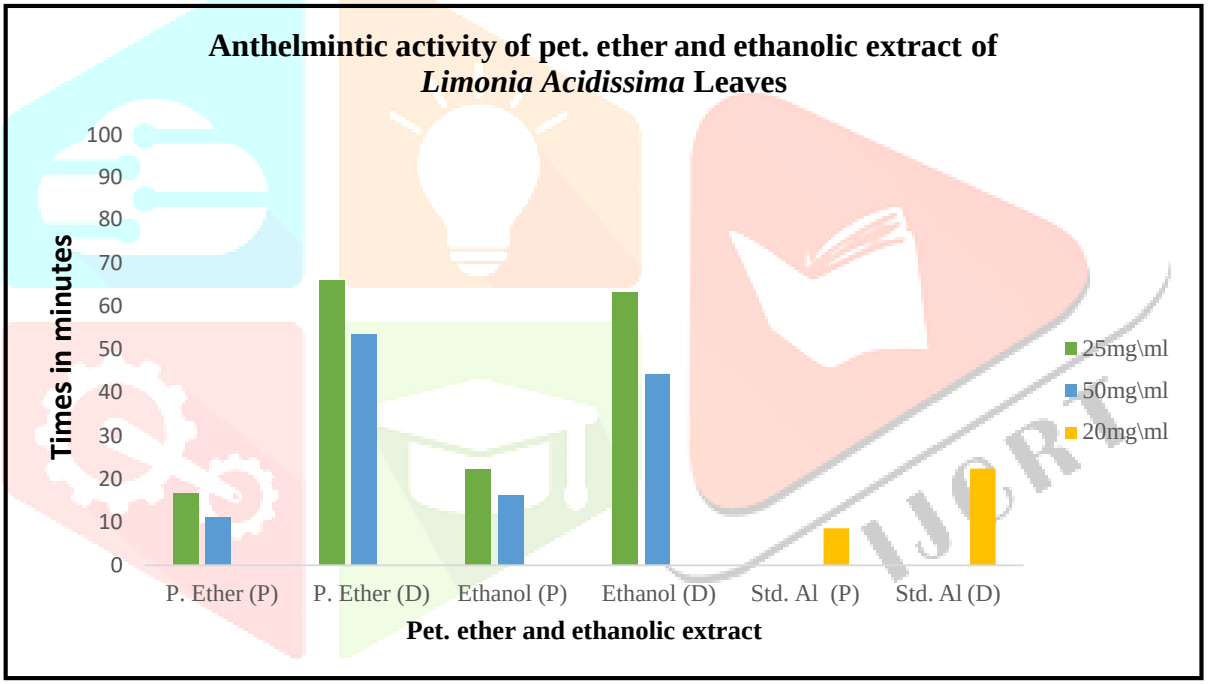

Fig 10: Anthelmintic activity of pet. ether and ethanolic extract of *Limonia Acidissima* Leaves

9. Result and Discussion

Some of phytoconstituents present in *Limonia Acidissima* may be responsible to show a potentanthelminthic 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 shows anthelminthic activity but Pet. ether extract show anthelmintic activity in dose dependentmanner giving shorter time of paralysis (P) and death (D) with 25,50 mg/ml concentration for worms. From Table 2, it is observed that 50 mg/ml conc. of leaves Feronia Limonia shown potent anthelmintic activity while 25 mg/ml conc. taken more time for death of worms. The results are compared with the standard drug Albendazole. The plant may be further explored for its phytochemical profile to recognize the active constitute accountable for anthelminthic activity.

10. Summary and Conclusion

As a result of the research exists, it's possible to conclude that the pet. ether extract of the plant*Limonia Acidissima* leaves exhibits strong anthelminthic activity in comparison to common anthelminthic drug. In order to establish the efficacy and pharmacological basis for the use of *Limonia Acidissima* as an anthelminthic drug, additional studies utilizing in vivo models must be conducted. The drug's potential for isolating and characterizing the active ingredient responsible for its anthelminthic efficacy can be further investigated.

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Conflict of interest:

The author declares no conflict of interest.

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