

## BIOLOGICAL EVALUATION OF ANTHELMINTIC ACTIVITY OF LEAVES EXTRACT OF *ATLANTIA MONOPHYLLA* IN INDIAN ADULT EARTHWORM

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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 2cm 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 and Ethanolic 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 every other way. *Atlantia monophylla* one such plant.<sup>[1]</sup>

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 all over India up to an altitude of 1000 m. It is found in India, Malaysia, Myanmar, Thailand, Cambodia<sup>[13][14]</sup>. It occurs throughout India from Maharashtra, Tamilnadu, Kerala and Karnataka in the south.

### Scientific Classification.

<b>Kingdom</b>	Plantae
<b>Order</b>	Sapindales
<b>Family</b>	Rutaceae
<b>Genus</b>	Atalantia
<b>Species</b>	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:** leaf 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 3 mm 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. Styleto 3 mm long.

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

**Seeds:** seeds are few, 6 mm long.<sup>[14]</sup>

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

### *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.<sup>[19]</sup>
- 2) The earthworms are collected and washed with normal saline with 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 groups were released into 25 mg/ml, 50 mg/ml, 100 mg/ml, of desired formulation.
- 7) Each group was treated with one of the following: Vehicle, Piperazine citrate, 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 (20mg/ml) was taken as reference drug.

**RESULT AND DISCUSSION****1. Parameters of Extracts.****Table No.1: Phytochemical Analysis.**

<b>Characteristics</b>	<b>Methanolic extract</b>
Colour	Greenish
Odour	Aromatic
Taste	Bitter

**Chemical Test**

<b>A.Test for Alkaloids</b>	<b>Methanolic extract</b>	<b>Ethanolic extract</b>
1. Mayer's Test	+	+
2. Dragndroff's Test	+	+
3. Wagner's Test	+	+
4. Hager's Test	+	+
<b>B.Test for Tannins</b>		
1. 5%FeCl <sub>3</sub>	+	+
2. Anthraquinone Glycoside	-	-
4.Coumarin Glycoside	-	-
<b>D.Test for Flavonoids</b>		
1. Shinoda Test	±	±
2. Sulphuric Acid Test	±	±
<b>E.Test for Carbohydrates</b>		
1. Fehling's Test	-	-
2. Benedict's Test	-	-
<b>Test for Resin</b>	-	-
<b>Test for Steroids</b>	-	-
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.



**Effect of Ethanolic extract of *Atlantia monophylla* on Indian adult earthworm.**



**Effect of Methanolic extract of *Atlantia monophylla* on Indian adult earthworm.**

### Observation Table

#### Anthelmintic Activity of Methanolic and Ethanolic Extract of *Anlantia Monophylla* Leaves.

Group	Concentration of extract in mg/ml	Time taken in minutes	
		Paralysis	Death
Methanolic extract	25	9.20	14.55
	50	9.30	10.45
	100	7.35	9.20
Ethanolic extract	25	11.20	19.55
	50	9.30	14.45
	100	7.35	11.20
Piperazine citrate	25	52.07	58.68
	50	30.10	33.06
	100	13.45	18.17

### RESULT

The activity was compared with standard drug Piperazine citrate. Methanolic and Ethanolic extract both show significant anthelmintic activity against Indian adult earthworms.

Methanolic extract shows more effect as compared to aqueous extract. The Methanolic extract *Atlantia monophylla* confirmed paralysis as well as death of worms in a good time as compared to Piperazine citrate especially in higher concentration of 100mg/ml. The Ethanolic extract also showed significant activity.

## CONCLUSION

From above study it is confirm that methanolic and aqueous extract of roots of *Tephrosia purpurea* shows anthelmintic activity In Indian adult earthworms.

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