



Original Research Article

Phytochemical Screening and Antioxidant Effects of Azadirachta Indica Leaves Extract

Article History:

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Received: 29-10-2025

Revised: 14-11-2025

Accepted: 23-11-2025

Published: 26-12-2025

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Abstract: Azadirachta indica is a traditional plant of great importance, cultivated worldwide, including in India, China, America, and Nigeria. It belongs to the Meliaceae family and is widely recognised as a medicinal plant in Chinese, Ayurvedic and Unani medicine in the Indian subcontinent, with its health benefits mainly due to its rich anti-microbial, antioxidant and anti-inflammatory properties. It is regarded as a safe medicinal plant that can modulate numerous biological and physiological processes without causing adverse effects. Fresh leaves of *A. indica* were collected, dried, ground, and extracted using aqueous, ethanol, ethyl acetate, methanol, chloroform, and petroleum ether. Phytochemical screening revealed the presence of various phytoconstituents such as flavonoids, phenols. The study aimed to evaluate the quantitative and antioxidant activity of the solvent extracts. Ethanolic extract of *A. indica* leaves showed better results among all the extracts. The phytochemical composition suggests that the pharmacologically active constituents of *A. indica* may contribute to its anti-inflammatory and antioxidant properties. These findings provide scientific validation for the traditional use of neem in the treatment of infectious and inflammatory disorders.

Keywords: Azadirachta indica, medicinal, qualitative, quantitative, anti-oxidant property.

INTRODUCTION

Medicinal plants have a long history of use in managing respiratory disorders (Firdaus et al., 2025). Even in contemporary medicine, plant-derived compounds play a crucial role in enhancing immune function and improving clinical outcomes (Rahman et al., 2022). Natural products continue to represent a major source of therapeutic agents, with more than 50% of clinically approved drugs originating from higher plants (Gohel et al., 2021). Numerous studies have demonstrated potent anti-inflammatory properties among various medicinal plant extracts and formulations, highlighting their promising potential as alternative or complementary therapeutic options for respiratory diseases.

Neem (*Azadirachta indica* A. Juss) is a native plant from India, originating from the Meliaceae family that is widespread throughout the world and can be grown in tropical and subtropical countries (Islas et al., 2020). Neem is known by the Sanskrit name "arista," which means "perfect, complete, and perishable" (Girish & Shankara, 2008). It is an attractive perennial tree that is native to the Indian region. In addition, it

is grown in numerous Latin American nations, Southeast Asia, Australia, East and SubSaharan Africa, Fiji, and Mauritius (Puvan et al., 2015). Neem has excellent medical benefits with various biological activities (Islas et al., 2020). Neem has been known for centuries and is used in traditional Indian medicine since ancient times. Neem can be used to treat multiple diseases in almost all parts of the plant (Rahmani et al., 2018). Several studies report that neem leaves have antioxidant, anti-inflammatory, anti-cancer, anti-diabetic, immunomodulatory, anticancer, wound healing, nephroprotective, hepatoprotective, antimalarial, antifungal, antibacterial, neuroprotective, antifertility and contraception (Kharwar et al., 2020).

The plant kingdom represents a rich storehouse of organic compounds, many of which have been used for medicinal purposes and could serve as a lead for the development of novel agents having good efficacy in various pathological disorders in the coming years. Neem plant is considered to be the richest sources of drugs for traditional medicine, modern medicine, nutraceuticals, food supplements, folk medicine,

pharmaceutical intermediates and chemical entities for synthetic drugs (Mahima et al., 2013). Global, agricultural, environmental, and health issues have long been addressed using neem (Mudele et al., 2021). Its leaves can be taken as medication to treat eczema, diabetes, and lower fever. Neem leaves are used to produce toothbrushes, and the plant's roots are insect- and disease-repelling. Neem tree seeds are extremely rich in oil. Many illnesses, including diabetes and tuberculosis, are treated with neem oil, which is also used as a lubricant, pesticide, and medication (Virshette et al., 2020). Some of the phytochemicals contained in neem plant have been isolated, quantified and identified through intensive studies. These bioactive chemicals have provided leads in the development of several lifesaving drugs, which are in use today (Padal et al., 2013). Active constituents of the neem leaf include nimbin, nimbidine, isomeldenin, β -sitosterol and quercetin (Lee et al., 2017). Quercetin, β -sitosterol and nimbidine have been shown to exert anti-inflammatory effects. These effects are due to the inhibition of pro-inflammatory molecules, such as TNF- α , iNOS and NF- κ B (Kim et al., 2012).

The present study builds on this foundation by investigating the anti-oxidant potential of *Azadirachta indica*, a traditionally used medicinal plant, and developing an ethanolic extract based aimed at enhancing its therapeutic efficacy for ARDS management.

MATERIALS AND METHODS

2.1. Collection of plant material and its extraction

The dried leaf powder was collected from Natural Remedies Private Limited (Bangalore, Karnataka, India). Leaf powder of *A. indica* (16 grams) was subjected to extraction with various organic solvents (250ml) (Chloroform, Ethanol, Methanol, Ethyl acetate, and Petroleum ether) for 7 days in rotary shaker at room temperature and distilled water (DW) for 6 hours in water bath at 37°C (Kant et al., 2013).

2.2. Preliminary Phytochemical Screening

Qualitative analysis was performed for the following extracts - Aqueous, ethanol, methanol, chloroform, petroleum ether, and ethyl acetate to identify various active constituents like phenols, flavonoids, steroids, alkaloids, glycosides, and tannins.

2.2.1. Carbohydrate Test

- Molisch's test: 1ml of extract is mixed with a few drops of alcoholic α -naphthol. 0.2 ml of concentrated H₂SO₄ was gradually added to this reaction mixture. The development of violet color at the intersection of two layers indicates the presence of carbohydrates (Veerachari et al., 2011).
- Benedict's test: a few drops of Benedict's

reagent was added to 1 ml extract, and the mixture was then boiled for a few seconds in a water bath. The appearance of a reddish-brown precipitate showed that reducing sugars were present (Veerachari et al., 2011).

- Fehling test: mix 1 ml of the extract with 1 ml of Fehling's A and B reagents. Then, boil the mixture for one minute. A brick-red residue shows that reducing sugar is present (Ramarajan et al., 2019).

2.2.2. Proteins Test

- Millon's test: 2 ml of millon's reagent was added to 1 ml of the extract. The white precipitate that forms and turns crimson when heated gently indicates a positive result (Karthikeyan et al., 2019).

2.2.3. Alkaloid Test

- Wagner's test: 1 ml of extract was mixed with a few drops of wagner's reagent. A reddish-brown precipitate suggests the presence of an alkaloid (Karthikeyan et al., 2019).

2.2.4. Phenolic Compounds

- Zinc-hydrochloride reduction test: A pinch of zinc dust and a few drops of strong hydrochloric acid (HCl) were added to one ml of the plant extract. The presence of phenols was indicated by the occurrence of yellowish, yellow-orange colouration on occasion and orange colouration after a few minutes (Ramya et al., 2019).
- Ferric Chloride test: 1 ml of plant extract was treated with few drops of ferric chloride (5%) reagent. The appearance of bluish-black or dark green colour confirms the test as positive (Ramya et al., 2019).

2.2.5. Anthocyanins test

- 1 ml extract, 1 ml 10% NaOH was mixed and heated for 5 mins in 100°C. Formation of blue color indicates the presence of anthocyanins (Redha et al., 2018).

2.2.6. Sterols and Triterpenoids Test:

- 1ml of plant extract is mixed with 1 ml of chloroform followed by a few drops of conc. H₂SO₄. This reaction mixture was shaken properly and left for a few minutes. Red colour lower layer indicates the presence of sterols and yellow coloured layer indicates the presence of triterpenoids (Rahimah et al., 2019).

2.2.7. Tannins

- Ferric chloride test – To 1 ml of the plant extract was added a few drops of 5% Ferric chloride (FeCl₃) solution. Blue color (for gallic Tannins); greenish-black color (catholic Tannins); greenish-brown color (condensed Tannins) (Ramya et al., 2019).
- Alkaline reagent test: In 1 ml of plant extract, a few drops of 5% ferric chloride solution were added. Greenish blue indicates the presence of catecholic tannins, blue colour (gallic tannins), and greenish-brown colour (condensed tannins) (Ramya et al., 2019).

2.2.8. Quinones Test

- 1 ml of concentrated sulphuric acid was added to 1 ml of extract. Red colour indicates the presence of quinones (Roghini et al., 2018)

2.2.9. Flavonoids Test

- Zinc-hydrochloride reduction test: To 1 ml of the plant extract, a pinch of zinc dust and a few drops of conc. HCl was added. The appearance of red colour indicates the presence of flavonoids (Pant et al., 2017).
- Alkaline reagent test: To 1ml of the plant extract, add a few drops of 10% sodium hydroxide or 10% ammonium hydroxide solution. Intense yellow colour turns into colourless on the addition of a few drops of dilute acetic acid or HCl (Pant et al., 2017).

2.3. Quantitative Analysis

2.3.1. Determination of total Flavonoid content (TFC)

TFC was estimated by the aluminium chloride colorimetric method. Plant extracts (50-250 µg/ml) were mixed with 5% NaNO₂ (30 µl) and incubated at 25°C for 5 minutes. Subsequently, 10% of an AlCl₃ solution (30 µl) was added, followed by 6 minutes of incubation at 37°C. Thereafter, 200 µl of 1 M NaOH

was added, and the reaction mixture volume was adjusted to 1 ml with distilled water. Absorbance was recorded at 510 nm. Flavonoid content was quantified using a quercetin calibration curve and expressed as quercetin equivalents (Madhu et al., 2016).

2.3.2. Determination of total tannin content (TTC)

TTC was estimated by the Folin-Ciocalteu method. Extracts (25-200 µg/ml) were treated with Folin-Ciocalteu reagent (50 µl) and 35 % Na₂CO₃ (100 µl), vortexed, and incubated at 37°C for 5 minutes. Absorbance was recorded at 700 nm, and tannin content was expressed as gallic acid equivalents using a calibration curve (Chandran et al., 2016).

2.5. Antioxidant Activity

2.5.1. Determination of DPPH radical scavenging activity

The antioxidant activity was assessed using the DPPH radical scavenging method described by Blois (1958) with slight modifications. Plant extracts and ascorbic acid (20-100 µg/ml) were mixed with 0.1 mM DPPH solution and incubated in the dark at room temperature for 30 minutes. Absorbance was recorded at 517 nm, and radical scavenging activity (%) was calculated using: % inhibition = [(A₀-A₁)/A₀] × 100 where, A₀ is the absorbance of the control, and A₁ is the absorbance sample

The inhibitory concentration (IC₅₀) DPPH values (the concentration of sample required for inhibition of 50% of DPPH radical) were obtained from the linear regression line. The antioxidant activity was evaluated based on this IC₅₀ value.

2.5.2. Determination of H₂O₂ radical scavenging activity

The assay was performed as described by Ruch et al. (1989). Plant extracts (20-100 µg/ ml) were mixed with phosphate buffer (pH 7.4) and 2 mM H₂O₂ solution, incubated for 10 minutes, and absorbance was measured at 230 nm. Ascorbic acid was used as the standard, and scavenging activity (%) was calculated using:

$$\text{scavenging percentage} = [(A_0 - A_1)/A_0] \times 100$$

RESULTS AND DISCUSSION

3.1. Phytochemical Screening

Phytochemical screening is used to evaluate the constituents of the plant extracts and their predomination, along with the search for bioactive constituents that may be helpful in the production of therapeutic drugs. In the current study, the qualitative phytochemical analysis of the plant extracts revealed the presence of various bioactive compounds across different solvents (see Table 1). In the ethanolic, methanolic, and aqueous extracts, carbohydrates, proteins, phenols, triterpenoids, tannins, quinones, and flavonoids were identified. The chloroform and petroleum ether extracts contained similar compounds, including carbohydrates, sterols, triterpenoids, quinones, and flavonoids. For the ethyl acetate extracts, quinones and flavonoids were detected. The study demonstrated that the ethanolic, methanolic, and aqueous extracts exhibited a higher diversity of phytoconstituents compared to the chloroform, ethyl acetate, and petroleum ether extracts. The therapeutic potential of *A. indica* might be due to the presence of these phytochemicals. Terpenoids show multiple pharmacological activities i.e., working as active agents

against inflammation, cancer, viruses, and bacteria along with hindering cholesterol synthesis. Flavonoids are known to have antioxidant effects, inhibiting the initiation, promotion, and progression of tumors. Tannins possess antiviral, antibacterial, and antitumor activity (Bhattacharya et al., 2016)

Consequently, the ethanolic, methanolic, and aqueous extracts were selected for further quantitative phytochemical analysis due to their richer bioactive compound profiles. This variation in phytochemical composition across solvents highlights the differential solubility and extractability of these bioactive compounds, which may influence their potential pharmacological properties.

Table 1: Qualitative analysis of A. indica extracts

Phytochemicals		Ethano lic extract	Methanoli c extract	Chlorofo r m extract	Petroleu m ether extract	Ethyl acetat e extrac t	Aqueou s extract
1. Carbohy drates	Molisch test	-	-	-	-	-	-
	Benedict test	+	+	-	-	-	+
	Fehling test	+	+	+	+	+	+
2. Proteins	Millon's test	+	-	-	-	-	+
3. Alkaloid s	Wagner's test	+	-	-	-	+	-
4. Phenolic compounds	Zinc- hydrochloride reduction test	-	+	+	-	-	-
	Ferric chloride test	+	+	+	+	-	+
5. Anthocy anins		-	-	-	-	-	-
6. Sterols		-	-	-	+	-	-
7. Triterpe noids		+	+	+	-	+	+
8. Tannins	Ferric chloride test	+	+	+	+	+	+
	Alkaline reagent test	-	-	-	-	-	-
9. Quinone s		+	+	+	+	-	+
10. Flavonoi ds	Zinc- hydrochloride reduction test	-	+	-	-	-	-
	Alkaline reagent test	+	+	+	+	+	+

Quantitative Analysis

Flavonoids, a prominent subclass of polyphenols, are widely recognized for their diverse pharmacological benefits. Their effects are often dependent on their specific structural composition and include antioxidant, antibacterial, hepatoprotective, anticancer, antiviral and anti-inflammatory properties (Shrestha, et al., 2024).

Tannins, another important category of phenolics, exhibit significant antibacterial and antiviral effects, while also possessing strong free radical scavenging activity, which supports their role in cellular protection and anti-ageing processes (Sun et al., 2023).

Among the six extracts, the ethanolic, methanolic, and aqueous extracts demonstrated a higher diversity of

phytoconstituents; thus, these were selected for quantitative analysis. The total flavonoid content (TFC), and total tannin content (TTC) of these extracts are detailed in Table 2. The ethanolic extract displayed the highest concentrations of flavonoid compounds (81.76 ± 0.22 mg QE/g), and tannin levels (56.41 ± 0.2 mg GAE/g). In contrast, the aqueous extract demonstrated the lowest concentrations of phenolic, and tannin compounds.

Table 2. Total flavonoid and tannin content

Extracts	TFC mg of Quercitin equivalents/g dry weight of plant extract	TTC mg of Gallic acid equivalents/g dry weight of plant extract
<i>A. indica</i> ethanolic extract	81.76 ± 0.22	56.41 ± 0.20
<i>A. indica</i> methanolic extract	62.71 ± 0.18	51.28 ± 0.18
<i>A. indica</i> aqueous extract	67.47 ± 0.19	41.02 ± 0.14

Antioxidant Activity

Natural antioxidants have gained lot of interest among consumers and the scientific community because epidemiological studies have indicated that frequent consumption of natural antioxidants is associated with reduction of cardiac and other disorders with no side effects. Several studies are going on throughout the world to identify pharmacologically potent source of antioxidant compounds. It is well known that *A. indica* is an important herb and largely used in Ayurveda for skin disorders, fever, burning sensation, respiratory diseases, hepatitis (Kant et al., 2013). The DPPH radical is a stable free radical commonly employed as a sensitive and rapid assay to evaluate the free radical scavenging activity of both hydrophilic and lipophilic antioxidants. Antioxidants neutralize free radicals by interacting with DPPH, either through electron transfer or by donating hydrogen atoms (Archana et al., 2005). This assay quantifies the antioxidant's radical scavenging capacity by monitoring the reduction in DPPH absorbance, which correlates with a color transition from purple to yellow, indicating the conversion of DPPH to its stable form via hydrogen donation (Matthaus, 2002; Bajpai et al., 2015).

Hydrogen peroxide is a weak oxidizing agent and can inactivate a few enzymes directly, usually by oxidation of essential thiol (-SH) groups. Hydrogen peroxide can cross cell membranes rapidly, once inside the cell, hydrogen peroxide can probably react with Fe^{2+} , and possibly Cu^{2+} to form hydroxyl radical and this may be the origin of many of its toxic effects. The scavenging of hydrogen peroxide by the extract may be attributed to active secondary metabolites, phenolics which neutralize hydrogen peroxide by donating electrons thereby neutralizing it to water (Vasanthakumari., 2017).

The ethanolic and methanolic extracts were further analyzed to evaluate their antioxidant activity. The IC₅₀ values were calculated as the concentration of plant extracts that could scavenge 50% of the free radicals. Table 4 shows the plant extracts' radical scavenging (%) activities against DPPH and H₂O₂ radicals. Lower IC₅₀ values indicate stronger antioxidant activity while high IC₅₀ values indicate weak antioxidant activities. Ascorbic acid was used as a reference standard for evaluating and comparing the antioxidant activity of the extracts. Ethanolic extracts showed lower IC₅₀ values in comparison to methanolic extracts which indicates that the ethanolic extract exhibit stronger antioxidant activity than methanolic extract.

Table 3: Antioxidant activity (IC₅₀) of ethanolic and methanolic extract of *A. indica* along with the standard ascorbic acid

Extracts	DPPH (IC ₅₀ µg/ml)	H ₂ O ₂ (IC ₅₀ µg/ml)
<i>A. indica</i> ethanolic extract	63.59	90.5
<i>A. indica</i> methanolic extract	73.33	109.7
Ascorbic acid	26.57	53.55

CONCLUSION

The present study indicated that the ethanol extract of *A. indica* may have potential use in medicine. From the previous study and our investigation, it may be concluded that ethanol extract possesses more pharmacological properties than n-hexane and

chloroform extracts. In our study, the ethanol extracts of *A. indica* showed significant dose-dependent inhibition of analgesic effect. These current studies suggested ethanol extract of *A. indica* demonstrated significant results and possesses analgesic properties. Now our next aim is to isolate

the leading compounds and to establish their chemical structure.

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