

Comparative Pharmacognostical and Phytochemical Evaluation of Dried Leaves of *Azadirachta indica* A. Juss.

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Abstract

Medicinal plants constitute an important source of bioactive compounds and play a significant role in traditional and modern healthcare systems. Among them, *Azadirachta indica* (Neem) has gained considerable attention due to its broad spectrum of biological activities. The present study was undertaken to investigate the pharmacognostical and phytochemical properties of dried neem leaves. Pharmacognostical evaluation included the assessment of macroscopic and microscopic characteristics, whereas phytochemical screening was performed using standard qualitative tests. The results revealed distinct morphological and anatomical features that can serve as diagnostic markers for identification of neem leaves. Phytochemical analysis demonstrated the presence of alkaloids, flavonoids, tannins, glycosides, saponins, terpenoids, and phenolic compounds. The occurrence of these secondary metabolites supports the therapeutic potential of neem and validates its traditional medicinal applications. The study emphasizes the significance of pharmacognostical and phytochemical investigations in ensuring the authenticity, quality, and efficacy of herbal drugs and formulations derived from neem leaves.

Keywords: Neem (*Azadirachta indica*), Pharmacognostical evaluation, Phytochemical screening, Medicinal plant, Secondary metabolites, Standardization, Authentication, Herbal formulation

INTRODUCTION

Since antiquity, human societies have relied on natural resources to meet their needs, particularly using plant derived remedies to prevent and treat disease. With limitations of some synthetic drugs, including treatment failures and the growing problem of antimicrobial resistance plants are once again being investigated as promising sources of novel lead compounds. Plants produce a wide array of constitutive secondary metabolites, such as alkaloids, phenolics, terpenoids, steroids, glycosides, and related derivatives [Yadav et al., 2023].

Taxonomic Rank	Classification
Kingdom	Plantae
Subkingdom	Viridiplantae
Division	Magnoliophyta (Angiosperms)
Class	Magnoliopsida (Dicotyledons)
Order	Sapindales
Family	Meliaceae
Subfamily	Melioideae
Tribe	Melieae
Genus	<i>Azadirachta</i>
Species	<i>Azadirachta indica</i> A. Juss.



The growing interest in herbal medicines has increased the need for scientific evaluation, standardization, and quality control of medicinal plants. Pharmacognostical and phytochemical investigations provide essential information regarding the identification, authentication, and medicinal potential of plant materials [Saleem et al., 2018].

Azadirachta indica A. Juss., commonly known as Neem, is a well-known medicinal plant belonging to the family Meliaceae. It is widely distributed in tropical and subtropical regions and has been extensively used in traditional systems of medicine such as Ayurveda, Unani, and Siddha. Due to its broad spectrum of therapeutic applications, neem is often referred to as the “Village Pharmacy.” Various parts of the plant, including leaves, bark, seeds, flowers, and roots, have been used for the treatment of numerous ailments such as skin diseases, microbial infections, inflammation, fever, and diabetes [Kadir et al., 2024; Yadav et al., 2023].

Neem leaves are particularly valued because they contain a wide range of biologically active phytoconstituents. Previous studies have reported the presence of alkaloids, flavonoids, tannins, glycosides, saponins, terpenoids, phenolic compounds, nimbin, nimbidin, and azadirachtin in neem leaves. These compounds are responsible for various pharmacological activities including antimicrobial, antioxidant, anti-inflammatory, antidiabetic, and anticancer

effects [Saleem et al., 2018; Atawodi and Atawodi, 2009]. The medicinal significance of neem has attracted considerable scientific attention, leading to extensive studies on its phytochemical composition and therapeutic applications.

Pharmacognostical evaluation is an important tool for the identification and quality assessment of medicinal plants. It involves the study of macroscopic and microscopic characteristics such as colour, odour, taste, shape, stomatal features, trichomes, and vascular tissues. These characteristics serve as reliable diagnostic markers for the authentication of crude drugs and help prevent adulteration [Patil et al., 2022]. Similarly, phytochemical screening plays a crucial role in detecting the major classes of secondary metabolites present in plant extracts and provides a scientific basis for their medicinal use.

Therefore, the present study aims to carry out a comparative pharmacognostical and phytochemical evaluation of dried leaves of *Azadirachta indica*. The study focuses on the assessment of morphological and microscopic characteristics along with preliminary phytochemical screening to identify major bioactive constituents. The findings may contribute to the standardization, quality control, and scientific validation of neem leaves for their effective use in herbal formulations.

MATERIALS AND METHODS

3.1 Collection of Plant Material

Fresh leaves of *Azadirachta indica* (Neem) were collected from healthy neem trees growing in the local area. The collected leaves were washed thoroughly with distilled water to remove dust and other impurities. The leaves were shade-dried at room temperature for 10–15 days until a constant weight was obtained. The dried leaves were then pulverized using a mechanical grinder to obtain a coarse powder and stored in airtight containers for further analysis (Yadav et al., 2023; Kadir et al., 2024).

3.2 Pharmacognostical Evaluation

3.2.1 Macroscopic Examination

The dried neem leaves were examined for various morphological characteristics including colour, odour, taste, shape, size, apex, margin, surface texture, and venation pattern. These characteristics were recorded and compared with standard pharmacognostical descriptions available in the literature (Kadir et al., 2024; Yadav et al., 2023).

3.2.2 Microscopic Examination

Microscopic studies were carried out using powdered leaf samples. Small quantities of powdered material were mounted on clean glass slides using glycerin and observed under a compound microscope. Diagnostic features such as epidermal cells, stomata, trichomes, vascular bundles, xylem vessels, and calcium oxalate crystals were examined and recorded (Yadav et al., 2023).

3.3 Preparation of Extract

Approximately 20 g of air-dried, pulverized *Azadirachta indica* leaves were subjected to maceration in ethanol. Ethanol was selected because it efficiently extracts a wide range of phytochemicals including flavonoids, phenolics, glycosides, and alkaloids. The plant material

was soaked in solvent for 24 hours with occasional agitation. After maceration, the mixture was filtered through Whatman No. 1 filter paper and the filtrate concentrated by gentle evaporation at ambient temperature. The concentrated extract was stored in a sealed container until use for phytochemical screening (Saleem et al., 2018; Patil et al., 2022).

Because solvent polarity determines which metabolites are extracted, polar solvents such as ethanol and methanol generally yield higher concentrations of phenolic compounds, flavonoids, and glycosides, whereas non-polar solvents preferentially extract lipophilic constituents. Previous studies have demonstrated that ethanolic and methanolic extracts of neem leaves possess higher phytochemical content and stronger biological activities than extracts obtained using less polar solvents (Saleem et al., 2018; Patil et al., 2022; Yadav et al., 2023).

3.4 Preliminary Phytochemical Screening

The ethanolic extract of neem leaves was subjected to qualitative phytochemical screening following standard procedures described in previous studies (Atawodi and Atawodi, 2009; Saleem et al., 2018; Yadav et al., 2023).

Alkaloids Test (Mayer's Test)

A few drops of Mayer's reagent were added to the extract. Formation of a creamy white precipitate indicated the presence of alkaloids (Yadav et al., 2023).

Flavonoids Test (Shinoda Test)

The extract was treated with magnesium turnings and concentrated hydrochloric acid. Development of a pink or red colour indicated the presence of flavonoids (Saleem et al., 2018; Yadav et al., 2023).

Tannins Test

Ferric chloride solution was added to the extract. Formation of a blue-black or greenish-black colour confirmed the presence of tannins (Saleem et al., 2018).

Saponins Test (Foam Test)

The extract was vigorously shaken with distilled water. Formation of persistent foam indicated the presence of saponins (Atawodi and Atawodi, 2009).

Glycosides Test

The extract was treated according to standard phytochemical procedures. Development of characteristic colour reactions indicated the presence of glycosides (Saleem et al., 2018; Yadav et al., 2023).

Terpenoids Test (Salkowski Test)

The extract was mixed with chloroform followed by concentrated sulfuric acid. Formation of a reddish-brown interface indicated the presence of terpenoids (Atawodi and Atawodi, 2009; Saleem et al., 2018).

Phenolic Compounds Test

The extract was treated with ferric chloride solution. Appearance of a dark blue or green colour confirmed the presence of phenolic compounds (Saleem et al., 2018).

3.5 Data Analysis

The observations obtained from pharmacognostical and phytochemical investigations were compiled, compared, and presented in tabular form. The results were interpreted with reference to previously published studies on neem leaves (Atawodi and Atawodi, 2009; Saleem et al., 2018; Patil et al., 2022; Yadav et al., 2023; Kadir et al., 2024).

RESULTS AND DISCUSSION

4.1 Pharmacognostical Evaluation

Macroscopic Characteristics of Dried Neem Leaves

The dried leaves of *Azadirachta indica* were examined for various morphological parameters. The leaves were found to be greenish-brown in colour with a characteristic odour and distinctly bitter taste. The leaflets were lanceolate in shape with serrated margins and pointed apex. These characteristics are consistent with the standard descriptions reported for neem leaves and can be used for preliminary identification of the crude drug.

Table 1. Macroscopic Characteristics of Dried Neem Leaves

Parameter	Observation
Colour	Greenish-brown
Odour	Characteristic
Taste	Bitter
Shape	Lanceolate
Margin	Serrated
Apex	Acute
Surface	Slightly rough

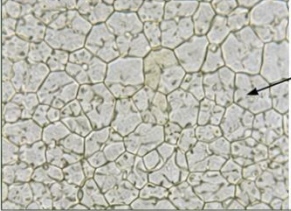

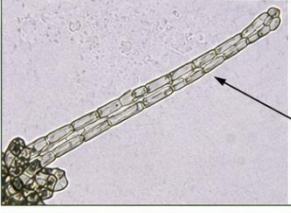
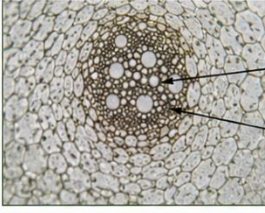
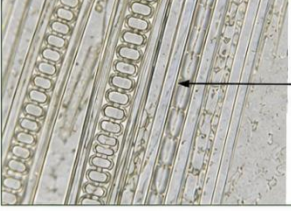
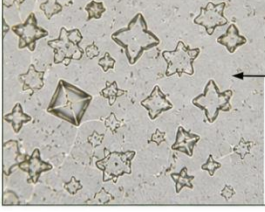
Microscopic Characteristics

Microscopic examination of the powdered leaf revealed the presence of epidermal cells, stomata, trichomes, vascular tissues, and calcium oxalate crystals. These features are considered important diagnostic markers for the authentication of neem leaf powder and are useful in distinguishing genuine plant material from adulterants.


The observed pharmacognostical characteristics are in agreement with previous studies, which reported similar morphological and anatomical features for neem leaves. Such parameters are essential for quality control and standardization of herbal raw materials.

Table 2. Microscopic Characteristics of Dried Neem Leaves

Microscopic Feature		Observation
Epidermal cells		Present
Stomata		Present
Trichomes		Present
Vascular bundles		Present
Xylem vessels		Present
Calcium oxalate crystals		Present

<p>1. Epidermal cells</p>  <p>Epidermal cells</p> <p>(40x)</p>	<p>2. Stomata</p>  <p>Stomatal pore</p> <p>Guard cells</p> <p>(40x)</p>
<p>3. Trichomes</p>  <p>Trichome</p> <p>(40x)</p>	<p>4. Vascular bundles (T.S.)</p>  <p>Xylem</p> <p>Phloem</p> <p>(40x)</p>
<p>5. Xylem vessels</p>  <p>Xylem vessels</p> <p>(40x)</p>	<p>6. Calcium oxalate crystals</p>  <p>Calcium oxalate crystals</p> <p>(40x)</p>

Microscopic examination of powdered dried neem leaves shows the presence of epidermal cells, stomata, trichomes, vascular bundles, xylem vessels and calcium oxalate crystals which are important diagnostic characters for the authentication of the plant material.



4.2 Phytochemical Screening








Preliminary phytochemical analysis of the ethanolic extract of neem leaves revealed the presence of several biologically active secondary metabolites. The qualitative tests confirmed the occurrence of alkaloids, flavonoids, tannins, saponins, glycosides, terpenoids, and phenolic compounds.

The presence of flavonoids and phenolic compounds suggests significant antioxidant potential of neem leaves. Tannins contribute to antimicrobial and astringent activities, while alkaloids and terpenoids are associated with various pharmacological effects including anti-inflammatory and antimicrobial properties. The occurrence of saponins and glycosides further supports the medicinal importance of neem as a valuable source of bioactive compounds.

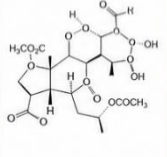
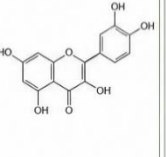
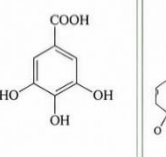
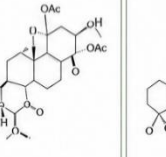
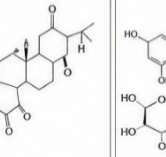
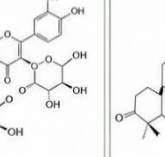
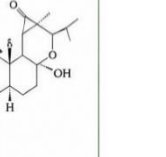
The phytochemical profile obtained in the present study is comparable to findings reported by previous researchers, who identified similar classes of secondary metabolites in aqueous, ethanolic, and methanolic extracts of neem leaves. Variations in phytochemical content may occur due to differences in geographical location, environmental conditions, drying procedures, and extraction methods.

Overall, the results demonstrate that dried neem leaves possess characteristic pharmacognostical features and a rich phytochemical composition. These findings support the traditional use of neem in herbal medicine and emphasize its potential for pharmaceutical and therapeutic applications.

Table 3. Preliminary Phytochemical Screening of Neem Leaf Extract

Phytochemical Constituent	Result	Visual Observation of Phytochemical Tests						
		Alkaloids (Mayer's Test)	Flavonoids (Shinoda Test)	Tannins (Ferric Chloride Test)	Saponins (Foam Test)	Glycosides (Keller–Killiani Test)	Terpenoids (Salkowski Test)	Phenolic compounds (Lead Acetate Test)
Alkaloids	+							
Flavonoids	+	Yellowish precipitate	Orange yellow color	Blue-black color	Persistent foam	Brown ring at interface	Reddish brown color	Blue-green color
Tannins	+							
Saponins	+							
Glycosides	+							
Terpenoids	+							
Phenolic compounds	+							
(+) = Present (-) = Absent								

Major Phytochemical Compounds Reported in Neem Leaves

Azadirachtin (Limonoid)	Quercetin (Flavonoid)	Gallic Acid (Phenolic Acid)	Nimbin (Limonoid)	Nimbidin (Limonoid)	Rutin (Flavonoid Glycoside)	Gedunin (Limonoid)
						
Molecular Formula: C ₃₃ H ₄₄ O ₁₆	Molecular Formula: C ₁₅ H ₁₀ O ₇	Molecular Formula: C ₇ H ₆ O ₅	Molecular Formula: C ₃₀ H ₃₆ O ₉	Molecular Formula: C ₃₀ H ₃₆ O ₉	Molecular Formula: C ₂₇ H ₃₀ O ₁₆	Molecular Formula: C ₃₀ H ₃₆ O ₇
Property: Antimicrobial, Insecticidal, Anti-inflammatory	Property: Antioxidant, Anti-inflammatory, Cardioprotective	Property: Antioxidant, Antimicrobial	Property: Anti-inflammatory, Antimicrobial	Property: Anticancer, Antimicrobial	Property: Antioxidant, Anti-inflammatory	Property: Antimalarial, Anticancer

CONCLUSION

The present study focused on the pharmacognostical and phytochemical evaluation of dried leaves of *Azadirachta indica* (Neem). Macroscopic examination revealed characteristic features such as greenish-brown colour, bitter taste, lanceolate shape, and serrated margins, which are useful for the identification of the crude drug. Microscopic analysis further confirmed the presence of important diagnostic characters including epidermal cells, stomata, trichomes, vascular tissues, and calcium oxalate crystals.

Preliminary phytochemical screening demonstrated the presence of various bioactive constituents such as alkaloids, flavonoids, tannins, saponins, glycosides, terpenoids, and phenolic compounds. These phytochemicals are known to contribute to the diverse pharmacological properties of neem, including antimicrobial, antioxidant, anti-inflammatory, and therapeutic activities.

The findings of the study support the traditional medicinal use of neem and highlight the importance of pharmacognostical and phytochemical investigations in the authentication, standardization, and quality control of herbal drugs. Thus, *Azadirachta indica* leaves may be considered a valuable source of natural bioactive compounds with potential applications in pharmaceutical and healthcare industries.

FUTURE SCOPE

Further studies may be carried out to isolate and characterize individual bioactive compounds present in neem leaves using advanced analytical techniques such as HPLC, GC-MS, and FTIR. Quantitative estimation of phytochemicals and evaluation of their pharmacological activities may provide additional scientific evidence for the development of neem-based herbal formulations and therapeutic products.

REFERENCES

- [1.] **Bem, A.A., Adamu, H. and Bem, L.S., 2025.** Comparative Phytochemical Analysis and Medicinal Properties of Neem (*Azadirachta indica*) and Eucalyptus (*Eucalyptus globulus*) Plant. *Sahel Journal of Life Sciences FUDMA*, 3(2), pp.304–310.
- [2.] **Kadir, A., Ghosh, T., Ilango, K., Deshmukh, V.N., Mahato, P., Krishna, K.V.V.S., Bali, S., Dhubkaria, Y. and Kumar, A., 2024.** In-depth review on taxonomy, phytochemistry, traditional uses and pharmacological significance of *Azadirachta indica* plant. *European Journal of Biomedical*, 11(6), pp.277–284.
- [3.] **Nwanekezie, M.N., Ndivi, J.N., Ogbonna, I.L. and Sebe, G.O., 2023.** Comprehensive physicochemical profiling and characterization of neem plant leaf extracts: insights for pharmaceutical and biomedical applications. *Advances in Chemical Engineering and Science*, 13(4), pp.382–399.
- [4.] **Yadav, M., Mishra, S., Tiwari, R., Kumari, B., Shukla, M., Dahiya, M., Teotia, A., Mehra, V., Kalaiselvan, V. and Raghuvanshi, R.S., 2023.** Investigating the Pharmacognostic and Pharmacological Activities of *Azadirachta indica* L. through Biochemical Assays. *Pharmacognosy Research*, 15(2).
- [5.] **Patil, S.M., Shirahatti, P.S. and Ramu, R., 2022.** *Azadirachta indica* A. Juss (Neem) against diabetes mellitus: A critical review on its phytochemistry, pharmacology, and toxicology. *Journal of Pharmacy and Pharmacology*, 74(5), pp.681–710.
- [6.] **Malar, T.J., Antonyswamy, J., Vijayaraghavan, P., Kim, Y.O., Al-Ghamdi, A.A., Elshikh, M.S., Hatamleh, A.A., Al-Dosary, M.A., Na, S.W. and Kim, H.J., 2020.** In-vitro phytochemical and pharmacological bio-efficacy studies on *Azadirachta indica* A. Juss and *Melia azedarach* Linn for anticancer activity. *Saudi Journal of Biological Sciences*, 27(2), pp.682–688.
- [7.] **Sharma, G., Thakur, A., Lal, S. and Nadda, R.K., 2019.** Comparative analysis of phytochemical, antibacterial, and antioxidant activity of different extracts of *Azadirachta indica* leaves. *Asian Journal of Pharmaceutical and Clinical Research*.
- [8.] **Saleem, S., Muhammad, G., Hussain, M.A. and Bukhari, S.N.A., 2018.** A comprehensive review of phytochemical profile, bioactives for pharmaceuticals, and pharmacological attributes of *Azadirachta indica*. *Phytotherapy Research*, 32(7), pp.1241–1272.
- [9.] **Kumar, V., Chakraborty, A., Kaur, M., Pandey, S. and Jena, M.K., 2018.** Comparative study on antimicrobial activity of tulsi (*Ocimum sanctum*) and neem (*Azadirachta indica*) methanol extract. *Asian Journal of Pharmaceutical and Clinical Research*, pp.514–517.

[10.] **Singh, V. and Chauhan, D., 2014.** Phytochemical evaluation of aqueous and ethanolic extract of neem leaves (*Azadirachta indica*). *Indo American Journal of Pharmaceutical Research*, 4(12), pp.5943–5948.

[11.] **Susmitha, S., Vidyamol, K.K., Ranganayaki, P. and Vijayaragavan, R., 2013.** Phytochemical extraction and antimicrobial properties of *Azadirachta indica* (Neem). *Global Journal of Pharmacology*, 7(3), pp.316–320.

[12.] **Atawodi, S.E. and Atawodi, J.C., 2009.** *Azadirachta indica* (Neem): a plant of multiple biological and pharmacological activities. *Phytochemistry Reviews*, 8(3), pp.601–620.

[13.] **Gupta, V., Sharma, S., Singh, D. and Mishra, A.** A review on comparative pharmacognostic and pharmacological aspects of Neem.