



PHYTOCHEMICAL SCREENING AND EVALUATION OF INVITRO ANTHELMINTIC ACTIVITY OF LEAF AND BARK EXTRACTS OF *TAMARINDUS INDICA*

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ABSTRACT

Some commonly occurring plants have anthelmintic activity. The leaves and bark of *Tamarindus Indica* was evaluated for anthelmintic activity using adult earthworms by using solvent extract of ethanol. There concentrations (5mg/ml, 10mg/ml, and 15mg/mg) of extract were studied in activity, which involved the determination of time of paralysis and death of the worm. The results were compared with albendazole as a positive control and normal saline as a negative control. Albendazole shows 100% effective on exposure, while worms exposed to normal saline are still alive even after 3 hours of exposure. Earthworms exposed to ethanolic extract of leaf and bark were paralysed and died proving the potency of *Tamarindus Indica*. Thus, Ethanol is more potent solvent extract. Finally, the extract show vermifuge and vermucidal

action.

INTRODUCTION

1. HELMINTHIASIS

The word "helminths" comes from the Greek meaning worm. The parasites that infect humans can be classified as heirlooms or souvenirs. Parasites that are inherited from ancestors in Africa are called Heirlooms, and those that are acquired from the animals during contact through our evolution, migrations, and agricultural practices are called souvenirs. In

developing countries, the most common infectious agents of humans are these helminthic infections. More than a quarter of the world's population, that means approximately 2 billion people are affected by the helminthic parasite, and it is one of the major burdens of developing countries, especially in children.

PARASITE RESPONSIBLE

There are two major phyla of helminths known as nematodes and platyhelminths. Nematodes are also known as roundworms that include soil-transmitted helminths and the filarial worms that cause lymphatic filariasis (LF) and onchocerciasis. Other phyla platyhelminths also called flatworms, which include flukes schistosomes and tapeworms such as the pork tapeworm that causes cysticercosis. Flukes are known as trematodes, and tapeworms are known as cestodes.

Soil-transmitted helminthiasis is a roundworm (*Ascaris lumbricoides*), whipworm (*Trichuris trichiura*), and hookworm (*Ancylostoma duodenale* and *Necator americanus*). The soil transmitted helminths (STHs), enter into the human body from contaminated soil that contains eggs of *A. lumbricoides* and *T. trichiura*. Some helminth can penetrate the skin directly (hookworm larvae). The diseases by helminths are neglected tropical diseases because they usually have insidious effects on growth and development. Also, the study of these diseases receives less than 1% of the global research budget.

ETIOLOGY

Intestinal parasite infections often cause morbidity and mortality, especially in children. The major risk factors of helminthiasis are rural areas, low socioeconomic status, poor sanitation, poor availability of clean water, poor personal hygiene, lack of nail trimming, crowded living conditions, lack of education, lack of access to health care, and inadequate dwelling conditions.

- *lumbricoides* and *T. trichiura* are transmitted through the fecal-oral route. Adult *Ascaris* is a long cylindrical worm, and its larvae can migrate into the pulmonary circulation, but *T. trichiura* cannot.
- *A. duodenale* and *N. americanus* are transmitted by penetration of the skin from where it goes into the lungs and crosses pulmonary capillaries to penetrate into alveolus and then to the intestine through the passing of larynx. *N. americanus* is globally predominant compared to *A. duodenale*. *S. stercoralis* can infect percutaneously and orally.

- Poor hygiene of mother or caregiver is also one of the most important risk factors for soil-transmitted helminths infection in preschool children.
- Schistosomiasis infection is usually transmitted from contact with freshwater snails during swimming or washing. Schistosomiasis causes chronic inflammation that produce oxygen-free radicals. These free radicals are responsible for different mutations and the formation of carcinogenic N-nitrosamines that cause bladder carcinoma and portal tract fibrosis.
- Diphyllorhynchiasis is most commonly occurs by species being *D. latum* from the ingestion of larva of the fish tapeworm.

PATHOPHYSIOLOGY

1.31 DIRECT DAMAGE

Direct damage is done by worm activity itself, such as internal organ blockage or direct pressure effects by growing parasites.

- Adult *Ascaris* blocks the intestine that leads to small bowel obstruction, volvulus, or intussusception, especially in children, or can invade orifices leading to appendicitis, cholecystitis, pancreatitis, and gastric ascariasis. Migrating *Ascaris* can also block the bile duct and may also alter the intestinal microbiota. Mucosal bleeding from the upper gastrointestinal tract or generalized inflammation leads to anemia.
- *Trichuris* lies in intestinal mucosa and can cause petechial lesions, blotchy mucosal hemorrhage, oozing, and colonic inflammation. It can also cause severe anemia in pregnant women.
- Schistosomiasis infection is acquired by contact with contaminated freshwater, especially during swimming or washing. Deposition of schistosome eggs within the liver and bladder may form granulomas around these eggs that can block blood flow in the liver that leads to pathological changes like periportal fibrosis and have been linked with neoplasia. Interestingly, this periportal fibrosis has retained hepatocellular function that is different from other causes of cirrhosis. These liver flukes can also cause bile duct hyperplasia.
- *Wuchereria bancrofti* causes lymphatic obstruction leads to elephantiasis. Hydatid cyst caused by the larval tapeworm infections (*Echinococcus granulosus*) leads to pressure atrophy.
- *Taenia solium*, the pork tapeworm, frequently develops in the intestine leads to taeniasis, and in the central nervous system leads to cysticercosis.

- *Ancylostoma* and *Necator* burrow their teeth into mucosa and submucosa, create negative pressure by contracting their muscular esophagi that lead to rupture of the capillaries and arterioles and actively suck blood. Blood vessels are ruptured by both mechanical compressions and hydrolytic enzymes secreted by these hookworms.
- *Diphyllobothrium latum* causes vitamin B12 deficiency through interfering with the absorption through the intestine. Migration through body tissue, many helminths cause direct tissue damage and also by hypersensitivity reactions, whereas most affected organs are skin, lungs, liver, and intestines.

1.3.2 INDIRECT DAMAGE

Indirect damage is done by the host immune response against helminth.

- All helminths are antigenic to the body because they are foreign bodies and stimulate the immune response. Lymphatic blockage by *W. bancrofti* and granuloma formation by schistosomes in the liver and bladder are associated with hypersensitivity reaction against these helminths.
- *Strongyloides* and *Trichinella* may induce prolonged inflammation of the intestine that causes villous atrophy; in severe cases, it may cause protein-losing enteropathy.
- *S. stercoralis* can cause Loeffler syndrome by type 1 hypersensitivity reaction. *Trichuris*, which is also known as whipworm, can cause inflammation of the colon that leads to blood loss and rectal prolapse. □
- Indirect damage depends on the severity of the inflammation and the duration of inflammation. If the duration is prolonged, many worms produce extensive inflammatory damage to tissues that is an irreversible and functional loss of the tissues, such as bile duct hyperplasia by long term infection with liver flukes, fibrosis caused by schistosomiasis and skin atrophy caused by onchocerciasis.

1.4 TREATMENT / MANAGEMENT

Proper hygiene maintenance is one of the most important measures to prevent helminth infection. For the treatment of *A. lumbricoides*, several drugs may be used, including albendazole, mebendazole, pyrantel pamoate, levamisole, and ivermectin. If patients develop intestinal obstruction, it requires proper treatment with intravenous support, anthelmintics, and antibiotic treatment. Laparotomy might be necessary in case of small bowel obstruction, intussusception, and volvulus. Hepatobiliary ascariasis can be treated with drug therapy. If conservative therapy fails, then endoscopic and surgical therapy may be required.

1.5 PHARMACOLOGY

1. For **trichuriasis**, available drugs that might be use are albendazole, mebendazole, pyrantel pamoate, and ivermectin. If the symptom is severe or the patient develops anemia, iron therapy is provided, and supportive therapy is needed if a patient develops dysentery.
2. For ***A. duodenale*, *N. americanus***, recommended treatment options include albendazole or mebendazole. Iron supplementation is provided for additional nutritional support.
3. For ***S. stercoralis*** treatment, benzimidazoles (thiabendazole, mebendazole, and albendazole) or ivermectin gives a very good response. In Strongyloides hyperinfection syndrome and disseminated strongyloidiasis, hydration, nutritional support, and antibiotics are needed as indicated; ivermectin should be continued for at least seven days or until sputum or stool samples are negative for the parasite for two weeks.
4. For **schistosomiasis**, the drug of choice is praziquantel. Repeated treatment is needed in severe cases or if Katayama fever develops. Steroids may also be given during Katayama fever to decrease symptoms.
5. **Lymphatic filariasis** can be treated by several drugs, including ivermectin, suramin, mebendazole, flubendazole, and diethylcarbamazine. Other available options are surgical, thermal, and herbal treatment.
6. Four different modalities are available for the management of **cystic echinococcosis**, which includes chemotherapy, percutaneous therapy, surgery, and observation without intervention. Benzimidazoles are the cornerstone of medical therapy.
7. Treatment options for **neurocysticercosis** are symptomatic therapy such as anticonvulsants, analgesics, antihelminthic therapy, and surgery, which include worm removal surgery or shunt placement.
8. Praziquantel is used for the treatment of diphyllbothriasis. Niclosamide is also another available drug.

1.6 COMPLICATIONS

A lot of complications can occur in helminth infection, which may include:

- Anemia
- Malnutrition
- Growth retardation
- Developmental retardation

- Intestinal obstruction
- Gastrointestinal hemorrhage
- Portal hypertension
- Urinary bladder carcinoma
- Neurological complications such as seizure, myelopathy
- Primary and secondary infertility
- Ectopic pregnancy and tubal pregnancy
- Cholangitis
- Cholecystitis
- Pancreatitis
- Cyst or Hydatid cyst rupture
- Chronic lymphatic damage
- Blindness

1.8 EARTHWORM

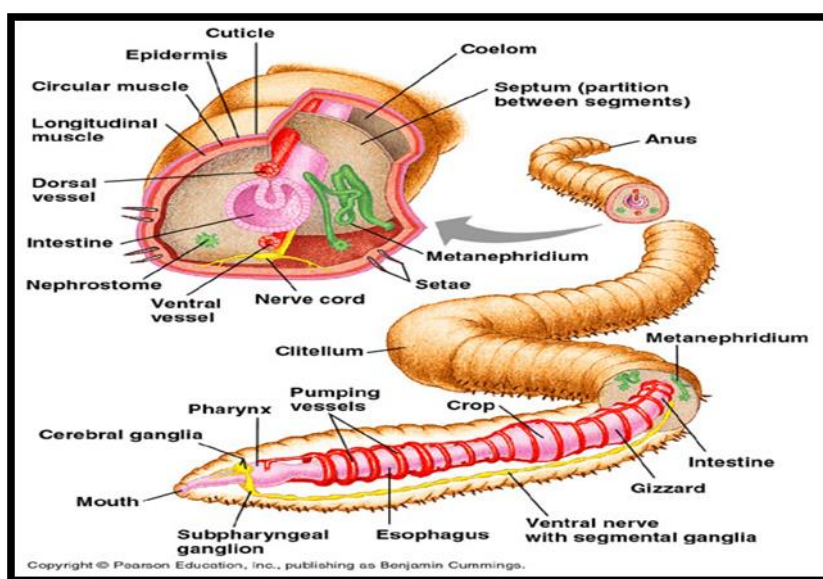
Earthworms are considered as the farmer's friend as they contribute a major role in biodiversity and are responsible for increasing the nutrient content of soil through various processes such as aggregates formation and nutrient cycling processes which involves nitrogen cycles, phosphorus and carbon, which ultimately helps in agriculture. The feeding, burrowing and other activities of earthworm are known to influence the soil fertility by undergoing several physiochemical and microbial changes. When the soil passes through the gut of the earthworm the plant nutrient availability is increased by the activity of the gut microflora. Interactions between earthworms and micro-organisms are seemed to be complex. Specific phylogenetic groups of bacteria such as *Aeromonas hydrophila* in *E. fetida*, fluorescent *Pseudomonas* in *L. terrestris*, and Actinobacteria in *L. rubellus* have been found in higher numbers in earthworm guts, casts, or burrow.

MORPHOLOGY OF EARTHWORM

Earthworms have a reddish-brown colour with a cylindrical body. The body is also elongated and is pointed in the anterior region, while the posterior is rounded. The body is segmented and there are about 100-120 metameres or short segments. There is a dark median mid-dorsal blood vessel that is seen on the dorsal surface of the body. The ventral surface of the body has genital openings or pores.

Peristomium is the first body segment that also has the mouth. The last segment has the anus. In between segments 14 to 16, there is a prominent dark band of glandular tissue called as the clitellum. These are the fused segments. Between the 5th, 6th, 7th, 8th, and 9th inter segment, there are four pairs of spermathecal apertures present. On the 14th segment, in the mid ventral line, a single female genital pore is present. A single pair of male genital pores is present on the 18th segment. There are many minute called the nephridiopores on the surface of the body. Except for the first segment, last segment, and clitellum, all other segments have S-shaped setae that are present embedded. These setae help in the locomotion.

BODY ANATOMY



TAXONOMY

Table No. 1.1 taxonomy of earthworm.

Kingdom	Animalia
Phylum	Annelida
Class	Clitellata
Order	Opisthopora
Suborder	Lumbricina
Family	Megascolecidae
Genus	<i>Pheretima</i>
Species	<i>posthuma</i>

NATURAL REMEDIES TO TREAT HELMENTHIASIS

Helminthes infections are the most common infectious in man which affects the large proportions of the world's population. In the treatment of parasitic diseases, the anthelmintic drugs are used indiscriminately. Recently the use of anthelmintics produces

toxicity in human beings. hence the development and discovery of new substances acting as anthelmintics are being derived through plants are considered to be the best source of bioactive substances. various plants were used in venereal diseases, to promote healing of wounds, swellings, abscesses, rheumatism and treating pain in lower extremities, skin diseases leucorrhoeas, dysentery, dysuria and fever. Anthelmintics are those drugs that are used in expelling out the worms that are parasitic in nature by either stunning them or by killing them. They are also known as vermifuges or vermicides.

Natural drugs having anthelmintic activities includes the following list of components:

- Garlic
- Pumpkin seeds
- Papaya
- Neem
- Ginger

REVIEW OF LITERATURE

1. Sharma *et. al* (2019)

Tamarindus indica: Origin, cultivation and antioxidant potential effects

The *T. indicus* includes a variety of bioactive compounds in the leaves, seeds, bark, pulp, and flowers with beneficial effects to human health and the possibility of application in the pharmaceutical industry. The drugs normally used to regulate glycaemia, dyslipidemia and other metabolic disorders are costly; if we consider that these diseases have reached epidemic proportions in many countries, it is necessary to find non-allopathic alternatives that minimize the risk factors of these diseases and help in the treatment or in the prevention of further complications and death. Further studies are necessary in order to elucidate all the properties of the tamarin in order to obtain information enough to provide validation for its medical use.

2. Dineshkumar *et.al* (2010)

Evaluated aqueous and ethanolic extracts of *T.indica* leaves

T. indica is a cheap and easily available plant. It is a rich source of essential amino acids, phytochemicals and vitamins. In traditional medicine, it has so many wellknown health benefits. With the aid of modern techniques it could be used in evidence based medicine in so

many health conditions. There is a need of further investigation about this plant and its potential antioxidant and anti-inflammatory properties that can help in many of the diseases.

3. Edewor –Tsiri et.al (2014)

Tamarind leaves are worldwide reported for their antioxidant and antimicrobial activity, but it hasn't been possible to establish a relationship with the chemical composition due to the scanty information available. In this study, we detected eight components not previously reported, and confirmed the high fatty acid and polyphenol production in *Tamarindus indica* L leaves. In addition, high concentration of the most prominent pro-oxidant/antioxidant cations is described. All this information give light to the pretended intention to find chemical proof that support the pharmacological activities previously described for tamarind leaves.

4. Richard komakech et al (2019)

Anti –inflammatory and analgesic potential of *Tamarindus Indica*

Anti-inflammatory and analgesic potential of *Tamarindus indica* Linn. Throughout the world, diseases caused by inflammation are a significant health burden and hence all possible measures have to be explored to tackle it. *T. indica* has a rich history of use as anti-inflammatory and analgesic medicinal plant in traditional medicine. In fact, the anti-inflammatory effects of *T. indica* may be due to its ability to inhibit a number of biological pathways including NF- κ B activation pathways, and leukotriene biosynthesis while its analgesic activity may be via the activation of the opioidergic mechanism at both the peripheral and central mechanisms of pain generation and inhibition of the prostaglandin pathways. And as observed in this study, although the anti-inflammatory and analgesic activities of the classes of bioactive compounds flavonoids, alkaloids, tannins, phenols, and saponins discussed in this study were from different sources and not directly isolated from *T. indica* and experimented, their presence in *T. indica* gives an insight into the Anti-inflammatory and be done to evaluate the safety associated with its long term use.

5. Julio César Escalona-Arran et al

Antimicrobial activity of extracts from *Tamarindus indica* L. Leaves

In the light of our results, we can suggest that some compounds found in the essential oils from leaves of the *T. indica* L. that grows in Cuba could be responsible for its antimicrobial activity. Anyway, in our climatic conditions, the poor yield of essential oil production, and in addition, the low levels of the most prominent antimicrobial compounds detected in the tamarind leaves' essence give us a possible explanation to the relatively poor ethnobotanical

reports in Cuba. Nevertheless, we cannot reject the fact that other compounds such as phenols could show some activity against particular kinds of microorganisms. Also, a combination of some of the metabolites found with others types of compounds extracted by other popular remedy preparations could form a natural mixture that reaches the final antimicrobial spectrum by some kind of synergy, as it could have happened with the hydroalcoholic extracts.

6. Joshi *et.al* (2019)

Tamarindus indica L. or Tamarind of the family Fabaceae, subfamily Caesalpinioideae, is an important food in the tropics. Every part of *Tamarindus indica* including root, wood, bark, leaves and fruits has either medicinal or nutritional value, with a number of commercial and industrial applications. Tamarind is a multipurpose tree having its uses in every field, either medicinal or nutritional. This plant is indigenous to tropical Africa but it has been introduced worldwide in more than 50 countries for its food flavoring, essential oil applications and in traditional medicines. Mostly tamarind contains oleic acid, palmitic acid, eicosanoic acid and linoleic acid. It also contains many minerals like calcium, copper, iron, manganese, phosphorus and zinc. It is also a source of Vitamin B, Vitamin C and carotene. The extent of these chemical constituents varies depending on its types and cultivation conditions. Tamarind is an essential component of several industrial applications. Tamarind is also well known for its anti-oxidant, antimicrobial, anti-inflammatory and anti-fungal activity that has been reported from various plant parts.

7. Abubakari A-H *et al* (2020)

study of nutritional potential of tamarind (*Tamarindus indica* L.) in Northern Ghana

Many authors have recognized the tamarind tree as an underutilized crop with a high potential. The benefits derived from this tree and its products are promising and numerous as evident in this study. The tamarind plant is an all-round, beneficial and nutritious fruit with a great potential. Almost every part of plant (fruit pulp, leaves, bark, root, stems, and seeds) has either some nutritional benefit or medicinal value, and it widely used domestically in Ghana with a number of industrial and commercial applications across the world. This study has exposed in different ways through the collection and reviewing of primary and secondary data, that the tamarind tree comes with enormous benefits, uses and has a great potential. From this study, it is clear that several authors have reported the use of this fruit tree for both local and industrial purposes. It can also be a very important remedy in parts of the world

where malnutrition is a prevalent problem. From its use in local food, drinks and medicinal purposes across northern Ghana and other countries stretching from Africa through Asia and some Latin American countries, it can serve a source of lowcost nutrient supplement. Industrially, the pulp is commonly used in the making of chilled drinks, jams, syrups, juices and other localized products. In its nonfood use potential, this study has shown that tamarind non-fruit parts such as leaves, bark and roots have various uses such as a source of fuelwood, charcoal making, source of timber and as fodder for animals. Based on the high levels of fats and oils in sourced pulps in this study, it could be explored as an alternative source. The potential of the tamarind tree, its products and utilization forms should be further investigated to enhance human nutritional and medicinal needs.

8. Brailson Mansingh (2021)

The raw material obtained from tamarind tree must be processed, extracted and modified before it is used in industrial applications to develop eco-friendly and sustainable product and processes. Modified tamarind fruit shell, seed coat and tamarind wood are efficiently used in treatment of toxic effluents from industries. Tamarind seed coat was also used as animal feed to reduce methane emission during rumen fermentation process and increase the availability of protein for ruminants. The pulp of tamarind fruit and its extract was used as food additive, preservative and to produce ready to eat foods by food industries. TSP a biopolymer extracted from endosperm of tamarind seed was used for controlled and targeted drug delivery applications in pharmaceutical industries, as thickener and gelling agent in food industries, as solid electrolyte in electrochemical industries and in the form of hydrogel in agricultural industries. TKP was used as adhesive in wood and electrochemical industries. Tamarind seedoil blended with diesel is used as biofuel in internal combustion engine to reduce harmful emissions that pollute the environment. The composite material with TFF as reinforcement was used in automobile, construction and packaging industries. The tamarind leaf extract was used to reduce metal salts into nanoparticles which finds application in textile industries.

9. SontayaSookying et al (2022)

Botanical aspects, phytochemicals, and toxicity of *Tamarindus indica* leaf and a systematic review of antioxidant capacities of *T. indica* leaf extracts.

In the present study, the antioxidant capacity of *T. indica* leaves was reviewed. *T. indica* leaf extracts exhibited *in vitro* antioxidant capacity through free radical scavenging capacity and

transition and heavy metal chelating capacity. There is a high possibility that the antioxidant capacities are responsible for the polyphenols and the elements. The polyphenols found in *T. indica* leaves are flavonoids and phenolic acids such as catechin, vitexin, orientin, apigenin, and luteolin. In addition, elements such as selenium, copper, manganese, and zinc are present. These chemicals and elements are well-known as strong antioxidants, which makes *T. indica* leaves a promising natural antioxidant mixture. The safety of *T. indica* leaves was investigated in erythrocytes and animals. The extracts were found to be safe after oral administration of 4,000 mg/kg BW for 14 days, and no death was observed after the ingestion of 5,000 mg/kg BW. The 50% lethal intraperitoneal dose was 566 mg/kg BW.

10. A .K. Singh et. al (2025)

The results demonstrate how varied germplasm might be used to improve tamarind cultivars. To overcome heterogeneity in desired features, a complete collection of 28 morphological descriptors is provided to characterize, evaluate, and identify tamarind genotypes. The results underscore the importance of phenotypic diversity for developing core collections with enhanced variability and for designing targeted tamarind tree breeding strategies. This study provides valuable insights for the improvement and conservation of tamarind germplasm, a valuable species with considerable potential for fruit production and other economic uses.

11. Michel Selassie et. al (2020)

The utility of *Tamarindus indica* in ethnomedicine is immense. This study has shown that both root and stem bark ethanolic extracts of the plant do possess anti-inflammatory and antioxidant activities. The stem bark extract showed better activity in reducing carrageenan-induced edema in chick compared to the root extract. The stem extract also showed much better scavenging potential than the root extract. Both root and stem extract, however, had similar total antioxidant capacities and total phenolic contents. The results of this study provide further support for the use of the plant in ethnomedicine.

12. Bathrinarayanan M 1 et al (2023)

It mainly focused on anthelmintic activity, which is exerted by the various easily available and effective phytoconstituent samples. In this study, we reviewed the research work and found promising results given by the samples (especially in the extracts obtained from leaves). Adult Indian earthworms, *Pheretima posthuma*, have been used as test worms in most of the anthelmintic screenings as they show anatomical and physiological resemblance to the

intestinal roundworm parasite in humans. Which is taken as a base for various research efforts to overcome the emerging resistance shown by conventional anthelmintic drugs.

PLANT PROFILE

Tamarind (*Tamarindus indica*) is a leguminous tree bearing edible fruit that is indigenous to tropical Africa and naturalized in Asia. The genus *Tamarindus* is monotypic, meaning that it contains only this species. It belongs to the family Fabaceae.

DESCRIPTION

The tamarind is a long-living, medium-growth tree, which attains a maximum crown height of 25 metres (80 feet). The crown has an irregular, vase-shaped outline of dense foliage. The tree grows well in full sun. It prefers clay, loam, sandy, and acidic soil types, with a high resistance to drought and aerosol salt (wind-borne salt as found in coastal areas).

The evergreen leaves are alternately arranged and paripinnately compound. The leaflets are bright green, elliptic-ovular, pinnately veined, and less than 5 centimetres (2 inches) in length. The branches droop from a single, central trunk as the tree matures, and are often pruned in agriculture to optimize tree density and ease of fruit harvest. At night, the leaflets close up.

As a tropical species, it is frost-sensitive. The pinnate leaves with opposite leaflets give a billowing effect in the wind. Tamarind timber consists of hard, dark red heartwood and softer, yellowish sapwood.

The tamarind flowers bloom (although inconspicuously), with red and yellow elongated flowers. Flowers are 2.5 cm (1 in) wide, five-petalled, borne in small racemes, and yellow with orange or red streaks. Buds are pink as the four sepals are pink and are lost when the flower blooms.

History

The name derives from Arabic: تمر هندي, romanized *tamrhindi*, "Indian date". Several early medieval herbalists and physicians wrote *tamarindi*, medieval Latin use was *tamarindus*, and Marco Polo wrote of *tamarandi*.

In Colombia, Nicaragua, Costa Rica, Ecuador, Cuba, the Dominican Republic, Guatemala, El Salvador, Honduras, Mexico, Peru, Puerto Rico, Venezuela, Italy, Spain, and throughout

the Lusosphere, it is called *tamarindo*. In those countries it is often used to make the beverage of the same name (or *agua de tamarindo*). In the Caribbean, tamarind is sometimes called *tamón*.

TAXONOMY

Table No. 3.1: Taxonomy of *Tamarindus Indica*.

Kingdom	Plantae
Phylum	Magnoliophyta
Class	Dicotylendons
Clade	Angiosperms
Order	Fabales
Family	Fabaceae
Sub family	Detarioideae
Genus	<i>Tamarindus. L</i>
Species	<i>T.Indica</i>

Morphological source

1. Tree

Size: A large tree, typically reaching 10-25 meters in height, with a trunk diameter of 30-50 cm (or up to 90 cm).

Bark: Rough and greyish, with an irregular, longitudinal splitting pattern.

Crown: Spreading and irregular, often described as vase or round-shaped, and dense.

2. Leaves

- **Arrangement:** Alternate.
- **Type:** Compound, meaning they are made up of multiple leaflets.
- **Leaflets:** Numerous (10-18 pairs), opposite, narrowly oblong, with obtuse bases and apexes. They are small, typically 1.2-3.2 cm long and 0.3-1.1 cm wide.
- **Texture:** The petiole and rachis (the central stalk of the leaf) are finely haired.

3. Flowers

- **Arrangement:** Terminal, in small, lax racemes (spikes).
- **Color:** Pale yellow or pinkish.
- **Structure:** Zygomorphic (bilaterally symmetrical), with 4 sepals, 5 petals (3 well-developed, 2 minute), and 3 fertile stamens. The lower 2 petals are fused to form a keel.

4. Fruit

- **Type:** An indehiscent pod (meaning it doesn't split open naturally).

- **Shape:** Subcylindrical, straight or curved, and often constricted.
- **Size:** Typically 10-18 cm long and 2-3 cm in diameter.
- **Texture:** Velvety or scurfy on the outside.
- **Color:** Rusty-brown when mature.
- **Content:** Contains a sticky, edible pulp and 3-10 seeds.
- **Seeds:** Irregularly shaped, with a hard, shiny, and smooth testa (seed coat).

BIOLOGY

Tamarind (*Tamarindus indica*) is a tropical tree that produces a pod-like fruit with a tangy, sweet-sour pulp. It's commonly grown for its fruit, which is used in cooking, especially in sauces, drinks, and curries. The reproduction of tamarind can be done through **seeds** or **vegetative propagation** (cuttings), although **seeds** are more commonly used in practice. Here's a breakdown of the reproductive methods:

1. Seed Propagation (Sexual Reproduction)

1. **Soak Seeds:** Soak tamarind seeds in water for 24 hours.
2. **Plant Seeds:** Plant seeds 1-2 inches deep in well-draining soil.
3. **Water:** Keep the soil moist, but not soggy.
4. **Germinate:** Place in a warm spot (25-30°C). Seeds will sprout in 1-2 weeks.
5. **Transplant:** Once seedlings have 2-3 leaves, transplant them into bigger pots or the ground.

2. Vegetative Propagation (Asexual Reproduction)

Tamarind can also be propagated by cuttings, though this method is less common and often more difficult than seed propagation.

Steps

- **Take Cuttings:** Select a healthy, semi-hardwood stem about 6-8 inches long.
- **Prepare the Cutting:** Remove lower leaves, and optionally dip the cut end in rooting hormone.
- **Plant Cutting:** Place the cutting in a pot with a well-draining mix (sand + soil or perlite).
- **Water:** Keep the cutting moist and place it in a shaded, humid area.
- **Root Development:** In about 4-6 weeks, the cutting should root. Then, transplant it into larger pots or the ground.

Cultivation

Tamarind (***Tamarindus indica***) thrives in hot, tropical climates with temperatures between 25-30°C and full sunlight. It grows best in well-drained, loamy soil with a pH between 5.5 and 7.0. Propagation is primarily done through seeds, which should be soaked for 24 hours before planting in nursery beds. Once the seedlings reach 10-15 cm, they are transplanted 10-12 meters apart. Tamarind trees are drought-resistant but require regular watering during the early stages of growth. Fertilization with balanced NPK or organic compost helps support healthy development. Pruning is essential to remove dead branches and promote good airflow. Pests like aphids and mealybugs can be managed with organic treatments, while root rot can be avoided by ensuring proper soil drainage. Harvesting typically begins after 6-8 years, when the pods turn brown and soft. Tamarind is used in cooking for its sour-sweet flavor in sauces, drinks, and curries, as well as in traditional medicine for its digestive benefits. Additionally, the wood is durable and used for furniture and crafts.

GROWTH AND DEVELOPMENT

The growth of ***Tamarindus indica*** (tamarind) starts with seed germination in 2–4 weeks. In the first 3 years, the tree develops a strong root system and trunk, requiring regular water and sunlight. By the 3rd–6th year, it begins flowering, and fruit production follows. Tamarind trees start bearing fruit at 6–8 years, with full fruiting at 10 years.

Mature trees, reaching 7–10 meters, can live for decades and produce 100+ kg of fruit annually. Regular pruning and pest control help ensure healthy growth, while the tree's wood is also valuable.

USES

1. Culinary Uses

- **Pulp:** The sour-sweet pulp inside tamarind pods is used in various dishes, such as curries, sauces, soups, and chutneys. It's a key ingredient in many Asian, African, and Latin American cuisines.
- **Beverages:** Tamarind is used to make refreshing drinks like "tamarind juice" or "tamarind soda" in many cultures.
- **Condiments:** Tamarind paste or concentrate is commonly used in making tangy condiments and marinades.

2. Medicinal Uses

- **Digestive Aid:** Tamarind is known for its digestive properties, helping to relieve constipation and improve appetite.
- **Antioxidant:** Rich in vitamins (like vitamin C) and antioxidants, tamarind is believed to promote general health and support immune function.
- **Anti-inflammatory:** The fruit has anti-inflammatory properties and has been used in traditional medicine to treat conditions like arthritis.

3. Industrial & Non-Culinary Uses

- **Wood:** Tamarind wood is strong, durable, and resistant to termites, making it ideal for furniture, tool handles, and construction.
- **Dye:** The seeds and pulp have been used in some cultures for making natural dyes.
- **Cosmetics:** Tamarind extracts are sometimes used in skincare products due to their antioxidant and exfoliating properties.

4. Agricultural Uses

Soil Enrichment: Tamarind trees can improve soil quality by preventing erosion and enriching the soil with organic matter when leaves and pods decompose.

4. AIM AND OBJECTIVES

Aim

To evaluate the Phytochemical screening and In vitro anthelmintic activity of Leaf and bark extracts of *Tamarindus Indica*.

Objectives

Objectives of present study

- Survey of Review of Literature
- Selection of plant
- Collection of plant material
- Extraction
- Preliminary phytochemical screening for secondary metabolites
- Thin Layer Chromatography
- In vitro Anthelmintic activity
- Results and Analysis

Hypothesis

As many experimental studies proved that *Tamarindus indica* plants have potent anthelmintic activity, we propose to investigate the strengths of leaves and bark extracts of *Tamarindus indica*.

5 MATERIALS AND METHODS

Plant collection and extraction

- *Tamarindus indica*, leaves are collected from loamy soil of narsapur, telangana in the month of april 2025.
- Plants are cleaned, leaves are separated and washed.
- These are shade dried.
- Dried leaves are powdered to attain a coarse powder.
- This powder is kept for soxhlation by using ethanol as a solvent.

Method of extraction

The leaves *tamarindus indica* were collected. These are washed with water for removal of dirt and dried in shade at room temperature for 25 consecutive days. The dried leaves were coarsely powdered. Then 30gm leaves powdered of *tamarindus indica* were kept for extraction by taking 500ml of ethanol as solvent using soxhlet apparatus. The dried residue was stored in a desiccators.

Soxhalation process

Though various methods are available for the extraction of secondary metabolites from the plants, effective extraction is influenced by the method and selection of solvent. Soxhlet extraction method is convenient and widely used extraction because of its continuous process, less time and less solvent consumption than other extraction processes 36gm powdered leaves material were placed in a Soxhlet apparatus, which is fitted on top of a collecting flask beneath and reflux condenser. A suitable solvent is added to flask and to set up is heated under reflux below the boiling point of the solvent. The steam of the solvent, which contacts with the material will dissolve metabolites and brings it back to RBF.

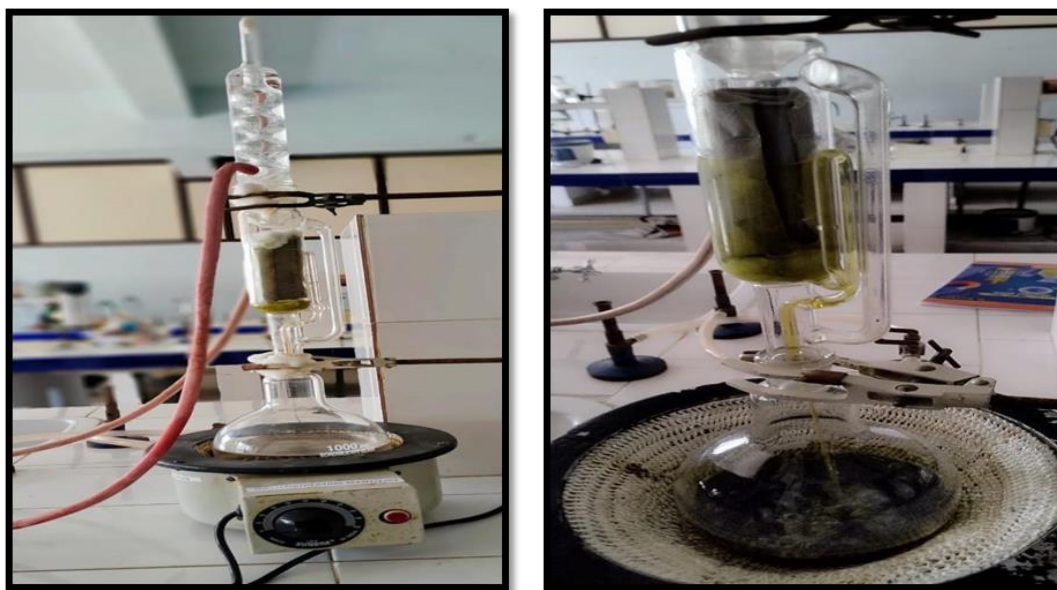


Figure No. 5.1: extraction by soxhlet Apparatus of *Tamarindus Indica*.



Figure No. 5.2: Extraction Residues of Leaf and Bark.

Residue of extracts were calculated by using below formula:

$$\text{Percentage (\%) yield} = \frac{\text{weight of extract}}{\text{weight of powder}} \times 100$$

1. Ethanolic extract of leaf

$$\text{Percentage (\%) yield} = \frac{\text{weight of extract}}{\text{weight of powder}} \times 100$$

$$0.52/30 \times 100 = 1.73\%$$

2. Ethanolic extract of bark

$$\text{Percentage (\%) yield} = \frac{\text{weight of extract}}{\text{weight of powder}} \times 100$$

$$0.65/30 \times 100 = 2.16\%$$

The results were tabulated in table no 6.1

5.3 PRELIMINARY PHYTOCHEMICAL SCREENING FOR SECONDARY METABOLITES

Different tests were performed as follows.

(a) Test for alkaloids

Mayer's test

The extract was treated with Mayer's reagent. Formation of a yellow cream precipitate indicates the presence of alkaloids.

Wagner's test

The extract was treated with Wagner's reagent. Formation of brown/ reddish brown precipitate indicates the presence of alkaloids.

(b) Test for flavonoids

Lead acetate test: Extract was treated with few drops of lead acetate solution. Formation of yellow color precipitate indicates the presence of flavonoids.

H₂SO₄ test: Extracts were treated with few drops of Conc. H₂SO₄. Formation of orange color indicates the presence of flavonoids.

(c) Test for steroids

2ml of acetic anhydride was added to 5ml of the extract and then added each with 2ml of Conc. H₂SO₄. The color was changed from violet to blue or green indicates the presence of steroids.

(d) Test for terpenoids

Salkowsk's Test: 5ml of the extract were mixed with 2ml of chloroform and then added carefully the 3ml of conc. H₂SO₄ to form a layer. An appearance of reddish brown color in the inner face indicates the presence of terpenoids.

(e) Test for phenols

Ferric chloride test: 10ml of the extract was treated with few drops of ferric chloride solution. Formation of bluish black color indicates the presence of phenol.

(f) Test for tannins: A small quantity of extract was mixed with and heated on a water bath. The mixture was filtered and ferric chloride was added to the filtrate. A dark green color was formed. It indicates the presence of tannins.

(g) Test for glycosides

Borntrager's test: To the test tubes containing 2ml of extract 2ml of dilute sulphuric acid was added, boil for 5 min and filtered. To the filtrates, equal volumes of chloroform was added and mixed well. Organic layers were separated and ammonia was added to this. Pinkish red color of the ammonia layer indicated the presence of glycosides.

(h) Test for protein

Biuret test: To 5ml of extract equal volume of 40 % NaOH solution and 2 drops of 1 % copper sulphate solution was added. The appearance of violet color indicates the presence of protein.

(i) Test for saponins

Foam test: The extract was shaken vigorously with equal volume of water and observed for persistent foam, which indicates the absence of Saponins.

(j) Test for carbohydrates

Few drops of Molisch's reagent were added to an aqueous solution of each extract followed by vigorous shaking. Thereafter, 1.0 ml of conc. H₂SO₄ was added carefully by sliding down the walls of the tube gently to form two layers. The solution was examined for the appearance of brown ring separating the solution into two layers.

(k) Test for amino acid

Ninhydrin test: About 0.5ml of extract was taken and 2 drops of freshly prepared 0.2% ninhydrin reagent was added and heated. The appearance of purple color indicates that the absence of peptides and amino acid.

5.4 THIN LAYER CHROMATOGRAPHY

Thin-layer chromatography (TLC) is a chromatography technique that separates components in non-volatile mixtures.

It is performed on a TLC plate made up of a non-reactive solid coated with a thin layer of adsorbent material. This is called the stationary phase. The sample is deposited on the plate, which is eluted with a solvent or solvent mixture known as the mobile phase (or eluent).

This solvent then moves up the plate via capillary action. As with all chromatography, some compounds are more attracted to the mobile phase, while others are more attracted to the stationary phase. Therefore, different compounds move up the TLC plate at different speeds and become separated. To visualize colourless compounds, the plate is viewed under UV-light or is stained. Testing different stationary and mobile phases is often necessary to obtain well-defined and separated spots. TLC is quick, simple, and gives high sensitivity for a relatively low cost. It can monitor reaction progress, identify compounds in a mixture, determine purity, or purify small amounts of compound.

5.4.1 Procedure

The process for TLC is similar to paper chromatography but provides faster runs, better separations, and the choice between different stationary phases. Plates can be labelled before or after the chromatography process with a pencil or other implement that will not interfere with the process. There are four main stages to running a thin-layer chromatography plate. Plate preparation: Using a capillary tube, a small amount of a concentrated solution of the sample is deposited near the bottom edge of a TLC plate. Solvent completely evaporate before the next step. A vacuum chamber may be necessary for non-volatile solvents.

To make sure there is sufficient compound to obtain a visible result, the spotting procedure can be repeated. Depending on the application, they may also place different samples in a row, the same distance from the bottom edge; Each sample will move up the plate in its own "lane."

5.4.1.1 Development chamber preparation: The solvent or solvent mixture is placed into a transparent container (separation/development chamber) to a depth of less than 1 centimetre. They then place a strip of filter paper (aka "wick") along the container wall. This filter paper should touch the solvent and almost reach the top of the container. The container is covered with a lid and the solvent vapors are allowed to saturate the atmosphere of the container. Failure to do so results in poor separation and non-reproducible results.

5.4.1.2 Development: The TLC plate is placed in the container such that the sample spot(s) are not submerged into the mobile phase. The container is covered to prevent solvent evaporation. The solvent migrates up the plate by capillary action, meets the sample mixture, and carries it up the plate (elutes the sample). The plate is removed from the container before the solvent reaches the top of the plate. Otherwise, the results will be misleading. Without delay, they mark the solvent front: the furthest extent of solvent up the plate.

5.4.1.3 Visualization: The solvent evaporates from the plate. Visualization methods include UV-light, staining, and many more.

5.5 SEPARATION PROCESS AND PRINCIPLE

The separation of compounds is due to the differences in their attraction to the stationary phase and because of differences in solubility in the solvent. As a result, the compounds and the mobile phase compete for binding sites on the stationary phase. Different compounds in the sample mixture travel at different rates due to the differences in their partition coefficients. Different solvents, or different solvent mixtures, gives different separation. The retardation factor (R_f), or retention factor, quantifies the results. It is the distance travelled by a given substance divided by the distance travelled by the mobile phase.

In normal-phase TLC, the stationary phase is polar. Silica gel is very common in normal-phase TLC. More polar compounds in a sample mixture interact more strongly with the polar stationary phase. As a result, more polar compounds move less (resulting in smaller R_f) while less polar compounds move higher up the plate (higher R_f). A more polar mobile phase also binds more strongly to the plate, competing more with the compound for binding sites; A more polar mobile phase also dissolves polar compounds more. As such, all compounds on the TLC plate move higher up the plate in polar solvent mixtures. "Strong" solvents move compounds higher up the plate, whereas "weak" solvents move them less.

If the stationary phase is non-polar, like C18-functionalized silica plates, it is called reverse-phase TLC. In this case, non-polar compounds move less, and polar compounds move more. The solvent mixture will also be much more polar than in normal-phase TLC.

5.6 PLATE PRODUCTION

TLC plates are usually commercially available, with standard particle size ranges to improve Reproducibility. They are prepared by mixing the adsorbent, such as silica gel, with a small

amount of inert binder like calcium sulphate (gypsum) and water. This mixture is spread as a thick slurry on an unreactive carrier sheet, usually glass, thick aluminium foil, or plastic. The resultant plate is dried and activated by heating in an oven for thirty minutes at 110 °C. The thickness of the adsorbent layer is typically around 0.1–0.25 mm for analytical purposes and around 0.5–2.0 mm for preparative TLC. Other adsorbent coatings include aluminium oxide (alumina), or cellulose.

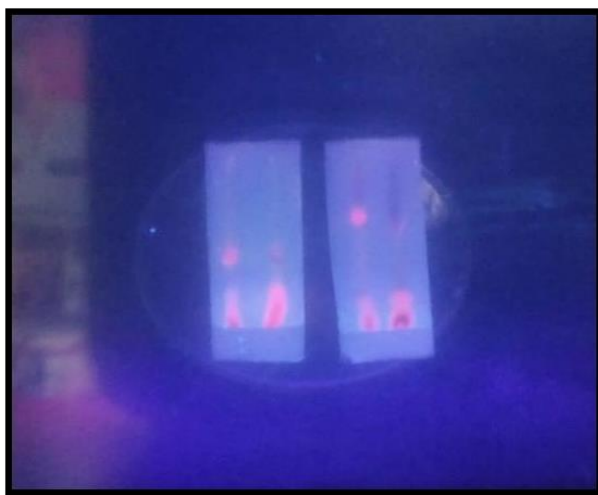


Figure No. 5: Development of TLC Plates in UV Chamber.

5.7 IN-VITRO ANTHELMINTHIC METHOD

5.7.1 Procedure of Anthelmintic Activity

For Anthelmintic activity of leaf extracts of *Tamarindus indica*. Indian adult earthworms (*Pheretina posthuma*) were collected from loamy soil, Narsapur, Telangana. Earthworms are used for evaluating the anthelmintic activity due to its anatomical and physiological resemblance with the intestinal round worm parasites of human beings. All earthworms were washed with normal saline solution in order to remove all the earthy matter.

Properly washed and sterilized petri plates are taken. Standard vehicle is prepared by mixing 5% Dimethyl Formamide in normal saline solution. All the leaf extracts are mixed with standard vehicle. The Earthworms are divided into 2 groups for leaf and bark extracts of different concentrations 5 mg/ml, 10 mg/ml, 15 mg/ml, and standard and control.

Group 1- Ethanolic extract of leaf (5 mg/ml, 10 mg/ml, 15 mg/ml)

Group 2- Ethanolic extract of bark (5 mg/ml, 10 mg/ml, 15 mg/ml)

Each group consisting of 3 earthworms. The 3 petri plates were taken in each group. In each

petri-plate 1 earthworm were placed, and the lids of petri plates are closed partially by leaving an air space. Time of earthworms placed is noted and the activity is to be monitored. Then after some time the motility of worms are being stopped which was checked by pinning with needle, then finally complete motility of worms was stopped and the time of death of earthworms are noted. This procedure is repeated for different concentrations of extracts i. e., 5 mg / ml, 10mg/ml, 15 mg/ml . All the times are noted, and the activity is calculated. The results were tabulated in table no.6.3

RESULTS AND DISCURSSION

6. RESULTS

6.1 Percentage yields of extract

Table No. 6.1: Percentage yield of extracts.

Extracts	Percentage yield
Leaf extract	1.73%
Bark extract	2.16%

6.2 Results for Phytochemical Screening For Secondary Metabolites

Table No. 6.2: Phytochemical screening for secondary metabolites.

Test	Leaf extract	Bark extract
Alkaloids	+	+
Flavanoids	+	+
Steroids	-	+
Terpenoids	+	+
Phenols	+	+
Tannins	+	+
Glycosides	-	+
Proteins	-	-
Saponins	+	+

Preliminary phytochemical investigations of the extracts of leaves and bark of *Tamarindus Indica* revealed the presence of alkaloids, flavonoids, saponins, Tannins, and phenolic compounds. some of these phytoconstituents are responsible for anthelmintic activity.

Table No. 6.3: effects of leaf and bark extracts on the motility of worms.

Conc	Time in Minutes			
	Leaf extract		Bark Extract	
	Paralysis	Death	Paralysis	Death
5mg/ml	36 Mins	38 Mins	23 Mins	27 Mins
10mg/ml	30 Mins	35 Mins	19 Mins	22 Mins
15mg/ml	26 Mins	29 Mins	13 Mins	18 Mins

From the above table it shows that bark extract shows more anthelmintic activity than the leaf extract. It also shows that as the concentration of drug increases the time required for the paralysis and death of worms will be decreased.

Table 6.4: Effect of Standard Drug Albendazole [suspension].

Concentration	Time
10mg/ml	18 minutes



Figure 6.1: Observation of leaf extract of *Tamarindus Indica*.



Figure 6.2: Observation of bark extract of *Tamarindus Indica*.



Figure 6.3: Observation of standard drug Albendazole.

7. SUMMARY AND CONCLUSION

The earthworm *Pheretimaposthuma* is one of the most important soil invertebrates in promoting soil fertility. These earthworms are important components of the diets of many higher animals. Helminthic infections of the gastrointestinal tract of human beings and animals have been recognised to have adverse effect on health standards with a consequent lowering of resistance to other diseases. In search of components with anti-helminthic activity, a few substances were screened using different species of worms, for example earthworm, ascaris, nippostrongylus and heterakia of all the species, earthworms have been used widely for the initial evaluation of anthelmintic compounds in vitro because they resemble intestinal 'worms' in their reaction to anti-helminthic and are easily available. It has been demonstrated that all anthelmintics are toxic to earthworms and substance toxic to earthworms is worthy for investigation has an anthelmintic. The results of the present study indicated that the Ethanolic extract of bark shows a better anthelmintic activity against Indian earthworms (*PheritemaPhostima*). Presence of Tannins and polyphenolic in the bark extract could be attribute to the anthelmintic activity. The Flavonoids, Saponins and Alkaloids in the leaf extract could be reason for the Anthelmintic property of *Tamarindus Indica*. These results support the anthelmintic properties of *Tamarindus Indica*. Therefore they could be brought to use Pharmacologically.

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