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**Pharmaceutical Sciences** **Research Article……!!!**

# "IN-VITRO ANTI-HELMINTIC ACTIVITY ON ANNONA RETICULATA LINN FRUIT AND MENTHA PIPERITA LEAF EXTRACTS"

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**KEYWORDS:**

Anthelmintic Efficacy, Phytochemical Examination, phenolic compounds, alkaloids, and flavonoids, *Mentha piperita*.

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**ABSTRACT**

The purpose of this study was to assess the anthelmintic efficacy of *Mentha piperita* leaf extract and *Annona reticulata* fruit extracts against helminth parasites in vitro. The extracts performed a qualitative phytochemical examination after being synthesised in accordance with established protocols. Using the adult earthworm (*Pheretima posthuma*) model, the anthelmintic activity was evaluated in accordance with published methods. The outcomes showed that both extracts had considerable dose-dependent anthelmintic activity when compared to the control group. Additionally, the phytochemical examination revealed the existence of bioactive substances that may be involved in the anthelmintic activity that was detected, including tannins, phenolic compounds, alkaloids, and flavonoids. These results point to the possibility of using *Mentha piperita* leaf extract and *Annona reticulata* fruit extracts as natural anti-helmintic drugs, which calls for more research on their safety and therapeutic efficacy profiles.

**INTRODUCTION:**

A substantial proportion of the global population suffers from helminth infections, which are among the most prevalent infections in humans. They are a major public health risk in developing nations, where they also raise the risk of pneumonia, eosinophilia, anaemia, and malnutrition. While the majority of worm-related infections are often restricted to tropical regions, travellers who have visited those areas may experience them, and certain infections may develop in conditions that are similar to those of the target region [1]. The world's population relies heavily on traditional medical remedies due to the restricted availability and high cost of pharmaceutical medicines. Approximately 20,000 species of higher plants are employed for medicinal purposes worldwide. Numerous well-known medications found in the current pharmacopoeia have natural sources.

**Classification of Helminths:**

Helminths can be classified as:

1. Flukes, or nematodes

2.common flukes(trematodes)

3.Tapeworms, or cestodes

4.roundworms, or nematodes

Invertebrates known as helminths are distinguished by their long, flat, or spherical bodies. Roundworms (nematodes), tapeworms (cestodes), and flukes (trematodes) are examples of flatworms (platyhelminths). The residing host organ (such as intestinal roundworms and lung flukes) designates further subdivision (2).

**Signs and symptoms:**

Certain varieties of helminthiasis can primarily cause lung damage and symptoms because they harbour various evolutionary forms of worms in the lungs or because they harbour parasitic antigens that are stuck in the pulmonary vasculature. Thus, there are two main reasons why helminths might be found in the lungs. The presence of eggs or larvae in the lung tissues, without transformation into another evolutive form, because they had been passively getting to this organ via blood or lymphatic circulation, or by erratic and occasional larval migrations. The requirement for larvae to migrate through the lungs (3).

**Treatment:**

By modifying host immune responses, parasitic helminths have evolved alongside the mammalian immune system to enhance their own survival. These worms trigger an immune response that is reliant on a Type 2 cytokine response, which involves the release of interleukin (IL)-4, IL-5, IL-9, and IL-13. Additionally, intestinal mast cells, eosinophils, goblet cells,

enterocyte proliferation, and intestinal contractility are activated. The next step is the creation of granulomas, which isolate the eggs and larvae and cause tissue repair. A primarily anti-inflammatory response results from the activation of additional accessory pathways, such as the increase of regulatory T cells, IL-10, and/or transforming growth factor beta levels. It has been demonstrated that mice with helminth infections that are IL-10 lacking have increased mortality and/or morbidity (4).

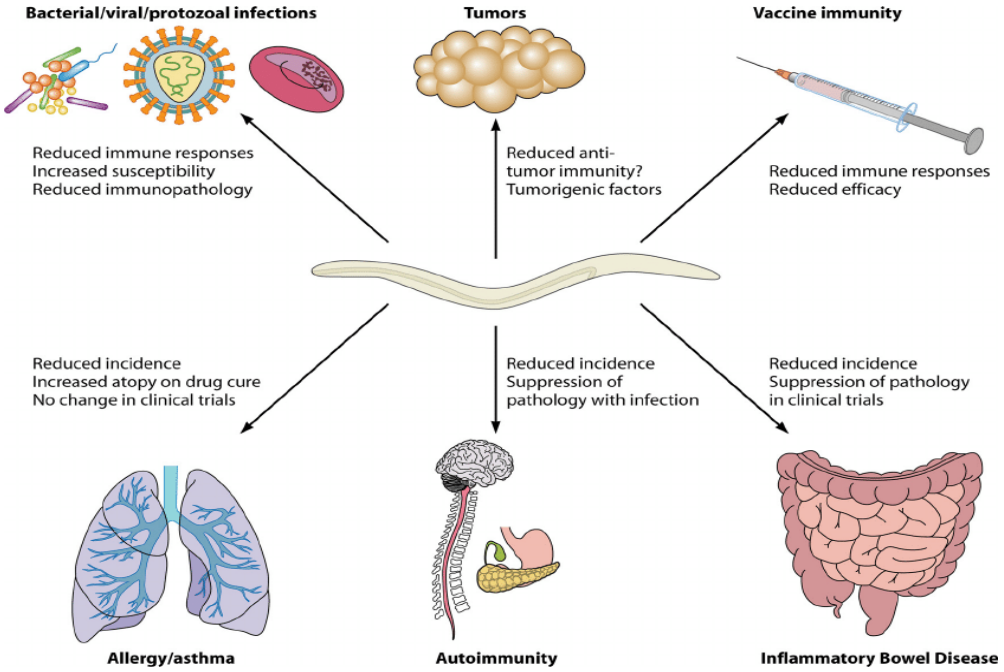
**Pathophysiology of helminths:**

The following is an explanation of pathological information found in biological sciences regarding the primary origins and consequences of disease-causing pathogens of certain intestinal parasites in humans and other mammals. Endoparasites and ectoparasites are two types of parasitic creatures that momentarily reside inside or on the bodies of other organisms as hosts. There are several ways to infect and spread intestinal parasitic worms, including mosquito bites, ingesting organisms' eggs, penetrating the skin, and when faecal matter enters the mouth through contaminated food or water. These routes are also the main ways that certain helminthes, protozoa, and microsporidia are transmitted to humans: through fecal -oral, direct contact .A disease with two phases can result from encysted larvae of a particular species: the gastrointestinal phase, which happens after humans consume contaminated meat, and the systemic (parenteral) phase, which happens when the larvae enter the lymphatic circulation and then go into the blood, skeletal muscles, myocardium, and brain, all of which have high oxygen content .

One of the main causes of gastrointestinal disorders, such as diarrhoea, dysentery, vomiting, anorexia, hematuria, abdominal distension, weight loss, nausea, and iron deficiency anaemia, is intestinal parasites. They can also cause pharyngeal irritation, itching and scratching around the perianal area, swelling of the lower limbs, and other symptoms (5). In the field of biological sciences, human parasite infections are the most common cause of illness worldwide, particularly in developing nations where they are the main cause of death and produce higher rates of morbidity and mortality than other infectious illnesses.

The two primary categories of parasitic organisms are metazoan organisms, which include nematodes, trematodes, and cestodes, and protozoans, which comprise helminth species and plasmodium species. Parasitic organisms are those that reside in a range of ecological environments, can be found on the inside or outside of other creatures, and some illnesses are important zoonotic agents that is, they can spread disease and cause potentially. The blood-feeding parasites of the two nematode species, Ancylostoma duodenale and Necator americanus, are the source of the hookworm infection. According to epidemiology, there are currently 576-

740 million hookworm infections; of them, 80 million have severe infections, which are associated with intestinal blood loss, anaemia, and protein deficiency. The reason hookworms get their name is because, as shown in figure 1, their anterior end curls dorsally to resemble a hook. Necatar Americanus is the primary cause of hookworm infection(6). The clinical and pathological consequences of parasite infections are very much dependent on the particular organs/tissues in which the infections or immune responses are localised. Within the various parasite species, parasitic helminths appear tofollow extremely varied and complicated routes of infection within the host tissues (reviewed in Soulsby, 1982). There are however, some patterns of similarity in the migratory routes of the various parasites, largely dictated by the anatomical (7)




**Plant profile:**

**Annona Reticulata**

**Plant introduction:**

Botanical name: Annona reticulata

Family: Annonaceae

Indian name: bullock's heart, Ramphal

Habitat: It grows from sea level to 1500 metres (5,000 ft) altitude in areas of central America that have alternating wet and dry seasons

Parts used: Fruit

Phytochemical constituents: tannins, flavonoids, saponins, alkaloids and steroids

**Botanical classification:**

Annona reticulata belongs to the family ''Annonaceae''

**Classification**:

Kingdom: plantae

Clade: Tracheophytes

Clade: Anginosperms

Clade: Magnolids

Order: Annonaceae

Genus: Annona

Species: Annona reticulata (8)

**Mentha Piperita:**

**Plant Introduction:**

Botanical name: Mentha piperita

Family: Lamiaceae

Indian name: peppermint, mint

Habitat: Mint is cultivated in large areas of the gangetic plains of uttar Pradesh, Punjab,

Parts used: Leaves

Phytochemical constituents: tannins, flavonoids, saponins, alkaloids and steroids

**Botanical Classification:**

Pepper mint belongs to the family ‘’Lamiaceae’’

**Classification**:

Kingdom: plantae

Clade: Tracheophytes

Clade: Anginosperms

Clade: Eudicots

Clade: Asterids

Order: Lamiales

Family: Lamiaceae

Genus: Mentha

Species: Mentha piperita (9)

**Materials and Methods:**

**Plant materials:** The botanical survey of India, Hyderabad, Telegana, India, authenticated the Annona reticulata fruit and Mentha piperita leaves that were gathered from Medchal District gardens, and a herbarium specimen was placed in the Department of pharmacogonosy. The powder After that, it was run through sieve number 20. The medication that had been defatted was extracted using methanol, and the extract was dried in a desiccator after the solvent was

distilled out. Following methanolic extraction, the marc was collected and treated to a seven-day sequential maceration process for aqueous extraction. The extract was then dried by evaporating the water and kept for future use 10)

**Extraction by using maceration:**

This process involves adding the solvent to a stoppered flask containing the whole or coarsely ground crude medication. It is then let to stand at room temperature for at least three days, stirring often, until the soluble material dissolves. The combination was strained, and the mixed liquids were then treated to filtering or decantation after standing. The mixture was made up of marc, or damp solid material (11)

**Experimental worms:**

The anthelmintic activity involved collecting adult Indian earth worms (Pheretima posthuma) from the wet and moist soil of the Medchal district and washing them with regular saline to get rid of all the feces. Because of their morphological and physiological similarities to human intestinal roundworm parasites, earth worms measuring 3-6 cm in length and 0.1-0.3 cm in width were included in the experimental technique (12)

**Drugs and chemicals:**

Isolated fruit and leaf extracts of Annona reticulata and Pippermint and standard drug albendazole

**Preparation of methanol extract**:

40gm of powder mixed with 150ml of methanol and extracted by using maceration for 24hrs. The filtrate was then evaporated at 600 cand stored at 400 c until further process

**Preparation of aqueous extract:**

40gm of powder mixed with 150ml of aqueous and extracted by using maceration for 24hrs. The filtrate was then evaporated at 600 cand stored at 400 c until further process (13)

**Phytochemical screening of Annona reticulata and Mentha piperita (14)**

**Test for saponins:**

Foam test: Take 3ml of each extract and add 2ml of distilled water in test tube to dilute it. Now shake the mixture vigorously. The formation (or) occurance of form results /the formation of foam indicates saponins

**Test for alkaloids:**

The plant extract was dissolved in 100 mL of water, filtered, and cooked in steam with 2 mL of the filtrate and three drops of 1% HCl. Then, 1 mL of the heated mixture was combined with 6 mL of the Mayer-Wagner reagent. The appearance of a cream or brown-red colored precipitate indicated the presence of alkaloids.

**Test for steroids**:

About 1 mL of the crude extract was combined with 10 mL of chloroform and 10 mL of sulfuric acid, and the formation of the bilayer (red top layer and greenish bottom layer) reveals the presence of steroids (15)

**Test for flavonoids:**

**Shinoda test:** A piece of metallic magnesium was added to 1ml of extract and 2 drops of HCL and heat the test tube in water bath. The occurance of orange, red/violet precipitate indicates presence of flavonoids

**Test for tannins**

Take 2ml of each extract in each test tubes and boil them for 2mins and allow the test tubes to cool after cooling add 3 drops of ferric chloride solution to each extract the colour changes to dark blue results presence of tannins (16)

**Phytochemical screening of Annona reticulata:**

| Tests | Methanol extract |
|---|---|
| Alkaloids | + |
| Tannins | - |
| Flavonoids | + |
| Steroids | + |
| Saponins | + |

**Phytochemical screening for Mentha piperita:**

| Tests | Methanol extract |
|---|---|
| Alkaloids | + |
| Tannins | + |
| Flavonoids | + |
| Steroids | + |
| Saponins | - |

**In Vitro Anti-Helmintic Activity:**

An anthelmintic activity screen was performed on the produced derivative chemicals. For this investigation, earthworms with almost comparable sizes (6 cm±1) were chosen at random. Worms were prior to testing, adjusted to the laboratory environment. Three groups of six earthworms each were created from the division of the earthworms. Six almost equal-sized earthworms were dissolved in room-temperature solutions of the test chemical and the conventional medication solution(17). Using regular saline as a control Standard medication and

test substances weighing 10mg, 50 mg, and 100 mg were dissolved in 2 ml of dimethyl formamide (DMF), the lowest quantity, and the volume using regular saline solution up to 15 millilitres. The amount of time needed for the earthworms to completely paralyze and die was used to compare the test compounds and standard Every test compound's mean lethal time was noted and contrasted with that of the reference medication (18) The term "paralysis time" refers to the amount of time worms take to stop moving. External stimuli were sometimes administered to the immobile worms in order to determine their death, as these stimuli would normally stimulate and cause the worms to move, if they were still alive (19). The findings portion included a tabulation of the earthworms' mean fatal time and paralysis time for various test compounds and standard medication, respectively (20).

**RESULTS:**

**STANDARD DRUG ALBENDAZOLE**

| Conc (mg/ml) | Paralysis time (Times in minutes) | Death time (Times in minutes) |
|---|---|---|
| 10mg/ml | 20.4min | 25.6min |
| 20mg/ml | 17.9min | 21.2min |
| 40mg/ml | 14.8min | 18.6min |
| 60mg/ml | 12.5min | 15.3min |

**Annona reticulata:**

| Annona Reticulata (Methanol extract) | | |
|---|---|---|
| Conc (mg/ml) | Paralysis Time (Times in mins) | Death Time (Times in mins) |
| 10mg/ml | 30.1min | 35.3min |
| 20mg/ml | 26.4min | 30.7min |
| 40mg/ml | 24.6min | 28.4min |
| 60mg/ml | 21.5min | 25.6min |

**Mentha piperita:**

| Mentha piperita (Methanolic extract) | | |
|---|---|---|
| Conc (mg/ml) | Paralysis time (Times in mins) | Death time (Times in mins) |
| 10mg/ml | 29.8min | 35.1min |
| 20mg/ml | 27.3min | 32.7min |

| | | |
|---|---|---|
| 40mg/ml | 25.1min | 30.8min |
| 60mg/ml | 23.8min | 28.22 min |

**Discussion:**

Antihelmintic refers to the ability of a substance to treat or prevent infections caused by parasitic worms,known as helminths. In vitro tests using free living stages of parasitic nematodes (egg and larval stages) have been used to evaluate the anthelmintic activity of new plant compounds.The current study aims to assess the phytochemical constiutents and perform the in vitro anti-helmintic activity. The results from the phytochemical studies of the methanolic extracts of Annona reticulata and Mentha piperita has shown the presence of several bioactive compounds such as alkaloids,tannina,flavonoids,saponins and steroids. The current study also revealed that both Annona reticulata and Mentha piperita has antihelmintic activity.

**Conclusion**:

As shown in the results methanolic extracts of Annona reticulata fruit and Mentha piperita leaf extracts showed more potent activity when compared to the aqueous extracts of the annona reticulata fruit and mentha piperita leaf and solvent fractions exhibiting considerable activity when compared with reference standard. The present research work showed the validity and the clinical use of methanolic extracts of annona reticulata and mentha piperita in the control of Anti-helmintic activity.Howeever further investigation required for chemical and pharmacological properties.

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