

**IN-VITRO ANTHELMINTIC ACTIVITY OF ATLANTIA
MONOPHYLLA LEAVES**

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ABSTRACT

Indian atlantia is a small, much-branched, evergreen tree growing up to 6 metres tall. The branches are usually armed with single, stout, sharp spines up to 2 cm long. The plant is harvested from the wild, usually for medicinal purposes. It's reported use of Anti-inflammatory Antibacterial, Antioxidant, larvicidal, pupicidal, antifeedant also used in chronic rheumatism and paralysis in our study Different extracts of *Atlantia monophylla* leaves were found to possess in-vitro Anthelmintic activity against Indian earthworms *Pheritima posthuma*,

using Piperazine citrate as reference standard. Dose dependent activity was observed in extracts of plant leaves. Methanolic extract shown better activity of *Atlantia monophylla* leaves, which is not reported.

KEYWORDS: *Atlantia monophylla*, *Pheritima posthuma*, Piperazine citrate, Anthelmintic activity, larvicidal, pupicidal, antifeedant.

INTRODUCTION

From pre-historic times to the modern era in many parts of the world and India, plants, animals and other natural objects have profound influence on culture and civilization of man. Since the beginning of civilization, human beings have worshiped plants and such plants are conserved as a genetic resource and used as food, fodder, fiber, fertilizer, fuel, and febrifuge and in everyother way. *Atlantia monophylla* one such plant.^[1]

In ancient ayurvedic medicine the plant *Atlantia monophylla* known as "wild lemon". A large thorny shrub grows up to 2.5 meters in height. Leaves simple, alternate, oblong, alternate,

entire or crenulate. Flowers small seen in axillary racemes. Fruits small, round berries contain small seeds. It is a native of India, Pakistan and Bangladesh and distributed in the following states of India Maharashtra, Karnataka, Andhra Pradesh, Tamilnadu, Kerala.

Plant Profile



Synonym: Makad Limbu, Ran Limbu, wild lemon

G.S.: *A. monophylla* whole plant found allover India upto an altitude of 1000 m. It Found in India, Malaysia, Myanmar Thiland, Cambodia.^[13,14] It occurs throughout India from Maharashtra, Tamilnadu, Kerala and Karnataka in the south.

Scientific Classification

Kingdom	Plantae
Order	Sapindales
Family	Rutaceae
Genus	Atalantia
Species	Monophylla

Morphological Characters

Parts Used

Habit: Shrub or a small glabrous armed tree up to 5 m height.

Root: Simple, branched, woody at base and covered with a fissured; corky bark; branches somewhat succulent and densely white tomentose; early glabrescent.

Leaves: leave are $3-7 \times 2-4$ cm, ovate or elliptic, base broadly cuneate, margins entire to obscurely undulate, apex obtuse or emarginated.

Flowers: Flowers are 1 cm across, pedicels to 1.5 cm long. Calyx irregularly lobed or spathiform 3mm long. Petals 4 or 5, white, $7-9 \times 3-4$ mm

Floral Characteristics

Inflorescence: A dense, multiflowered, umbellate, pedicled cymes, arising from the nodes and appearing axillary or terminal.

Calyx: Sepal 5, 5 lobed, shortly united at the base, spathiform.

Corolla: Petals five, white $7-9 \times 3-4$ obovate.

Androecium: Stamens 8 or 10, unequal, base connate and forming a tube.

Gynoecium: ovary 4-5 mm long, oblong, 4 celled. Ovule 1 or 2 per cell. Style to 3 mm long.

Fruit: 1.5 cm across, globose, yellowish green, glandular dotted.

Seeds: seeds are few, 6 mm long.^[14]

Cultivation and Collection: Plant succeed in warm subtropical to tropical climates. This species can be grafted on Citrus species, and vice versa.

Uses: larvicidal, antibacterial, antioxidant, pupicidal, antifeedant, also used in chronic rheumatism and paralysis.

Need of Work: The active principles responsible for the Anthelmintic activity of Methanolic extract of *Atlantia monophylla* leaves need to be explored and exact mechanism of action need to be studied in detail.

Standard Drugs Used As Anthelmintic Activity: Piperazine Citrate.

Objectives: To evaluate Anthelmintic activity of Methanolic extract of *Atlantia monophylla* by using earthworms.

MATERIAL AND METHODS

A. Selection of Plant: The fresh leaves of the plant was collected from the Bhilar village, Maharashtra.

B. Authentication of Plant: The Plant was authenticated by head of department of Yashvantrao Chavan Institute of Sciences, Satara.

C. Extraction: Extraction of plant material by using combined method of Maceration and Ultra sonication.

D. Plant Material

- 1) The fresh leaves of the plant were collected from the Bhilar Village, Maharashtra
- 2) The leaves are cleaned by washing with running water and shade dry and then milled to coarse powder by mechanical grinder.

E. Preparation of Extracts

- 1) The dried powdered leaves were extracted by Ultra sonication and maceration method combination process.
- 2) Drug macerated for seven days with Methanol and simultaneously everyday 45 mins. Ultra sonication extraction was carried out on the same extract.
- 3) On the 7th day the solvent portion was evaporated under reduced pressure
- 4) The prepared extracts were kept under refrigeration for screening of Anthelmintic activity.^[19]

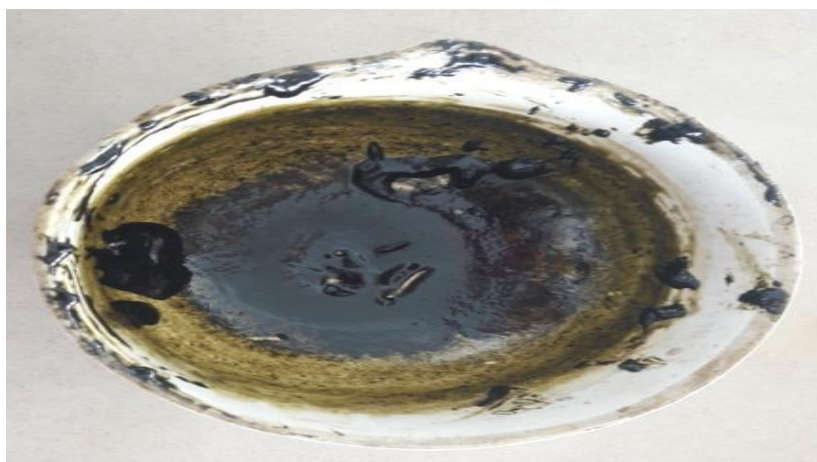
In-Vitro Anthelmintic Activity

- 1) The Anthelmintic activity was evaluated on adult Indian earthworm *Pheretima posthuma* due to its anatomical and physiological resemblance with the intestinal roundworm parasites of human beings.^[19]
- 2) The earthworms are collected and washed with normal saline to removal of fecal matter.
- 3) The earthworms are 5 to 6 cm length and 0.2 - 0.3 cm widths were used for experiment protocol.
- 4) Methanolic extracts that were prepared from *Atlantia monophylla* leaves were examined systematically for their *in-vitro* Anthelmintic activity against *Pheretima posthuma*.
- 5) The *in-vitro* Anthelmintic assay procedures were carried out. With slight modifications.
- 6) Five groups of equal size Indian earthworm consisting of six earthworms in each group were released into 15 mg/ml, 30 mg/ml, 60 mg/ml, of desired formulation.
- 7) Each group was treated with one of the following: Vehicle, Piperazine citrate (20 mg/ml), and different extracts of in normal saline.

8) Observations were made for the paralysis time and subsequently for death time of the worms. The mean paralysis and/or death time for each group was recorded (each reading taken for 6 times). The time taken by the worms to become motionless, consider as paralysis was recorded and the lethal time was recorded by observing the time taken to become motionless on application of external stimuli by pricking with pin. Piperazine citrate (20 mg/ml) was taken as reference drug.

RESULT AND DISCUSSION

1. Parameters of Extracts



Methanolic Extract

Table No.1.

Characteristics	Methanolic extract
Colour	Greenish
Odour	Aromatic
Taste	Bitter

Phytochemical Analysis

Chemical Test

A.Test for Alkaloids	Methanolic extract
1.Mayer's Test	+
2.Dragndroff's Test	+
3.Wagner's Test	+
4.Hager's Test	+
B.Test for Tannins	
1.5%FeCl ₃	+
2.Anthraquinone Glycoside	-
3.Saponin Glycoside	-
4. Coumarin Glycoside	-
D.Test for Flavonoids	
1.Shinoda Test	+

2.Sulphuric Acid Test	+
E.Test for Carbohydrates	
1.Fehling's Test	-
2.Benedict's Test	-
F. Test for Resin	-
G. Test for Steroids	-
a.Libermann test	-
b.Salkowski test	-

Anthelmintic Screening

Observations was 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.



Observation Table: Anthelmintic Activity of Methanolic Extract of *Anlantia Monophylla* Leaves.

Treatment	Concentration	Paralysis time(Min)	Death time(Min)
Vehicle	-	-	-
Piperazine citrate	20 mg/ml	50.7 min	58.30 min
Methanolic extract	15 mg/ml	11.20 min	19.55 min
Methanolic extract	30 mg/ml	9.30 min	14.45 min
Methanolic extract	60 mg/ml	7.35 min	11.20 min

RESULT

Methanolic extract shows significant Anthelmintic activity against earthworms (*Pheritima posthuma*). The methanolic extract demonstrated paralysis as well as death of worms (*Pheritima posthuma*) in a comparative less time as compared to Piperazine citrate especially in higher concentration of 60 mg/ml in case of *Atlantia monophylla*.

CONCLUSION

The Methanolic extract of leaves of *Atlantia monophylla* 60 mg/ml dose shows significant anthelmintic activity against *Pheritima posthuma* worms by comparing with 20 mg/ml dose of standard Piperazine citrate. It is observed that time required for paralysis and death of worm (*Pheritima posthuma*) by extract is less than the standard Piperazine citrate.

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