ejpmr, 2024, 11(11), 267-272

SJIF Impact Factor 7.065

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

"PHARMACOGNOSTICAL, PHYTOCHEMICAL AND INVITRO BIOLOGICAL EVALUATION OF ANTIOXIDANT AND ANTIDIABETIC ACTIVITY OF THE MELIA DUBIA"

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Article Received on 07/09/2024 **Article Revised on 27/09/2024** **Article Accepted on 17/10/2024**

ABSTRACT

India has a rich traditional of plant based knowledge in health care. Among the large number of herbal drug existing in india, very few have been studied systematically so far, The study on Melia dubia, a native Indian tree, focused on evaluating its medicinal properties, particularly its antioxidant and antidiabetic activities. The leaves, collected from the Mandya region, were analyzed for various phytochemical constituents including alkaloids, carbohydrates, steroids, tannins, flavonoids, saponins, and glycosides. Thin layer chromatography (TLC) was used to detect biomolecules in the methanolic extracts. The research found that the ethanolic extract of Melia dubia exhibited significant antioxidant and antidiabetic activities. Specifically, the methanolic extract demonstrated effective α-amylase inhibition at lower concentrations compared to the standard drug Acarbose. Ddditionally, the antioxidant activity of the extracts was notably high, likely due to the high levels of phenolic and flavonoid compounds in the methanolic fraction. These findings suggest that Melia dubia holds potential for development into plant-based medicines, leveraging its natural bioactive compounds for therapeutic purposes.

KEYWORDS: Melia dubia, anti-oxidant and anti-diabetic activity.

INTRODUCTION

Meliadubia L, also known as Hill Neem, Malai Vembu, and Munnattikaraka, is traditionally used for its anthelmintic properties and for treating gastrointestinal and colic disorders. The extensive use of chemical pesticides, antibiotics, and fungicides presents serious risks to human health and adversely affects plant health. Rising concerns about drug-resistant microbes have intensified the search for safer, more sustainable alternatives. The growing problem of microbial resistance raises significant concerns about the future effectiveness of antimicrobial treatments.[1]

India is facing a severe imbalance between wood demand and supply, driven by shrinking forest areas and low productivity. Indian forests yield only 0.5-0.7 m^3/ha annually, far below the global average of 2.1 m^3/ha.[2]

Figure 1: MELIA DUBIA LEAVES.

Taxonomical study

- Kingdom: Plantae
- Class: Magnoliposida
- Order: Sapindales
- Family: Meliaceae
- Genus: Melia
- Species: Meliadubia
- General name: *Melia dubia*
- Botanicals name :melia dubia cav[4]

MEDICINAL USES

Melia dubia, also known as Malabar neem or Brodiri, has been used in traditional medicine for various purposes across different cultures. Here are some of its ethnomedical uses.

- **Antimicrobial and antifungal:** Used to treat wounds, skin infections, and fungal diseases.
- **Anti-inflammatory:** Applied topically to reduce swelling and pain.
- **Antipyretic:** Used to lower fever.
- **Digestive issues:** Treats diarrhea, dysentery, and stomach problems.
- **Snake bites:** Used as an antidote for snake venom.

Chemical constituents

Alkaloid, tannins, flavonoids, phenolic compounds are present.[4-5]

MATERIAL AND METHODOLOGY

Figure 2: Fresh leaves.

Figure 3: Dried leaves.

Figure 4: Dried powder.

1. Collection and authentication

The fresh plant leaves were collected from a home garden in Mandya, India, during JUNE - JULY. They were identified and authenticated by Dr. Thejesh Kumar MP, Coordinator of the Department of Botany at Bharathi College. After collection, the plants were washed thoroughly with running tap water, cut into small pieces, and shade-dried. The dried material was then ground into a coarse powder using a mechanical grinder. A voucher specimen was preserved in the laboratory for future reference.[7]

1. Extraction of crude drugs

The process involves in the preparation of crude extract from plant leaves using methanol as a solvent.

2. Preparation of Plant Material

Plant leaves are washed in running tap water to remove soil and debris. They are then shade-dried for 14 days and ground into a fine powder.

3. Extraction Process

Soaking: 50 grams of the powdered plant material is soaked in 100 milliliters of methanol at room temperature for 12 hours.

Shaking: The mixture is then shaken for few minutes to ensure thorough extraction.

4. Filtration

The initial filtrate is obtained by filtering through muslin cloth to remove larger particles.

The filtrate is further filtered through Whatman filter paper No. 1 in a funnel to obtain a clear final filtrate.

5. Evaporation

The final filtrate is evaporated to dryness, resulting in the crude extract of M. dubia.

This crude extract can then be used for further analysis or applications.[8-9]

PHYTOCHEMICAL SCREENING

- **Test for proteins**

Biuret test: Test solution was treated with equal volum of 10% sodium hydroxide solution and two drops of 1% copper sulphate solution, mixed well and observed no formation of violet/pink colour. So it indicates that absence of proteins.

Xanthoproteic test: Two ml of extracts were treated with few drops of conc. Nitric acid. Mixed well. Formation of light to dark yellow color was not observed which which indicates the absence of proteins.

- **Test for carbohydrates**

Benedicstest: Test solution was mixed with few drops of Benedicts reagent and boiled in water bath, observed for the no formation of reddish brown precipitate which indicates that absence of carbohydrates.

Molisch`s test: Filtrates were treated with 2 drops of alcoholic a-naphthol solution in a test tube. No Formation of the violet ring at the junction indicates that the absence of carbohydrates.

**Fehling`s Test:** Filtrates were hydrolysed with dil.HCl, neutralized with alkali and heated with Fehling`s A & B solution there is no red precipitate indicates the absence of reducing sugars.

- **Test for Alkaloids**

**Dragendroff`s Test:** A fraction of extract was treated with 3-5 drops of dragendroff`s reagent and observed for the formation of reddish of reddish brown precipitate which indicates the presence of alkaloids.

**Hager`s Test:** Extract was treated with hager`s reagent. Formation of a yellow coloredprecipitate indicates the presence of alkaloids.

- **Test for Saponins**

Foam Test: Test solution was mixed with water and shaken and observed for the formation of froth, which should be stable for 15 minuts. This result indicates the absence of Saponins.

- **Test for Glycosides**

Liebermann`s test: Crude extract was mixed with each of 2mi of chloroform and 2ml of chloroform and 2ml acetic acid. The mixture was cooled in ice. Carefully conc. H2SO4 was added. No colour change from violet to blue to green indicated the absence of steroidal nucleus, i.e., glycine portion of glycoside.

- **Test for Flavonoids**

Alkaline reagent test: crude extract was mixed with 2ml of 2% solution of NaOH. An intense yellow colour was formed which turned colourless on addition of few drops of diluted acid which indicated the presence of flavonoids.

- **Test for Steroids**

Libermann Burchard test: Crude extract mixed with few drops of acetic anhydride, boiled and cooled. Concentrated sulphuric acid was then added from the sides of thetest tube and observed for the formation of a brown ring at the junction of two layers. No Green coloration of the upper layer indicate a negative test for steroids.

- **Test for phenolic**

Bromine water test: Add bromide water to the sample, obesrved Decolorazation its indicates the presence of phenolic compounds.[9]

Determination of Total Ash value

About 2gm of powdered drug was weighed accurately and placed in tarred silica crucible and incinerated at 450^0C in muffle furnace until free from carbon. Crucible was cooled, kept in a desiccator and weighed. Same procedure was repeated to arrive at constant weight. The percentage of total ash obtained was calculated with reference to the air-dried drug. Total Ash value of powdered crude drug was recorded.

Total Ash value of the sample = 100(Z─X) / Y
Z = Weight of the dish + ash (after complete incineration)
X = Weight of the empty dish
Y = Weight of the drug taken

a. Acid insoluble ash
Ash obtained from the total ash was boiled with 25ml of 2N HCl for a few minutes and filtered. The filter paper was transferred into a silica crucible. Incinerated at 450°C in a muffle furnace until free from carbon. The crucible was cooled and weighed. Percentage of acid insoluble ash was calculated with reference to air-dried substance.

b. Water soluble ash
Ash obtained from the total ash was boiled with 25 ml of distilled water for a few minutes. And filtered through an ash less filter paper. The filter paper was transferred into a tarred silica crucible. Incinerated at 450°C in a muffle furnace until free from carbon. The crucible was cooled and weighed. Percentage of water-soluble ash was calculated with reference to air-dried substance.

Determination of extractive value
Extractive value of crude drug determines the amount of active constituent extracted with solvents from a given amount of plant material.

a) Alcohol soluble extractive value
About 5g of coarse powder of the crude drug was weighed and macerated in iodine flask with 100 ml of 70% v/v alcohol, for a duration OG 24 hrs. With frequent shaking. Solution was filtered rapidly. Taking precaution against loss of alcohol, 25 ml of filterd solution was evaporated to dryness at 105ºC in a tarred flat bottom petridish. The percentage of alcohol soluble extract was determined with reference to shade dried drug.

b) Water soluble extractive value
About 5g of coarse powder of the crude drug was weighed and macerated in iodine flask with 100 ml of water. For a duration of 24 hrs., with frequent shaking. Solution was filtered rapidly; taking precaution against loss of water, 25 ml of filtered solution was evaporated to dryness at 105ºC in a tarred flat bottomed petridish. The percentage of water soluble extractive was determined with reference to the shade dried drug.[4]

TLC STUDIES OF PLANT EXTRACT
The extract was dissolved in methanol and it is spotted on the TLC plate the plate was developed using solvent system methanol: water (4:1). After reaching ¾ th height of the plate, then the plate was removed from the solvent system and subjected to drying. During drying the orange colour chromatogram were obtained and its RF value was determined by comparing the obtained test result with standard. Hence it is confirmed that the sample extract contains Limuloid.[3-6]

Determination of Anti diabetic activity
α-amylase (0.5 mg/ml) was mixed with the sample at various concentrations (100-500 µg/ml) to which 1% of starch solution and 100 µl of 0.2 mm of phosphate buffer (pH -6.9) were added. The reaction was allowed to be carried out at 37°C for 5 min and terminated by addition of 2 ml of 3, 5-dinitrosalicylic acid reagent. The reaction mixture was heated for 15 min at 100°C and diluted with 10 ml of distilled water in an ice bath. α-amylase activity was determined by measuring colour intensity at 540 nm in spectrophotometer.[8]

RESULT
Leaves extract
The weight of Melia dubia leaves powder =15gm
Weight of the extract obtained = 1.4g
Percentage yield = (Weight of the extract ÷ Weight of the powder) × 100 = (1.4÷15) × 100 = 9.3% w/w.

EXTRACTIVE VALUE OF MELIA DUBIA
Table 1: Determination of extractive value of leaves.

Sl No	slovents	Extractive value(w/w)
01	Alcohol soluble	11.3%
02	Water soluble	19.65%

Table 2: Determination of ash value of leaves.

Sl No	Parts of the plant	Total ash (w/w)	Water soluble ash (w/w)
01	leaves	7.5%	4.5%

Preliminary phytochemical screening of extract
The methanolic extract of leaves of Melia dubia were screened for various tests mentioned in methodology.

SL NO.	PHYTOCHEMICAL	METHNOL EXTRACT
1	**Alkaloid**	
	Mayer`s test	+
	Wagner`s test	+

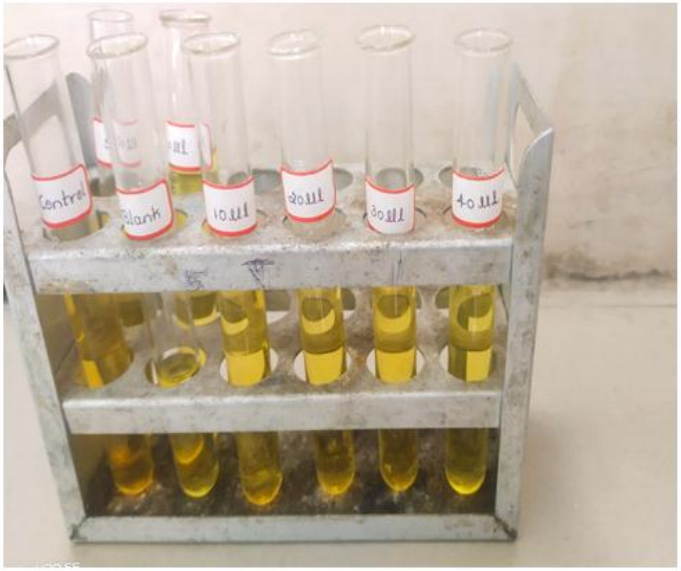
2	**Carbohydrates** Molisch `s test Fehling`s test	 – –
3	**Tannins** Ferric chloride test Gelatin test	 + +
4	**Terpenoids**	–
5	**Glycosides**	–
6	**Steroids** Salkowski reaction Liberman - Burchard test	 − −
7	**Saponins**	–
8	**Flavonoids** Alkaline reagent test Ammonium hydroxide test	+ + +
9	**Proteins** Biuret test Million`s test	 − −
10	**Mucilages**	–
11	**Phenolics**	+

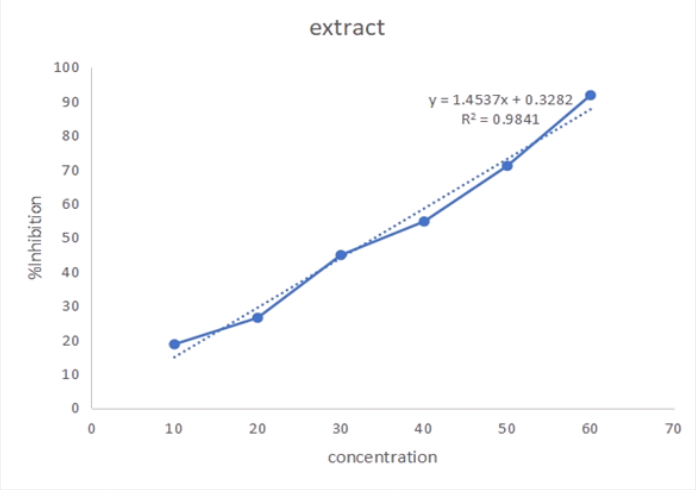
Anti-Diabetic Activity

The extracts demonstrated varying anti-diabetic activity, as indicated by mortality rates at different concentrations. Notably, at concentrations of 10 µg/ml, 20 µg/ml, 30 µg/ml, 40 µg/ml, 50 µg/ml, and 60 µg/ml, the inhibition of the α-amylase enzyme was 18.65%, 26.42%, 44.81%, 54.66%, 70.98%, and 91.70%, respectively, with the highest inhibition observed at 60 µg/ml. This suggests that the methanolic extracts of Melia dubia effectively inhibit the α-amylase enzyme, indicating potential for anti-diabetic activity. The presence of flavonoids, particularly limonoids, is likely a significant factor contributing to this activity, as reported in various studies.

Sl. No.	Concentration (µl)	Absorbance (nm)	% Inhibition
1	10	0.314	18.65285
2	20	0.284	26.42487
3	30	0.213	44.81865
4	40	0.175	54.66321
5	50	0.112	70.98446
6	60	0.032	91.70984

X	Y	C	M
34, 16922	50	O.3282	1.4537





Y=MX+C X=Y-C/M

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