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Assessment of bioactivities of ethanolic extract of *Melia azedarach* (Meliaceae) leaves

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PEER REVIEW

Peer reviewer

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Comments

The authors make the study interesting by evaluating the antioxidative, analgesic and antibacterial effects of *M. azedarach* extract in association with doing phytochemical screening. Materials and methods are well planned. Findings are attention-grabbing and the discussion section contains scientific interpretation. Details on Page 121

ABSTRACT

Objective: To assess ethanolic extract of the leaves of *Melia azedarach* L. (Family–Meliaceae) for its possible antioxidant, analgesic and antibacterial activities in association with performing phytochemical evaluation.

Methods: A number of phytochemical tests of this extract were utilized to distinguish the existence of different sorts of compounds. Qualitative and quantitative antioxidant activities were assessed by TLC and DPPH scavenging assay respectively. Acetic acid induced writhing test in mice and disk diffusion assay of the leaves extract were carried out to demonstrate the analgesic and antibacterial activities respectively.

Results: The phytochemical assessment revealed the existence of alkaloids, flavonoids, glycosides, saponins and tannins like compounds. Ethanolic extract of the leaves demonstrated antioxidant, analgesic and antibacterial activities *in vitro*.

Conclusion: Considering the study, this could justify the leaves extract's bioactivities but, to substantiate the activity of individual compound further investigation is necessary.

KEYWORDS

Melia azedarach L., Meliaceae, Antioxidant, Analgesic, Antibacterial, Phytochemical, DPPH, Acetic acid, Disk diffusion, Flavonoid

1. Introduction

Melia azedarach L. (*M. azedarach*) belongs to the family Meliaceae, also placed in Celtidaceae, Ulmaceae and it is a small tree. There are about 50 shrubs or trees in the genus *Melia*, widely distributed in tropical and subtropical regions of the world, of which most of them are traditionally used in different therapeutic purposes.

M. azedarach L. is a around 45–meter–tall deciduous tree. During young age, bark is smooth, greenish–brown and with age turning grey and fissured. Leaves, being bipinnate or occasionally tripinnate, are 20–40 m long. Fruits are small, 15 mm in diameter and smooth. A tree of the subtropical climatic zone, the natural habitat of *M.*

azedarach L. is seasonal forest, including bamboo thickets, Tamarindus woodland. It can tolerate an ambit sort of conditions. It is native to Bangladesh, India, Indonesia, Laos, Malaysia, Myanmar, Nepal, Pakistan, Papua New Guinea, Sri–Lanka, Thailand, and Vietnam.

In the earlier studies, ethanolic extract of *M. azedarach* L. showed activity against fever, nausea, vomiting, thirst and skin diseases^[1,2]. It has showed antioxidant activity^[3]. *M. azedarach* L. has also demonstrated analgesic activity^[4]. Leaves and fruits showed antifeedant activity^[5,6]. The plant has also showed antibacterial, antifungal, antimalarial, and cytotoxic activities^[7–10].

In this study, an endeavor was made to defend the conventional uses as per scientific research. Furthermore,

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the presence of reported compounds was distinguished by using a variety of standard chemical tests. Upon adequate literature review, found that a slight study has been executed to evaluate the rationale uses of this plant in traditional medicine. In our study, we therefore tried to assess the antioxidant, analgesic and antibacterial activities of the ethanolic extract of *M. azedarach* L. leaves.

2. Materials and methods

2.1. Plants collection and extraction

The fresh leaves of *M. azedarach* L. was collected from Naogaon, Bangladesh and branded by the specialists at National Herbarium, Mirpur, Dhaka, Bangladesh (Accession No.: 35542). The leaves were shade dried for one week. After adequate drying, the leaves were ground into a crude powder with the help of a suitable mechanical grinder and stored in a suitable airtight container and kept in a cool, dark and dry place to avoid any possible fungal attack.

For extracting, cold extraction method was used. About 350 g of powdered material was taken in a suitable container and soaked in 1200 mL of ethanol 99–100%. The container containing the contents was preserved and set aside for 15 d with occasional stirring and shaking. The whole mixture was filtered with clear cotton plug to remove plant garbage. Finally the extract was filtered through filter paper. The ethanol was evaporated with an electric fan at room temperature to find the dried crude extract (yield value 3.73%). Then the dried crude extract was stored in refrigerator.

2.2. Test animals

For the investigation of analgesic activity Swiss–Albino mice of either sex (22–25) g were collected from animal resources department of the ICDDR, B (International Center for Diarrhoeal Disease Research, Bangladesh). The mice were kept in the polypropylene cages for acclimatizing by providing with proper rodent foods in animal house, Pharmacy Discipline, Khulna University under the proper laboratory condition [room temperature (25±2) °C, relative humidity (55±5)%, and 12 h light: dark cycle] for period of 7 d prior to use.

2.3. Chemicals, reagents and standard drugs

Ascorbic acid, acetic acid, and dimethyl sulfoxide (DMSO) were purchased from Merck, Germany. 1, diphenyl–2–pycridylhydrazyl (DPPH) was obtained from Sigma Chemical Co. Ltd., (St. Louis, MO, USA). Tween–80 was purchased from Loba Chemie Pvt. Ltd., India. Solvents and all other

reagents were of analytical grade. Beximco Pharmaceuticals Ltd, Bangladesh supplied diclofenac sodium. Sterile blank discs of BBL, Cocksville, USA & Standard antibiotic discs (Ciprofloxacin 5 µg/disc) of Oxoid Ltd., UK were used.

2.4. Phytochemical tests

For the identification of foremost functional groups in the crude extract preliminary phytochemical screening was performed^[11–13]. The leaves extract demonstrated the presence of alkaloids, flavonoids, glycosides, saponins and tannins.

2.5. In vitro antioxidant activity test

Stable free radical DPPH (1,1–Diphenyl–2–picrylhydrazyl) scavenging method was applied for estimation of antioxidant activity of the ethanolic extract of sample both qualitatively and quantitatively^[14–16].

2.6. Qualitative test

For qualitative estimation of antioxidant property of test sample thin layer chromatographic (TLC) technique was applied^[16]. This test was performed with polar, medium polar, and non–polar solvent systems by using TLC plate to determine compounds of different polarities. Bleaching of DPPH (yellow on purple background) radical was observed after spraying 0.02% DPPH in ethanol to TLC plates for the period of 30 min and noted.

2.7. Quantitative test

Quantitative estimation of antioxidant potential of the extract was estimated on the basis of the extract's scavenging activity of the stable DPPH radical^[16]. Firstly, 10 mg extract was dissolved to 25 mL ethanol to prepare 400 µg/mL solution of the sample that is used as stock solution. Then 200, 100, 50, 25, 12.5, 6.25, 3.13, and 1.57 µg/mL solution was prepared by serial dilution technique. Add 2 mL of each sample solution into nine separate test tubes intended for each concentration. Then 6 mL of 0.004% DPPH solution was taken into these test tubes separately, and kept at dark place for 30 min for allowing reaction. After that, absorbance was measured by using UV spectrophotometer at 517 nm against blank preparation. Similarly prepared ascorbic acid was used as standard. Calculated the percent inhibition by using following formula:

Percent inhibition = $\frac{(\text{Absorbance of blank} - \text{Absorbance of sample or standard})}{\text{Absorbance of blank}} \times 100$.

IC₅₀ was found from percent inhibition *vs.* concentration curve.

2.8. Assessment of analgesic activity

To determine the analgesic activity of the crude sample, acetic acid induced writhing test in mice was used [16–18]. For this test, randomly experimental animals (mice) were selected, and split into four represented groups as control, positive control, and test group I and II. Each group contained of five mice. Control group was treated with 1% Tween-80 at the dose of 10 mL/kg body weight in distilled water, and diclofenac sodium at the dose of 25 mg/kg body weight was given to positive control group. Test group I and II were treated at the doses of 250 and 500 mg/kg body weight respectively with the test sample. Oral route was subjected to these treatments. To ensure proper absorption of the administered substances, thirty minutes interval was given. Then writhing inducer acetic acid solution (0.6%) was injected using intra-peritoneal route to each mice. To ensure absorption of administered acetic acid properly, a five minutes interval was given. At that time writhing number was counted for 10 min. Inhibition of writhing was calculated as percent for both test and control groups and compared.

2.9. Antibacterial activity

2.9.1 Disc diffusion assay

Antibacterial activity of the ethanolic extract of *M. azedarach* L. was assessed by disc diffusion assay [19]. The extract was prepared using DMSO at desired concentration. Sterile blank discs (BBL, Cockville, USA) were impregnated with the test extract at the concentrations of 250 and 500 µg/disc using micropipette. Then discs were dried. Dried sample discs, standard antibiotic discs (Ciprofloxacin 5 µg/disc, Oxoid Ltd., UK) and control discs (contain DMSO) were placed on nutrient agar medium seeded with bacteria in Petri dishes using sterile forceps. Then Petri dishes were incubated at 37 °C for 16 h. After incubation period, zone of inhibition was measured using digital slide calipers.

2.10. Statistical analysis

For determining significant differences between test and control group student's t-test was used. Standard deviations (SD) were carried out using Kirkman, T.W. (1996) Statistics to Use (<http://www.physics.csbsju.edu/stats/>), and GraphPad Software (<http://graphpad.com/quickcalcs/ttest2/>) was used to calculate the *P* value.

3. Results

3.1. Phytochemical tests

For identification of different bioactive groups in the ethanolic extract, a number of qualitative phytochemical tests were performed and results are cited in the Table 1.

Table 1

Phytochemical tests of leaves of *M. azedarach* L.

Phytochemical groups	Results
Alkaloids	+
Flavonoids	+
Glycosides	+
Gums	–
Reducing sugars	–
Saponins	+
Steroids	–
Tannins	+

+: Presence; -: Absence.

3.2. DPPH scavenging activity

The sample demonstrated DPPH scavenging activity with IC₅₀ value of 19 µg/mL approximately that is comparable to the standard ascorbic acid having IC₅₀ value of 60 µg/mL approximately (Figure 1).

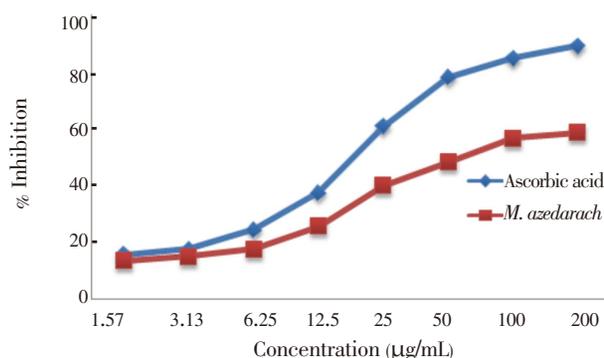


Figure 1. DPPH scavenging activity of *M. azedarach* L. leaves

DPPH scavenging activity was performed for plant extract as well as for ascorbic acid. Measuring absorbance by using UV spectrophotometer at 517, percent inhibition was calculated. Graphing the value, IC₅₀ for plant extract and standard ascorbic acid were found.

3.3. Acetic acid-induced writhing test

The sample demonstrated 45.45% ($P < 0.001$) and 67.05% ($P < 0.001$) writhing inhibition at the doses of 250 and 500 mg/kg body weight respectively in a dose dependent manner, which is extremely comparable with standard diclofenac sodium 69.32% ($P < 0.001$) at the dose of 25 mg/kg body weight (Table 2).

Table 2

Effect of *M. azedarach* L. leaves on acetic acid induced writhing in mice.

Treatment (n=5)	Dose (mg/kg)	No. of writhes	% Inhibition
Control	---	17.60±0.93	---
Diclofenac sodium	25	5.40±0.68*	69.32
Extract	250	10.20±0.95*	45.45
Extract	500	6.80±0.86*	67.05

Results are expressed as mean±SEM, SEM=Standard error of mean
*: $P < 0.001$ versus control, Student's *t*-test.

3.4. Activity in disc diffusion assay

The ethanolic extract of leaves of *M. azedarach* showed antibacterial activity against all the tested bacterial strains

with zone of inhibition ranging from 8.40 mm to 10.39 mm and 11.56 mm to 15.87 mm for 250 µg/disc and 500 µg/disc of extract respectively (Table 3).

Table 3

Antibacterial activity of *Melia azedarach* leaves in disk diffusion assay

Bacterial strains	Type of bacteria	Diameter of zone of inhibition (mm)			
		Blank	A	B	C
<i>Escherichia coli</i>	Gram (-)	-	24.65	10.39	15.87
<i>Salmonella typhi</i>	Gram (-)	-	23.30	8.54	12.15
<i>Shigella dysenteriae</i>	Gram (-)	-	22.12	8.40	11.56
<i>Staphylococcus aureus</i>	Gram (+)	-	22.15	9.45	14.05
<i>Staphylococcus epidermidis</i>	Gram (+)	-	24.34	9.21	12.55

A: Ciprofloxacin (5 µg/disc), B: Extract (250 µg/disc), C: Extract (500 µg/disc).

4. Discussion

The leaves extract of *M. azedarach* exhibited some potential phytochemicals, like alkaloids, flavonoids, glycosides, saponins and tannins.

The antioxidant activity was demonstrated by most popular DPPH scavenging assay method. This *in-vitro* antioxidant activity test shows that the investigated plant contains some potential antioxidant compounds. In this method, deep violet color of DPPH is renewed to light yellow color by converting free radical, DPPH to stable DPPH-H by accepting hydrogen radical, or electron. UV spectrophotometer detect an odd electron of DPPH radical at 517 nm against blank and the absorption decreases upon reduction with an antioxidant due to the development of its stable form, DPPH-H[20]. It was a concentration dependent manner and was analogous to well establish antioxidant ascorbic acid. Flavonoids delineated by the phytochemical investigation of plant extract may be accountable for this antioxidant activity[21,22].

Most widely used acetic acid induced writhing method in mice was applied for assessing *in-vivo* analgesic activity. In writhing test, peripherally acting analgesic activity of the sample is evaluated by inducing writhing through the sensitization of pain receptors by prostaglandins release[23,24]. Writhing is induced by the release of endogenous substances. Several endogenous substances like serotonin, bradykinins, histamine, prostaglandins (PGs), and substance P released by acetic acid are liable for producing pain by accelerating nerve endings. The abdominal constrictions response is activated by locally sensitize peritoneal receptors by administering acetic acid is a responsive pathway to approximate the peripherally acting analgesics[25]. The most probable mechanism of peripheral analgesic activity may be the inhibition of prostaglandins (PGE2 and PGE2 α) synthesis[26].

In disk diffusion assay, the ethanolic extract was profound to inhibit bacterial growth against all the tested bacterial strains with considerable zone of inhibition. But in disk diffusion assay, non polar compounds are not evaluated properly, because agar media is prepared with water, so poor

diffusion of non polar compounds results[27]. As a result, the antibacterial activity was mainly the attribution of polar compounds present in the bark extract. But it is must to declare that antibacterial activity was promising against all the tested bacterial strains.

Alkaloids, flavonoids, glycosides, saponins and tannins presence in the plant extract may be responsible for the investigated activities, because it is well established that a wide range of bioactivities are dependable to these phytochemicals[28–31]. Possible antioxidant, analgesic and antibacterial activities were exhibited in the crude leaves extract of *M. azedarach*, which justify its medicinal activities. To classify fundamental active constituents responsible for bioactivities as well as its mechanism, it should be required further investigations.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

In traditional medicinal system, several plants of medicinal properties are useful for antioxidant, analgesic & antibacterial purposes. However, *M. azedarach* L. is one of the vastly used species in ethnomedicine. This work objective consists the phytochemical screening of ethanol extract from *M. azedarach* leaves, as well as to assess its possible antioxidant, analgesic and antibacterial activities.

Research frontiers

This study is about to evaluate the potentiality of leaves of *M. azedarach* L., which is a local medicinal plant of Bangladesh. The authors have presented bioactivities test as well as phytochemical screening with crude ethanol extract of this plant leaves.

Related reports

Although, some researches have carried out the test with different solvents and different strains for antimicrobial test, same activities have been documented for the plant species.

Innovations and breakthroughs

This careful study showed that the ethanol extract of leaves of this plant has antioxidant, analgesic and antibacterial activities. The phytochemical screening also confirms alkaloids, flavonoids, glycosides, saponins and tannins like compounds.

Applications

M. azedarach leaves may be used strongly as a local source of antioxidant, analgesic and antibacterial agents.

Peer review

The authors make the study interesting by evaluating the antioxidative, analgesic and antibacterial effects of *M. azedarach* extract in association with doing phytochemical screening. Materials and methods are well planned. Findings are attention-grabbing and the discussion section contains scientific interpretation.

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