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Evaluation of Anti-Microbial and Anthelmintic Activity of Leaves of Melia Dubia

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Abstract: This study evaluates the anti-microbial and anthelmintic potential of Melia dubia leaf extracts. The plant is traditionally known for its medicinal properties and contains various bioactive phytochemicals. Leaves were collected, shade-dried and subjected to Soxhlet extraction using petroleum ether and methanol. Preliminary phytochemical screening revealed the presence of alkaloids, flavonoids, tannins, saponins, terpenoids, glycosides, phenols, steroids, carbohydrates and proteins. The anti-microbial activity was assessed using the paper disc diffusion method against Escherichia coli, Bacillus subtilis and Candida albicans. The methanolic extract exhibited higher activity compared to the petroleum ether extract, with maximum inhibition observed at higher concentrations, although standard drugs showed greater efficacy. Anthelmintic activity was evaluated using Pheretima posthuma earthworms. The methanolic extract demonstrated significant dose-dependent activity, reducing both paralysis and death time of worms. At higher concentrations (10–12 mg/ml), the extract showed effects comparable to the standard drug mebendazole. The findings suggest that Melia dubia leaves possess promising anti-microbial and anthelmintic properties, likely due to the presence of bioactive phytoconstituents. This supports its potential use as a natural therapeutic agent for microbial infections and parasitic diseases, though further studies are required to isolate active compounds and validate clinical efficacy.

Keywords: Melia dubia, Anti-microbial activity, Anthelmintic activity, Phytochemical screening, Medicinal plants, Soxhlet extraction.

1. INTRODUCTION

Infectious diseases caused by microorganisms such as bacteria and fungi continue to pose a major threat to global public health. Despite significant advances in modern medicine, the emergence and rapid spread of antimicrobial resistance (AMR) have reduced the effectiveness of many conventional drugs, leading to increased morbidity, mortality and healthcare costs. This growing challenge has necessitated the exploration of alternative sources of antimicrobial agents, particularly from natural products.

Helminthiasis is another major public health problem, especially in developing countries, affecting a large proportion of the global population. It is caused by parasitic worms such as nematodes and platyhelminths, leading to chronic infections and various complications

including malnutrition and organ damage. Although several anthelmintic drugs are available, their limited efficacy and the risk of resistance highlight the need for alternative treatments.

Medicinal plants have long been recognized as valuable sources of bioactive compounds and continue to play a crucial role in drug discovery. Phytochemicals such as alkaloids, flavonoids, tannins, saponins and terpenoids exhibit a wide range of pharmacological activities, including anti-microbial and anti-parasitic effects. These natural compounds often provide therapeutic benefits with fewer side effects compared to synthetic drugs.

Melia dubia (family Meliaceae), commonly known as Malabar neem, is a fast-growing medicinal plant widely distributed in tropical regions. It has been traditionally used for the treatment of various ailments, including infections, inflammation and parasitic diseases. Previous studies have reported that *Melia dubia* contains diverse phytoconstituents with notable biological activities such as anti-oxidant,anti-diabetics and anti-cancer properties.

However, comprehensive studies evaluating both the anti-microbial and anthelmintic activities of *Melia dubia* leaf extracts remain limited. Therefore, the present study aims to investigate the phytochemical constituents and to evaluate the anti-microbial and anthelmintic activities of *Melia dubia* leaves, thereby exploring its potential as a natural therapeutic agent.

PLANT PROFILE

Description:

- *Melia dubia* is the tropical deciduous tree that grows up to 20 m.
- It is one of the fast-growing species under irrigated condition.
- Suitable for cultivation as a monocrop as well as under agroforestry.
- Deciduous- December.
- New flush of leaves Feb to march.

Taxonomy:

- Kingdom : Plantae
- Subkingdom : Tracheobinto
- Super division : Spermatophyta
- Division : Magnoliophyta
- Class : Magnoliopsida
- Order : Sapindales

Vernacularnames:

- English : Malabar Neem
- Hindi : Mahanim
- Tamil : Malaivembu
- Telugu : Munnatikaraka
- Kannada : Arabevu mara
- Malayalam : Valiyaveppu

Fig.1: Leaves and Fruits of *Melia dubia*

2. MATERIALS AND METHODS

Drying of the Plant:

The collected *Melia dubia* leaves were shade dried in a well-ventilated area at ambient temperature to preserve their bioactive constituents. This method was adopted to retain secondary metabolites and prevent the loss of active compounds. The samples were regularly monitored to avoid mold growth and insect contamination. Direct sunlight was avoided to prevent chemical degradation caused by ultraviolet radiation. Dry conditions were maintained throughout the process to minimize fungal contamination and microbial fermentation.

Grinding:

The aim of grinding is to make fragments of the leaves into smaller particles and improves the subsequent extraction by rendering the sample more homogenous, increasing the surface area and facilitating the penetration of solvent into the cells. Mechanical grinder should be employed to shred the plant tissues to various particle sizes.

Preparation of plant extracts:

The leaves of Melia dubia were collected and dried in shade. 200g of the powder was extracted with petroleum ether (60-80°C) to remove chlorophyll. It was then filtered and the filtrate was discarded. The Marc was extracted with methanol by Soxhlet extraction.

Soxhlet extraction:

The coarsely powdered leaves of Melia dubia (200 g) were subjected to Soxhlet extraction for continuous extraction. The solvent was repeatedly evaporated, condensed, and circulated through the sample to ensure efficient extraction. Prior to heating, the solvent was allowed to siphon once for proper settling, and porcelain chips were added to prevent bumping. The extraction cycle was continued until complete extraction was achieved. The obtained extract was then collected and stored in an airtight container for further analysis.

3. PRELIMINARYPHYTOCHEMICAL SCREENING

Table 1: Phytochemical analysis of leaves of *Melia dubia*

S. No	Phytochemical	Test Name	Procedure	Observation
1.	Alkaloids	Dragendroff's Test	Add 1 ml of Dragendroff's reagent to extract	Orange-red precipitate
2.	Flavonoids	Lead Acetate Test	Add few drops of 10% lead acetate solution	Yellow precipitate

3.	Saponins	Foam Test	Dilute 1 ml extract to 20 ml with water and shake for 15 min	1 cm foam layer
4.	Terpenoids	Chloroform + H_2SO_4 Test	Add chloroform, evaporate, add sulphuric acid, heat	Greyish color
5.	Tannins	Braymer's Test	Add water and ferric chloride solution	Blue-green color
6.	Carbohydrates	Molisch's Test	Add Molisch reagent and sulphuric acid	Violet ring
7.	Glycosides	Keller-Killani Test	Add acetic acid + ferric chloride, heat, then add H_2SO_4	Blue color
8.	Phenols	Ferric Chloride Test	Add ferric chloride solution	Bluish-black color
9.	Steroids	Liebermann-Burchard Test	Add chloroform, acetic anhydride, and H_2SO_4	Red → Blue → Bluish-green
10.	Proteins	Biuret Test	Add Biuret reagent, wait 5 minutes	Violet color

3.1 ANTI-MICROBIAL ACTIVITY

Anti-microbial activity by paper disc diffusion method:

The paper disc diffusion method is used to evaluate the anti-microbial activity of substances by measuring the inhibition of microbial growth around discs impregnated with test samples. The anti-microbial agent diffuses from the disc into the agar medium and inhibits the growth of microorganisms, producing a clear zone called the zone of inhibition.

Procedure:

The anti-microbial activity of Melia dubia leaf extracts was evaluated using the paper disc diffusion method. The test microorganisms included Escherichia coli, Bacillus subtilis and Candida albicans. Mueller–Hinton agar plates were prepared, sterilized, and inoculated with freshly prepared microbial cultures adjusted to 0.5 McFarland standard.Sterile filter paper discs (6 mm) were impregnated with different concentrations of petroleum ether and methanolic extracts (10 µg/ml and 20 µg/ml) and placed on the inoculated agar plates. Standard drugs (Gentamycin for bacteria and Fluconazole for fungi) and a control were also included for comparison. The plates were incubated at 37°C for 18–24 hours for bacterial cultures and 25–28°C for fungal cultures.

After incubation, the zones of inhibition were measured in millimetres. The methanolic extract exhibited higher anti-microbial activity compared to the petroleum ether extract, with maximum inhibition observed at 20 µg/ml, particularly against Bacillus subtilis. However, the activity was lower than that of the standard drugs, indicating moderate anti-microbial potential of Melia dubia leaf extracts.

3.2 ANTHELMINTIC ACTIVITY

The anthelmintic activity was assessed using *Pheretima posthuma* earthworms. The worms were thoroughly washed with normal saline to remove any fecal matter. Earthworms measuring 4-5 cm in length and 0.1-0.2 cm in width, weighing between 0.8 and 3.04 grams, were used for the experiments. These earthworms were chosen due to their anatomical and physiological similarity to human intestinal roundworm parasites, making them suitable for studying anthelmintic activity.

Procedure:

Preparation of test solution:1.5 gm of the extract was accurately weighed and dissolved in 50 ml of methanol to prepare the stock solution. From this stock solution, aliquots of 2, 4, 6, 8, 10 and 12 ml were pipette out separately and transferred into different volumetric beakers.

Each solution was then make upto 20 mg/ml with normal saline to obtain different concentration for further analysis.

Preparation of standard solution: Mebendazole drug was used as a standard drug. Mebendazole was dissolved in 10ml of DMSO, then diluted to a final volume of 20 ml with normal saline to achieve a concentration of 10 mg/ml. The control was prepared similarly but without the drug. Six healthy earthworms of similar size were selected for each group and washed with saline. The worms were placed in Petri dishes containing test, standard or control solutions and maintained at room temperature (~37°C).

Paralysis was recorded when no movement was observed even after vigorous shaking, while death was confirmed by the absence of movement upon exposure to warm water (50°C). The time taken for paralysis and death was noted, with a maximum observation time of 120 minutes.

4. RESULT AND DISCUSSION

Percentage yield:

Table 2: Percentage yield of leaves of *Melia dubia*

EXTRACTS	% YIELD (% w/w)
Petroleum ether	8.11
Methanol	15.05

The % yield of leaves of *Melia dubia* using petroleum ether and methanol was found to be 8.11 % w/w and 15.05% w/w respectively.

Phytochemical studies:

The effective crude extract of *Melia dubia* was evaluated for the phytochemical constituents. The results of preliminary phytochemical investigation of various crude extract of leaves of *Melia dubia*. Was shown in **Table-3:**

Table-3: Preliminary phytochemical screening of crude extract of leaves of *Melia dubia*

PHYTOCONSTIUENTS	EXTRACT	
	PETROLEUM ETHER	METHANOL
ALKALOIDS	-	+
FLAVONOIDS	+	+
SAPONINS	+	+
TERPENOIDS	+	+
TANNINS	+	+
CARBOHYDRATES	-	-
GLYCOSIDES	-	+
PHENOLS	+	+
STEROIDS	+	+
PROTEINS	-	+

+ denotes – presence of constituent

- denotes – absence of constituent

The phytochemical screening of the petroleum ether extract revealed the presence of flavonoids, saponins, terpenoids, tannins, phenols,steroids and proteins.

The Methanolic extract showed the presence of alkaloids, flavonoids, saponins, terpenoids, tannins, glycosides, phenols, steroids and proteins.

Anti-Microbial Activity: The petroleum ether and methanolic extract of *Melia dubia* (leaves) was evaluated for the anti-bacterial and anti-fungal activity by **paper disc method**.The results of anti-Microbial activity were shown in **Table-4**

Table-4: Anti-microbial activity of crude extract of leaves of *Melia dubia*

Microorganisms	Zone of inhibition (in mm)				
	Methanol		Petroleum ether		Standard (10µg/ml)
	10µg/ml	20µg/ml	10µg/ml	20µg/ml	
Escherichia coli	9	13	7	11	27
Bacillius subtilis	8	17	6	7	25
Candida albicans	12	14	8	9	6.5

Standard discs:

Gentamycin (Bacteria)

Fluconazole (*candida albicans)*

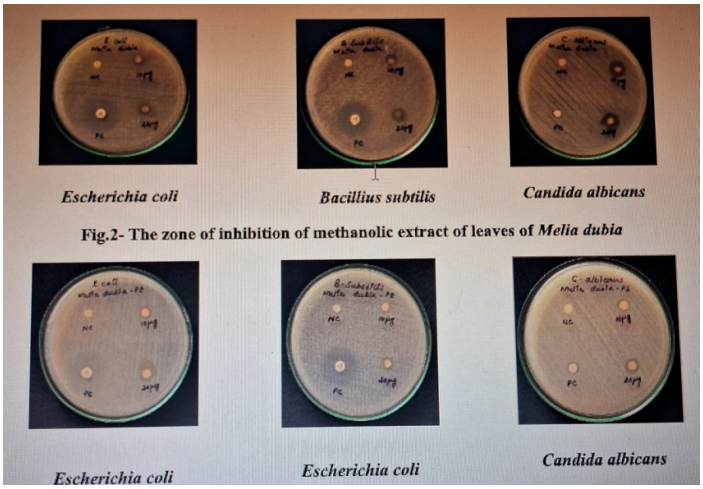

Fig.2- The zone of inhibition of methanolic extract of leaves of *Melia dubia*

Fig.3- The zone of inhibition of petroleum ether extract of leaves of Melia dubia

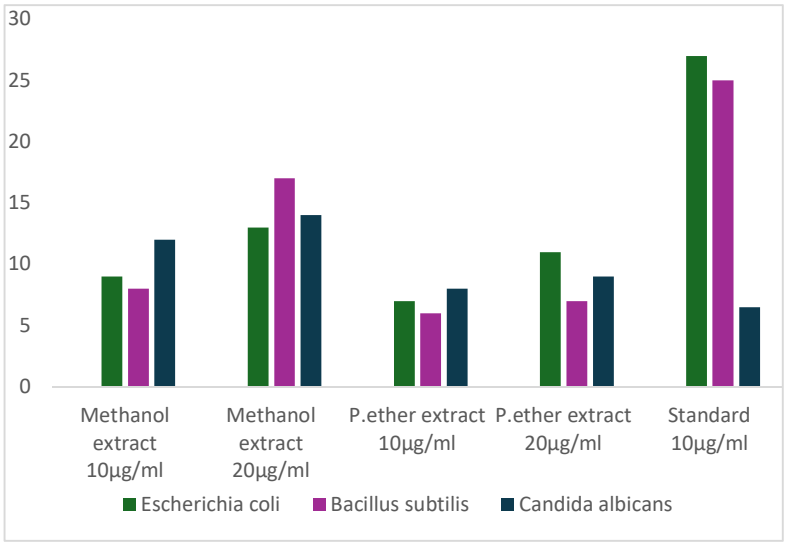

Fig.4: Anti-microbial activity of crude extract of leaves of Melia dubia

The methanolic extract of *Melia dubia* leaves exhibited higher anti-microbial activity compared to the petroleum ether extract against *Escherichia coli, Bacillus subtilis* and *Candida albicans.* Maximum zone of inhibition was observed with methanol extract at 20 µg/ml, particularly against *Bacillus subtilis* (17 mm), while petroleum ether showed comparatively lower activity. However, the standard drugs (Gentamycin and Fluconazole) produced greater inhibition zones, confirming the moderate anti-microbial potential of the plant extract.

Anthelmintic Activity

The anthelmintic activity was performed with methanolic extract of *Melia dubia* (Leaves) using Indian earthworm (*Pheretima posthuma)* as the test subject.The time taken for paralysis and Death were observed and noted in minutes.The result was shown in,

Table-5: The anthelmintic activity of methanolic extract of leaves of *Melia dubia*

DRUG	CONCENTRATION (mg/ml)	TIME TAKEN IN MINUTES	
		For paralysis	For death
Control	Normal saline	40 mins.	90 mins.
Mebendazole (standard)	10 mg/ml	2 mins.	45 mins.
	2 mg/ml	25 mins.	130 mins.
	4 mg/ml	8 mins.	100 mins.
	6 mg/ml	3 mins.	75 mins.

Methanolic extract of *Melia dubia*	8 mg/ml	2 mins.	56 mins.
	10 mg/ml	1 min	44 mins.
	12 mg/ml	0.31min	31 mins.

- The methanolic extract of *Melia dubia* showed dose-dependent anthelmintic activity, with increased concentration reducing the time taken for paralysis and death of worms.
- At 12 mg/ml, the extract produced paralysis (0.31 min) and death (31 min) comparable to the standard drug Mebendazole (10 mg/ml).
- These results indicate significant anthelmintic potential of the methanolic extract compared to control and standard drug.

Conclusion:

The leaves of *Melia dubia* were selected for study to confirm the anti-microbial and anthelmintic activity.

The preliminary phytochemical screening of *Melia dubia* revealed the presence of

- Alkaloids
- Flavonoids
- Tannins
- Saponin
- Terpenoids
- Glycosides
- Phenols
- Steroids
- Proteins

The extract of *Melia dubia* showed the highest zone of inhibition against *Escherichia coli, bacillus subtilis and candida albicans.* The leaves of *Melia dubia* belongs to the Family Meliaceae was taken to study the Anthelmintic activity with Indian earthworm which is anatomically similar to the human parasitic worm. The anthelmintic activity was studied for different concentration of methanolic extract of *Melia dubia* and was compared with the standard (Mebendazole). From the results 8 mg/ml, 10mg/ml concentration of the methanolic extract of *Melia dubia* (Leaves) shows action which is similar to the 10 mg/ml of the standard (Mebendazole). So, we concluded that the methanolic extract of *Melia dubia* (Leaves) has good Anti-microbial and anthelmintic activity and can be used to eliminate parasitic worm in the stomach for humans.

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