

In-Vitro Evaluation of Anthelmintic activity of Leaf Extract of *Mangifera indica* against *Pheretima posthuma*

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ABSTRACT:

Mangifera indica belongs to the family of Anacardiaceae and is an important fruit from South and Southeast Asia. India, China, Thailand, Indonesia, Pakistan, Mexico, Brazil, Bangladesh, Nigeria, and the Philippines are among the top mango producer countries. Leaves of the mango plant have been studied for their health benefits, which are attributed to a large amount of phytochemicals such as mangiferin, followed by phenolic acids, benzophenones, and other antioxidants such as flavonoids, ascorbic acid, carotenoids, and tocopherols. Various chemical tests have been performed to confirm their presence. Specifically, thin layer chromatography of ethanolic extract of *Mangifera indica* leaves powder has been performed to confirm its presence. The extracts from mango leaves have been studied for their biological activities, including anthelmintic activity. *Pheretima posthuma* has been used to study anthelmintic activity of mangiferin as they are anatomically related to intestinal parasites.

Other activities such as anti-cancer, anti-diabetic, anti-oxidant, anti-microbial, anti-obesity, lipid-lowering, hepato-protection, and anti-diarrheal has been reviewed.

Further, various bioactivities of the Mango leaf extracts are also critically discussed.

Key words: *Mangifera indica*, mangiferin, anthelmintic activity, TLC process.

INTRODUCTION:

Anthelmintic are drugs that are used to treat infections caused by parasitic worms, also known as helminthes. These drugs work by either killing the worms or preventing them from reproducing and spreading throughout the host's body.

India is a rich country in collection of registered and widely popular medicinal plants.

In recent years, the use of Ayurvedic (herbal) medicines to treat diseases has also attracted people's interest in western countries. Mango (*Mangifera indica*), which is the national fruit of India, is cultivated in several tropical and subtropical regions.

Mangiferin is a major component of mango leaves and important natural drug which has wide range of applications in pharmaceutical & other related industries. Mango (*Mangifera indica* L.) ascribed to the family Anacardiaceae has been adjudged as the vital traditionally significant and one of the most economically important tropical fruit crop globally ^[1].

Mango is an evergreen tree with a lot of traditional medicinal resources apart from its very famous fruits. Mangoes are native to the South and Southeast Asia, and in 2018, the global production of mangoes (the report includes guavas and mangosteens) was 55.4 million tonnes. The largest mango producing countries are India, China, Thailand, Indonesia, Pakistan, Mexico, Brazil, Bangladesh, Nigeria, and the Philippines.

Apart from its economically important portion (fruit), large amounts of crop residues such as leaves, flowers, stem, and bark are generated during pruning, which causes complications of disposal to the farmers. Mango leaves are the potential source of minerals, viz. nitrogen, potassium, phosphorus, iron, sodium, calcium, magnesium, and vitamins, viz. A, B, E, and C. A major bio-macromolecule present in mango leaves is protein. MLs can be utilized as an alternative source of livestock feeding in developing countries for alleviating the food shortage for livestock. Extracts of the Mango leaves have been utilized for traditional medicines to cure diabetes, bronchitis, diarrhea, asthma, kidney, scabies, respiratory problems, syphilis, and urinary disorders ^[2,3].

The most active biological constituent of Mango leaves is mangiferin, followed by phenolic acids, benzophenones, and other antioxidants such as flavonoids, carotenoids, quercetin, isoquercetin, ascorbic acid, and tocopherols.

Mangiferin is the main contributor of most of the biological activities of Mango leaves extract. Mango leaves have a great scope of valorization as they are recognized to possess varied phytochemical, biological, and pharmacological properties, viz. anti-microbial, antioxidant, anti-diabetic, anti-tumour, and immunomodulatory effects.

Mango leaves oil contains monoterpenes, sesquiterpenes, minor quantities of other analogues, and trace amounts of non-terpenoid hydrocarbons and oxygenated hydrocarbons.

The essential oil from Mango leaves also possesses bacteriostatic properties and contains several antimicrobial constituents such as α -gurjunene, trans-caryophyllene, α -humulene, α -selinene, and camphor. The benzophenone derivatives in Mango leaves possess significant α glucosidase inhibitory and immunosuppressive activities.

There are several reviews that have been developed to discuss the bioactive compounds and health promoting effect of mango fruit/pulp ^[4-7], whereas others contain a scattered compilation of literature on mango seeds, Mango leaves, and mango bark ^[8,9].

There is no critical compilation on the crucial information on Mango leaves bio-actives and associated bioactivities. Therefore, the current review will be focused on the nutritional and ANTI-HELMENTIC profile of the Mango leaves. The review also delivers important health promoting activities of the Mango leaves extracts.

MATERIAL AND METHODS:

MATERIALS: Plant selected - In the present study, *Mangifera indica*, was selected because of its therapeutic uses, the part used was leaves.

Chemicals and reagents used: Ethanol, Methanol, Normal saline water, Mayer's reagent, Ferric chloride solution, Ammonia solution, Magnesium metal, Conc. Hydrochloric acid, sulphuric acid and Chloroform

Drug: Albendazole

Animal used: *Pheretima postuma* (Indian adult earth worms)

METHOD:

Collection of plant material ^[10]

Mangifera indica leaves were collected in the month of August 2023 from Koratagere village, Dist of Tumkur, in the state of Karnataka, India.

The fresh leaves were collected, removed all the dirty matter, washed, dried under sun light and powdered by using mixer grinder.

Preparation of plant extract: The fresh leaves were collected, removed all the dirty matter, washed, dried under sun light and powdered by using mixer grinder.



FIGURE 01: [A] Fresh leaves of *Mangifera indica*, [B] Wet leaves, [C] Dried leaves, [D] Powdered leaves.

The different methods used in extraction of phytochemicals are:

- 1) **Extraction using organic solvents**
 - Cold maceration method
 - Decoction method (methanolic method of extraction)
- 2) **Extraction using aqueous solvent (distilled iodine free water)**
 - Boiling water method (infusion method)

Cold maceration: It was performed by using ethanol, methanol.

10 gm of powdered leaves was weighed and soaked in 100ml of ethanol and 100ml of methanol, separately in a stoppered container for about 7 days at room temperature with occasional stirring, until the soluble matter has dissolved.

After 7 days the solution was filtered through filter paper. And the filtrate thus obtained was collected in a beaker.



FIGURE 02: *Cold maceration extraction method*

Decoction method:

In this method, 10g of powdered leaves was weighed, added 100ml of methanol in a beaker, then boiled the mixture in a specified volume of water in a water bath for a defined time period (one hour), and then it is cooled and filtered by the filter paper. The filtrate obtained was collected into another beaker.



FIGURE 03: *Decoction extraction method*

Boiling water method (Infusion method):

In this method, 20g of powdered leaves was weighed, added 200ml of iodine free distilled water in a beaker, then boiled the mixture in a specified volume of water in a water bath for a defined time period (one hour), and then it is cooled and filtered by the filter paper. The filtrate obtained was collected into another beaker.



FIGURE 04: *Infusion method*

Collection of Worms:

Indian Adult Earthworms (*Pheretima postuma*) were used to evaluate the anthelmintic activity of plant extract due to its anatomical and physiological resemblance with intestinal round worm parasites present in human beings and also due to their abundant and easy availability. The worms were collected from damp soil and from manure, and then they were washed with normal saline water in order to remove all kinds of dirt and fecal matters.



FIGURE 05: freshly collected worms

EVALUATION:

- 1 Phytochemical Screening:** The aqueous extract (Methanolic and Ethanolic) of powder of dried *Mangifera indica* leaves were subjected for the following chemical tests for the identification of various active constituents ^[11].

1.1 Phenolic compounds (Ferric chloride test):

50 mg aqueous extract were dissolved in 5 ml of distilled water. To this few drops of 5.0% neutral ferric chloride solution was added and the development of dark green colour indicated the presence of phenolic compounds.

1.2 Flavonoids:

200 mg of the aqueous extract were dissolved in 2ml methanol and heated. Few turnings of magnesium metal were added to the mixture followed by the addition of a few drops of concentrated hydrochloric acid. The appearance of an orange to red coloration was considered as indicative of the presence of flavonoids.

1.2 Saponins:

50 mg extracts were diluted in 20 ml distilled water. The solution was then shaken in graduated cylinder for 15 minutes to see a layer of foam up to 2 cm which indicated saponins.

1.3 Tannins:

50 mg of extract was boiled in 20 ml of distilled water. A brownish green or blue-black colouration resulting upon the addition of 0.1% ferric chloride solution indicated the presence of tannins in the extract.

1.4 Terpenoids:

About 0.5g of the extracts were dissolved in 3ml of chloroform and filtered. Concentrated sulphuric acid was slowly added to the filtrate to form a lower layer. A reddish brown colour at the interface indicated the presence of terpenoids.

1.5 Glycosides-Borntrage's test:

50 mg of aqueous extract were hydrolyzed with 5 ml concentrated HCL for 2 hours in water bath and filtered. 2 ml of filtered hydrolysates were taken in a test tube and 3ml of chloroform was added. The chloroform layer was separated by shaking and 10% ammonia solution was added to this to see the appearance of pink colour which indicates the presence of glycosides.

1.6 Carbohydrates and sugars -Molish's test:

100 mg of dried extracts were weighed and dissolved in 5 ml distilled water and subjected to the following tests. 2 ml of filtrates were taken in a test tube and two drops of alcoholic solution of α -naphthol was added. The solutions were mixed properly and 1 ml concentrated H_2SO_4 was added slowly along the sides of the test tubes. A violet ring indicated the presence of carbohydrates.

1.7 Fixed oils and fats -Spot test:

The presence of fixed oil was described by pressing small quantity of extract between fingers and the presence of oil stain indicated the presence of fixed oil.

1.8 Alkaloids-Mayer's test:

For detection of alkaloids, 1.0 g of dried extract was dissolved in 10 ml of 1% HCl by stirring under steam bath. The solution was then filtered and the filtrate was tested with Mayer's reagent. 2 ml of filtrate were taken in a test tube and one or two drops of Mayer's reagent was added gently to the test tube. A white creamy precipitate indicates presence of alkaloid.

2 Estimation of Mangiferin by TLC: Thin layer chromatography uses affinity based method to separate and detect compounds present in a mixture. The various steps involved are:

2.1 Preparation of samples:

The ethanolic drug extract was prepared as discussed previously.

Solvent system:

Ethyl acetate: methanol: water: formic acid (10:1:1:0.5 v/v)

2.2 Preparation of TLC chamber and plate [12]:

Preparation of TLC chamber

A TLC chamber was made using a glass beaker and watch glass as its lid.

The solvent was added about 1/4th of the chamber and closed the lid for saturation about 30 minutes.

Preparation of TLC plate:

A clean glass slide was used.

Paste of silica gel G and ethanol was made and TLC plate was made by using pouring method.

The silica gel G plate was pre activated.

2.3 Spotting of TLC plate: 1 cm above the edge of the plate a line was marked. A spot of the drug extract was marked using capillary tube .

2.4 Placing the TLC plate for elution:

After spotting the plate was transferred carefully into the chamber and the lid was closed. It was left until the solvent had reached the upper marked line.

2.5 Removing the TLC plate and drying:

After completion of elution, the plate was removed and dried.

2.6 Detection under UV spectroscopy:

The dried TLC plate was detected under 240nm.

3. Evaluation of *In-vitro* Anthelmintic activity: The anthelmintic activity was carried according to the standard method. Adult Indian earth-worms *P. posthuma* has anatomical and physiological resemblance to the intestinal round worm parasites of human beings. Twenty-six Indian adult earthworms were collected and groups were divided into ten containing two earthworms in each group.

4.

Each group was treated with any one of 10 ml of standard drug Albendazole (10mg/ml);

Ethanolic extract of *Mangifera indica* (10mg/ml, 40mg/ml, 100mg/ml, 140mg/ml),

Methanolic extract of *Mangifera indica* (10mg/ml, 40mg/ml, 100mg/ml, 140mg/ml) and

Aqueous extract of *Mangifera indica* (10mg/ml, 40mg/ml, 100mg/ml, 140mg/ml) in eight

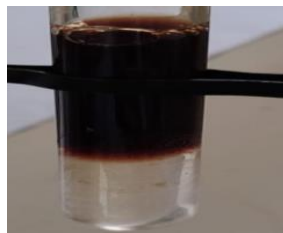
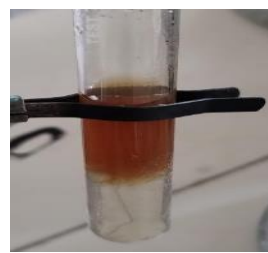
different petridish. The earthworms were placed in the petri-dish containing the different concentrations of

methanolic, aqueous extract of *Mangifera indica* and the Std. drug Albendazole. Each Time of paralysis recorded when slow movement was observed on vigorous shaking. Time of death was recorded when the worms neither moved when shaken.

Finally, the test results were compared with Std. reference compound Albendazole.

RESULTS:**1.PHYTOCHEMICAL ANALYSIS OF MAGNIFERA INDICA**

Sl.no.	Functional groups	Ethanolic extract	Methanolic extract	Aqueous extract
1	Carbohydrates	-	-	+
2	Alkaloids	+	+	+
3	Phenolic compounds	-	-	+
4	Tannins	-	-	+
5	Saponins	-	-	+
6	Fat oils	-	-	+
7	Glycosides	+	+	+
8	Terpenoids	+	+	+
9	Mangiferin	+	+	+

Table 01: Phytochemical test for aqueous extract of powder of dried *Mangifera indica* leaves*Phenolic test**flavonoid test**saponin test**alkaloids test**glycosides tests**terpenoids test**Carbohydrates test***FIGURE 06:** *Phytochemical investigation*

Phytochemical tests of alcoholic extract (ethanolic and methanolic) confirms the presence of alkaloids, glycosides, terpenoids and mangiferin while aqueous extract contains alkaloids, glycosides, carbohydrates, phenolic compounds, tannins, saponins and fat oils.

2. ESTIMATION OF MANGIFERIN BY TLC:

The TLC plate was detected under UV around 240 nm and the result was observed.

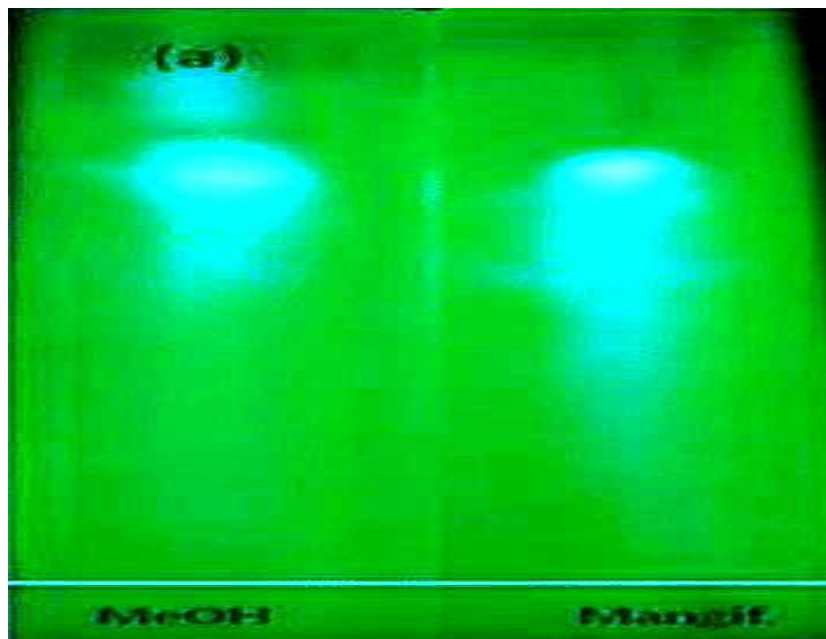


FIGURE 13: Observation of *TLC* under UV 240 nm.

The UV results of ethanolic extract matches with the standard values.

3. IN-VITRO EVALUATION OF ANTHELMINTIC ACTIVITY OF MAGNIFERA INDICA



In-vitro analysis of anthelmintic activity of aqueous extract with standard Albendazole



In-vitro analysis of anthelmintic activity of methanolic extract



In-vitro analysis of anthelmintic activity of ethanolic extract

Figure 07: *Invitro experimental model setup to evaluate the anthelmintic activity*

Table 02:

Anthelmintic activity of methanolic and aqueous extract of *Mangifera indica* leaves and standard Albendazole.

Extracts/Drug(Treatment)	Concentrations(mg/ml)	Time Taken for paralysis(min)	Time taken for death(min)
Standard drug(Albendazole)	10 mg/ml	35 ± 4	50 ± 4
Ethanolic extract of <i>Mangifera indica</i> leaves	10mg/ml	45 ± 4	63 ± 4
	40 mg/ml	40 ± 4	55 ± 4
	100 mg/ml	30 ± 4	41 ± 4
	140 mg/ml	19 ± 4	33 ± 4
Methanolic extract of <i>Mangifera indica</i> leaves	10mg/ml	75 ± 4	109 ± 4
	40 mg/ml	61 ± 4	83 ± 4
	100 mg/ml	52 ± 4	64 ± 4
	140 mg/ml	47 ± 4	59 ± 4
Aqueous extract of <i>Mangifera indica</i> leaves	10mg/ml	240 ± 4	281 ± 4
	40 mg/ml	163 ± 4	207 ± 4
	100 mg/ml	149 ± 4	180 ± 4
	140 mg/ml	138.5 ± 4	156 ± 4

Ethanolic leaf extract of *Mangifera indica* shows significant effect on *Pheretima posthuma* higher concentrations of extracts produce a paralytic effect much easier and time taken for death was shorter. It shows the maximum efficacy at 140mg/ml, as compared to standard drug Albendazole.

Conclusion:

It can be concluded that the ethanolic leaf extract of *Mangifera indica* produces better anthelmintic activity against Indian earthworm *Pheretima posthuma* than compared to aqueous extract of *Mangifera indica* and standard drug Albendazole.

At higher concentrations, the ethanolic extract showed higher activity.

From the biological assay performed and observations noted, it can be concluded that *Mangifera indica*, possesses significant anthelmintic activity when compared with the normally used drug. Hence it suggests that this medicinal plant is worthy to be investigated as an effort to yield a new anthelmintic agent

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