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"IN-VITRO ANTHELMINITIC ACTIVITY OF EXTRACT OF ATLANTIA MONOPHYLLA LEAVES"

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ABSTRACT

Indian *atlantia* is a small, much-branched, evergreen tree growing up to 6 meters 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 is source of antibacterial, antioxidant, larvicidal, pupicidal, antifeedant, also used in chronic rheumatism and paralysis. Different extracts of *Atlantia monophylla* leaves were found to possess in-vitro anthelminthic activity against Indian earthworms Pheritima posthuma, using Albendazol as reference standard. Dose dependent activity was observed in extracts of plant leaves. Ethanollic extract shown better activity of Atlantia monophylla leaves. No were reported on the part of leaves of Atlantia monophylla shows Anthelmintic activity.

Keywords: Atlantia monophylla, Albendazol, Anthelminthic 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. A monophylla one such plant. Kirtikar KR, Basu BD et al., (1999). In ancient ayurvedic medicine the plant Atlatia 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, and Kerala.

History of Helminthic:

Helminths (the word is derived from the Greek meaning "worms" [Faust, E. C., et al., 1970]. Have plagued humans since before the era of our earliest recorded history. The eggs of intestinal helminthic can be found in the mummified feces of humans dating back thousands of years [Hotez, P.J. et al., 2002] and we can recognize many of the characteristic clinical features of helminth infections from the ancient writings of Hippocrates, Egyptian medical papyri , and the Bible [Hotez, P.J., et al., 2002]. These same helminthiases markedly altered the course of modern 12th century world history [Hotez, P.J. et al., 2002], especially in china during the cold war, when the schistosome was known as "the blood-fluke that saves Formosa" because acute schistosomiasis sickened Mao's troops and aborted their amphibious assault of Taiwan (historically known as Formosa) just long enough for American ships to enter the Straits of Taiwan. [de Silva. N. R. et al., 2003]. There are two major phyla of helminthis. The nematodes (also known as roundworms) include the major intestinal worms (also known as soil transmitted helminthis) and the filarial worms that cause lymphatic filariasis (LF) and onchocerciasis, whereas the Platyhelminthes (also known as flatworms) include the flukes (also known as trematodes), such as the schistosomes, and the tap worms (also known as the cestodes), such as the pork tap worms that causes cysticercosis. The most common helminthiases are those caused by infection with intestinal helminthes, ascariasis, trichuriasis, and hookworm, followed by schistomiasis and LF.

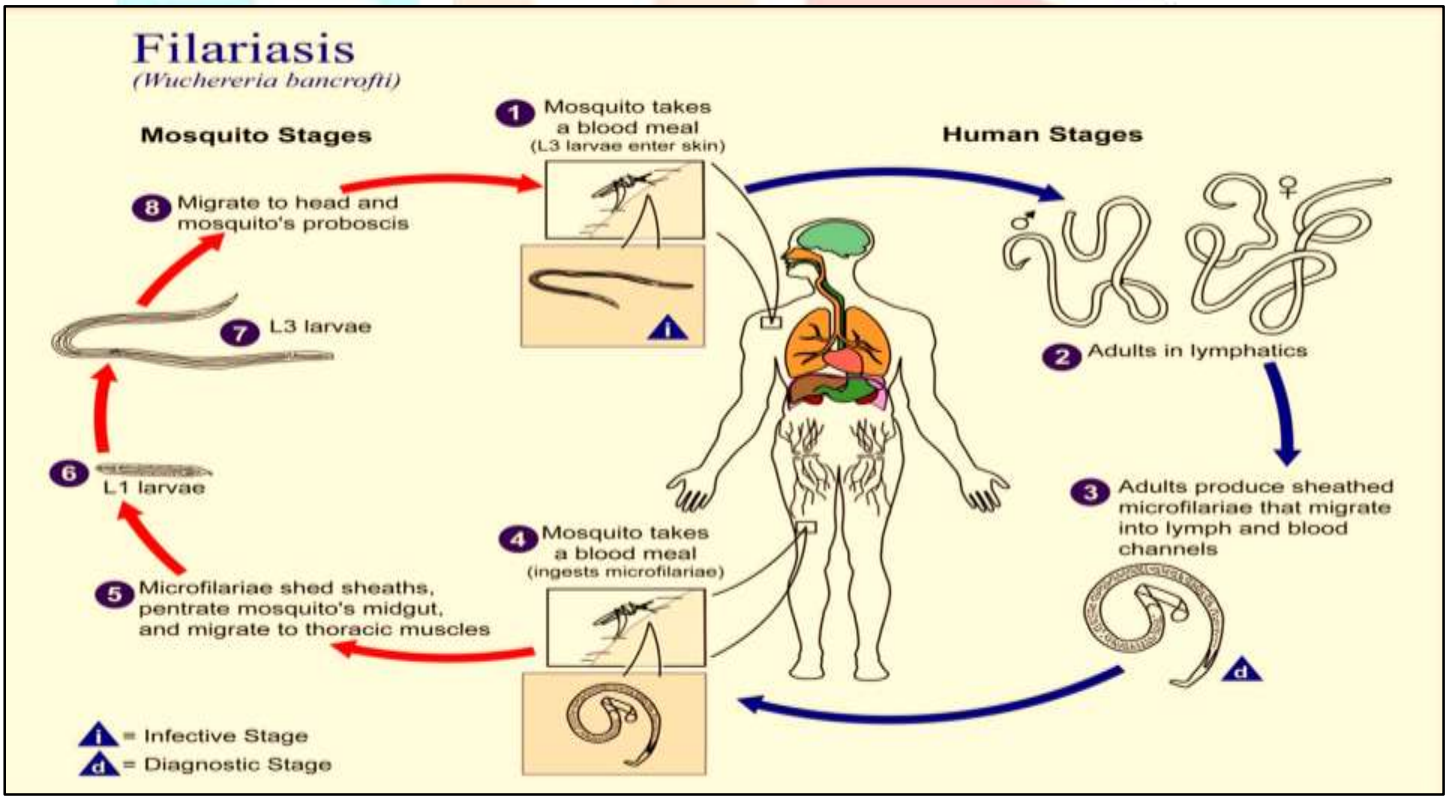

Fig No.1 Life Cycle of Helminth Disease. (www.infectionlandscapes.org).

Fig No.2 Taxonomy

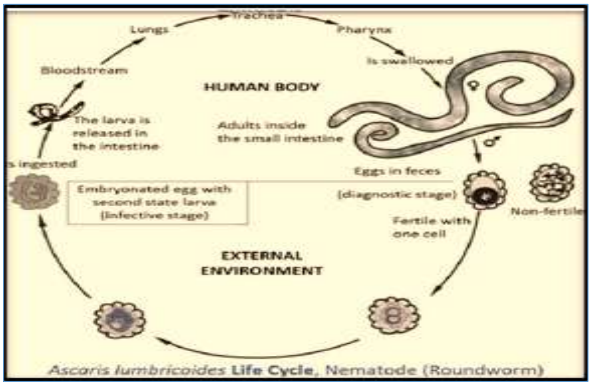

Fig No.3 Ascaris lumbricoides life cycle

Image showing life cycle inside and outside of the human body of one fairly typical and well described helminth Ascaris lumbricoides. There is no real consensus on the taxonomy (or groupings) of the helminths, particularly with the nematodes. The term "helminth" contains a number of phyla, many of which are completely unrelated. However, for practical considerations the term is still used nowadays to describe four groups with superficial similarities, the phyla Annelida, Platyhelminthes, Nematodes and Acanthocephala. There is in fact no helminth classification; it is an "artificial" term. The most important helminths in the sanitation field are the human parasites, which is why most people relate the term helminth to them, where they are classified as nemathelminthes (nematodes) and platyhelminthes, depending on whether they possess a round or flat-shaped body respectively. The latter are further divided into cestodes and trematodes depending on whether or not they have a segmented body. Ringworm (dermatophytosis) is actually caused by various fungi and not by a parasitic worm.

Common characteristics:

Helminths are a group of evolutionarily unrelated organisms which share a similar form. The number of different helminth species is vast: it is estimated to be around one million species. The nematodes are the most diverse of all the helminths with the highest number of species. There may be as many as 300,000 species of parasites affecting vertebrates, and as many as 300 affecting humans alone.

Life time:

The life time of adult worms varies tremendously from one species to another but is generally in the range of 1 to 8 years. This life time of several years is a result of their ability to manipulate the immune response of their hosts by secreting immunomodulatory products.

Causes of helminthic infection:

Some of the diseases caused by helminths are described below. Hookworm disease is a common worm infestation in the developing world caused by Ancylostoma duodenale or Necator americanus. The illness leads to anemia and malnutrition. Dracunculiasis is caused by the guinea worm or Dracunculus medinensis, which is transmitted through contaminated water. It lies burrowed within the skin and causes severe inflammatory reactions. Loiasis or African eye worm disease is caused by the filaria Loa loa worm, which is contracted through Deer fly or Mango fly bites. The adult worms move through subcutaneous tissue towards the subconjunctiva of the eye. The illness causes red, itchy swellings in the skin referred to as Calabar swellings. Cysticercosis is caused by the pork tapeworm or Taenia solium. Symptoms often do not present for years, but eventually painless bumps develop in the skin and muscles or cause neurological problems. Echinococcosis is caused by Echinococcus tapeworms. The liver is usually affected first, followed by the lungs and brain.

Sign and symptoms of helminthic infection:

The list of signs and symptoms mentioned in various sources for Helminth infections listed below:

Subcutaneous nodules, Conjunctivitis, Retinitis, Blindness, Dysentery, Urticaria, Diarrhoea, Cough, Wheeze, Hepatosplenomegaly, Fever, Abdominal pain

PLANT PROFILE:

Synonym:

Makad limbu, Ran Limbu, wild lemon

Biological Source:

The **lemon**, *Citrus limon* (L.) Osbeck, is a species of small evergreen tree in the flowering plant. family : Rutaceae, native to Asia.

Geographical Souce: ***A.*** Monophylla whole plant found all over India upto an altitude of 1000 m. It found in India, Malaysia, Myanmar, Thiland. *Sukumaran S, Raj ADS et al., (2010).* It occurs throughout india from Maharashtra Kerala and Karnataka in the south.

Uses:

Asthma, Abortifacient, Anti-cancer, Anthelmintic, CNS activity, Epilepsy, Eczema, Expectorant, Fever, Leprosy, Migraine.

MATERIAL AND METHODS:

A. Selection of plant:

The fresh leaves of the plant will be collected from the Satara city, Maharashtra.

B. Authentication of Plant:

The Plant will be authenticated head of department of Yashvantrao chavan institute of sciences, Satara

C. Extraction of plant material by using combined method of Maceration and Ultrasonication.

D. Plant Material

1. The fresh leaves of the plant will be collected from the Satara city, Maharashtra

2. The leaves were 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 70% hydro ethanol and simultaneously everyday 1hr 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.

In-vitro Anthelmintic activity

1) The anthelmintic activity was evaluated on adult Indian earthworm Phaeritimaposthuma due to its anatomical and physiological resemblance with the intestinal roundworm parasites of human beings. [Herbert, D. R., et al., 2004].

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) 70% hydro ethanolic extracts that were prepared from Calotropisgigantea leaves were examined systematically for their in-vitro anthelmintic activity against Phaeritimaposthuma.

5) The in-vitro anthelmintic assay procedures were carried out. With slight modifications.

[Decca Raman M, et al., 2010].

6) Five groups of equal size Indian earthworm consisting of six earthworms in each groups were released into 15 mg/mL, 30 mg/mL, 60 mg/mL l, of desired formulation.

7) Each group was treated with one of the following: Vehicle, albendazol (100mg/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. Albendazol (100mg/mL) was taken as reference drug.

PARAMETERS OF EXTRACTS:

1. Observation of Ethanollic Extract:

CHARACTERISTIC	70% HYDRO ETHANOLIC EXTRACT
Colour	Brown
Odour	Acrid
Taste	Bitter

2. Chemical Test:

SR	TEST	OBSERVATION	INFERENCE
1	Dragendorff's test: 2- 3ml extract with few drops of Dragendorff'reagent	Orange braown ppt form	Alkaloids present
2	Mayer's test: 2-3ml extract with few drops Mayer's reagent.	ppt form	Alkaloids present
3	Hager's test:2-3ml Extract with few drops Hager's reagent.	Yellow ppt	Alkaloids present
4	Wagner's test: 2-3ml Extract with few drops Wagner's reagent.	Reddish brown ppt	Alkaloids present

3. Test for Flavonoids:

TEST	OBSERVATION	INFERENCE
1. Shinoda Test	Orange, pink red to purple colour appears.	Flavonoids Present.
2. Sulphuric Acid Test:	Flavanes give orange to red colours.	Flavonoids Present.

Anthelmintic screening:

Observations were 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 color

Fig No.4 Anthelmintic Screening of Ethanollic Extract.

RESULT AND DISCUSSION:

The result in following table shows that the time taken for paralysis and death of worms after treating with extract of *Atlantia Monophylla.* Ethanollic extract shows significant Anthelmintic activity against earthworms *(Pheritima posthuma)*. Ethanollic found to be more active as compared to water extract. The ethanollic extract demonstrated paralysis as well as death of worms *(Pheritima posthuma)* in a comparative less time as compared to Albendazol especially higher concentration of 60 mg/ml in case of *Atlantia monophylla.*

Treatment	Concentration(mg/ml)	Paralysis Time (min)	Death time (min)
Vehicle	-	-	-
Albendazole	100mg/ml	10.30 min	17.23 min
70% Hydro-Ethanollic Extract	15 mg/ml	4.12 min	5.48 min
70% Hydro-Ethanollic Extract	30 mg/ml	3.58 min	6.30 min
70% Hydro-Ethanollic Extract	60 mg/ml	3.30 min	5.40min

Anthelmintic activity of Ethanollic extract of Atlantia monophylla leaves

CONCLUSION:

The Ethanollic extract of leaves of Atlantia monophylla 60 mg/ml dose shows significant anthelmintic activity against Pheritima posthuma worms by comparing with 100 mg/ml dose of standard Albendazol. It is observed that time required for paralysis and death of worm (Pheritima posthuma) by extract is less than the standard Albendazol.

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