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Qualitative and Quantitative Phytochemical Profiling of *Azadirachta indica* Leaves and Their Potential Therapeutic Implications

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Abstract

Background: Neem (*Azadirachta indica*) is widely recognized in traditional medicine for its diverse therapeutic properties. This study investigated the qualitative and quantitative phytochemical composition of neem leaves to provide a scientific basis for its pharmacological potential. **Methods**: Fresh leaves were collected from Sagamu, Nigeria, identified at the Department of Pharmacology, Olabisi Onabanjo University, and subjected to standard extraction and phytochemical screening techniques. **Results**: Qualitative analysis revealed the presence of alkaloids, flavonoids, saponins, terpenoids, steroids, polyphenols, and tannins. Quantitative assessment showed flavonoids (13.8 ± 0.17%) and terpenoids (13.13 ± 0.50%) as the predominant constituents, followed by alkaloids (10.67 ± 0.46%) and saponins (2.43 ± 0.32%). The high concentration of flavonoids and terpenoids supports neem's potential antioxidant and anti-inflammatory activities, while alkaloids, saponins, and tannins may contribute to its antimicrobial, anticancer, and immune-modulatory properties. **Conclusion**: *A. indica* leaves are a valuable source of bioactive compounds with significant therapeutic promise, justifying their continued use in traditional medicine and their exploration in modern drug development.

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Introduction

Medicinal plants have long been recognized as valuable sources of bioactive compounds with diverse therapeutic potentials. Among them, *Azadirachta indica* A. Juss., commonly known as neem, has been widely used in traditional medicine across Asia and Africa due to its broad pharmacological properties, including antimicrobial, anti-inflammatory, antioxidant, and antidiabetic effects (Alzohairy, 2016; Sidat et al., 2023). The medicinal value of neem is largely attributed to its rich phytochemical composition, which includes alkaloids, flavonoids, tannins, saponins, phenolic compounds, and terpenoids (Rafi et al., 2023).

Phytochemical investigations serve as a vital step in correlating the therapeutic efficacy of plant extracts with their chemical constituents. Qualitative phytochemical screening allows for the detection of various classes of secondary metabolites, while quantitative analysis provides an estimation of their concentrations, which is essential for dosage standardization and pharmacological evaluation (Alamu et al., 2020).

Several studies have highlighted the potential bioactive constituents of neem leaves, reporting significant antioxidant capacity linked to their high content of flavonoids and phenolics (Sarkar et al., 2021). These compounds have been shown to modulate oxidative stress, a critical factor in the pathophysiology of chronic diseases (Zahid et al., 2025). However, variations in phytochemical content may arise due to geographical, seasonal, and extraction method differences, necessitating location-specific profiling.

Ethanol was selected as the extraction solvent in this study because it is a widely accepted solvent in phytochemical research, capable of extracting a broad range of both polar and moderately nonpolar compounds. In addition, ethanol is considered relatively safe for human use compared to methanol, making it suitable for studies with potential translational relevance to herbal formulations (Harborne, 1998; Tiwari et al., 2011; Sasidharan et al., 2011).

The present study aims to perform both qualitative and quantitative phytochemical analyses of neem leaves, thereby contributing to a better understanding of its bioactive profile and potential health benefits. This will provide baseline data to support its continued use in herbal medicine and potential integration into modern therapeutic formulations.

Materials and methods

Materials

Fresh leaves of *Azadirachta indica* (neem) were collected from Sagamu, Ogun State, Nigeria. The plant material was identified in the Department of Pharmacology, Olabisi Onabanjo University, Sagamu Campus, Ogun State, Nigeria, by a qualified taxonomist and deposited in the departmental herbarium for future reference.

The collected leaves were washed thoroughly with clean distilled water to remove dust and other surface contaminants. They were then air-dried at ambient laboratory temperature for 14 days to prevent degradation of heat-sensitive phytochemicals. The dried leaves were ground into a fine powder using an electric blender and stored in airtight amber-coloured glass containers at room temperature (25 ^{0}C) in a cool, dry place protected from direct sunlight until further analysis. This method of preparation was adopted to preserve the phytochemical integrity of the plant material for both qualitative and quantitative analyses.

Method

Extraction of Plant Material

The powdered *Azadirachta indica* leaves were subjected to extraction using the cold maceration method as described by Harborne (1998) with slight modifications. Briefly, 200 g of the powdered leaves were soaked in 1.5 L of 95% ethanol in an amber glass container and agitated intermittently for 72 hours at room temperature. The mixture was filtered first through muslin cloth to remove coarse particles, followed by filtration with Whatman filter

paper to obtain a clear filtrate free of fine particulate matter. The filtrate was concentrated under reduced pressure using a rotary evaporator at 40 °C to obtain the crude ethanolic extract. The concentrated extract was further dried in a water bath at 40 °C to remove residual solvent, yielding 49.2 g of crude extract.

The dried extract was weighed, and the percentage yield was calculated using the formula:

$$\text{Percentage Yield (\%)} = \frac{weight\ of\ extract}{weight\ of\ dried\ powder} x\ 100$$

$$\text{Percentage Yield (\%)} = \frac{49.2\ g}{200\ g} x\ 100$$

Percentage Yield (%) = 24.6% w/w

The extract was stored in an airtight amber bottle until further use for phytochemical analysis.

Qualitative Phytochemical Screening

The crude ethanolic extract of *Azadirachta indica* leaves was subjected to preliminary phytochemical screening to identify the presence of major secondary metabolites using standard methods described by Harborne, (1998) and Evans, (2009). All qualitative phytochemical screening was performed in triplicate for reproducibility. The tests carried out included:

Test for Alkaloids (Mayer's and Dragendorff's tests):

A portion of the extract (2 mL) was treated separately with a few drops of Mayer's reagent (potassium mercuric iodide) and Dragendorff's reagent (potassium bismuth iodide). The formation of a cream or reddish-brown precipitate, respectively, indicated the presence of alkaloids.

Test for Flavonoids (Shinoda test):

About 2 mL of extract was mixed with a few magnesium turnings (approximately 0.5 g) followed by the addition of few drops of concentrated hydrochloric acid (37%). The appearance of a pink or red coloration indicated the presence of flavonoids.

Test for Tannins (Ferric chloride test):

A small portion of the extract was boiled in 20 mL of distilled water, cooled, and filtered. A few drops of 0.1% ferric chloride solution were added to 2 mL of the filtrate. A blue-black or greenish coloration indicated the presence of hydrolysable or condensed tannins.

Test for Saponins (Froth test):

About 5 mL of extract was vigorously shaken with 10 mL of distilled water in a test tube and left to stand for 10 minutes. Persistent frothing indicated the presence of saponins.

Test for Phenols (Ferric chloride test):

Two mL of the extract was treated with a few drops of 5% ferric chloride solution. The formation of an intense blue, green, or black coloration indicated the presence of phenolic compounds.

Test for Terpenoids (Salkowski's test):

Five mL of extract was mixed with 2 mL of chloroform and 3 mL of concentrated sulfuric acid added carefully along the side of the test tube to form a layer. A reddish-brown coloration at the interface indicated the presence of terpenoids.

Test for Steroids

Neem leave crude extract was mixed with 2 ml of chloroform and concentrated sulfuric acid (H_2SO_4, 98%) was added sidewise. A red colour produced in the lower chloroform layer indicates the presence of steroids. Another test was performed by mixing crude extract with 2 ml of chloroform. Then 2 ml of each of concentrated H_2SO_4 (98%) and acetic acid was poured into the mixture. The development of a greenish coloration indicates the presence of steroids.

Test for Coumarins

Neem leave extract solution is concentrated under reduced pressure using rotary evaporator at 40 ^{0}C to yield a residue. The residue was dissolved in hot water and after cooling, the solution was divided equally into two test tubes. To one test tube 10%

(w/v) Ammonium Hydroxide was added. Other test tube was used as control. The development of fluorescence colour under UV light indicated the presence of coumarins.

Quantitative Phytochemical Analysis

Quantitative estimation of major phytochemicals in the ethanolic extract of *Azadirachta indica* leaves was carried out following standard procedures as described by Harborne, (1998); Evans, (2009), and Sofowora, (1993). All analyses were conducted in triplicate, and results were expressed as percentage (%) composition of extract.

Determination of Alkaloid Content

5 gram of sample dust was dissolved in 100 ml of 10% acetic acid. It was Well shaken and left for 4 hours. The solution was then filtered in Whatman No. 42 filter paper. Filtrate was evaporated to ¼th of its original volume using hot plate with magnetic stirrer. Concentrated Ammonium hydroxide (NH_40H) was added drop wise to precipitate the alkaloid content. Solution was filtered again and washed with 1% NH_4OH. Filter paper containing precipitate was dried in oven at 60 ^{0}C for 30 minutes and weighed after allowed to cool for few minutes.

$$\text{Alkaloid \%} = \frac{W2-W1}{W1}\ x\ 100$$

W_1= weight of empty filter paper

W_2=Weight of paper+ alkaloid precipitate

Determination of Flavonoid Content

The powdered sample i.e. 5 gram was placed into a conical flask with 100 ml of water and 2ml HCL solution was added. The solution was allowed to boil for 30 minutes and allowed to cool before filtered into Whattman filter paper. Aqueous layer was discarded and filtered with preweighted filter paper. Residue of filter paper was dried in an oven for 30 minutes at 60 ^{0}C. Weight of flavonoids was calculated by using following formula.

$$\text{\% Flavonoid} = \frac{W2-W1}{weight\ of\ sample}\ x\ 100$$

W_1= weight of empty filter paper

W_2= weight of paper + Flavonoid extract

Test for Terpenoids

Dried plant extract 10 gram (Wi) was taken and soaked in 90 ml of ethanol (Indumathi et al., 2014). The extract after filtration was mixed with 10 ml of petroleum ether and again filtrated using separating funnel. The extract was waited for its complete drying and measurement is taken (Wf). The yield (%) of total terpenoids contents was measured by the formula:

$$\text{Total terpenoids} = \frac{Wi-Wf}{Wi}\ X\ 100$$

W_i= dried plant extracts,

W_f= extracts after drying

Test for Saponins

The plant extract i.e. 25 ml was placed in a round bottom flask.100 ml of 50% alcohol was added and boiled for 30 minutes and filtered while hot through a filter paper. 2 gram of charcoal was added to the filtrate and it is boiled and filtered while hot. The filtrate was cooled and an equal volume of acetone was added to completely precipitate the saponins. The precipitated saponins were collected (Muhammad and Abubakar, 2016).

$$\text{\% of true saponins} = \frac{W2-W2}{W1}\ x\ 100$$

W_1=Weight of filter paper

W_2=Weight of residue

Data Analysis

The results obtained from the qualitative and quantitative phytochemical analyses were expressed as mean ± standard error of mean (SEM) of triplicate determinations. Data were analyzed using Statistical Package for the Social Sciences (SPSS) version 25.0 (IBM Corp., Armonk, NY, USA)

Results and discussion

Extraction yield of neem leaf

In Table 1 below, from 200 g of dried neem leaf powder, ethanol extraction yielded 49.2 g of crude extract, corresponding to 24.6% w/w. This is consistent with previous reports on neem, where ethanol extraction yields typically range between 20–30% depending on solvent polarity, duration, and drying method (Hashim et al., 2021; Hismath et al., 2011).

Table 1: Extraction yield of neem leaf

Weight of dried leaf powder (g)	Weight of crude extract (g)	% Yield w/w
200 g	49.2 g	24.6%

Qualitative phytochemical screening of neem leaf

As seen in Table 2 below, the qualitative phytochemical screening of *Azadirachta indica* leaves in this study revealed the presence of alkaloids, flavonoids, saponins, terpenoids, steroids, polyphenols, and tannins. These findings are in agreement with earlier reports indicating that neem leaves are rich in a wide range of secondary metabolites with diverse biological activities (Sarkar et al., 2021; Sidat et al., 2023). The presence of these phytochemicals supports the traditional use of neem in ethnomedicine for its antimicrobial, anti-inflammatory, antioxidant, and antidiabetic properties.

Table 2: Qualitative phytochemical screening of neem leaf

Qualitative Phytochemical test	Neem leaf
Alkaloid test	Present
Flavonoid test	Present
Saponin test	Present
Terpenoid test	Present
Steroid test	Present
Polyphenols test	Present
Tannin test	Present
Cumarin test	Absent

Quantitative phytochemical screening of neem leaf

In Table 3, quantitative analysis demonstrated that flavonoids (13.8 ± 0.17%) and terpenoids (13.13 ± 0.5%) were present in the highest concentrations, followed closely by alkaloids (10.67 ± 0.46%), while saponins (2.43 ± 0.32%) were relatively lower. These values are comparable to reports from Nigerian neem accessions, where flavonoid content ranged between 10–15% and terpenoids between 11–14% (Erhabor & Erhabor, 2024), but higher than those observed in Indian neem leaves extracted with aqueous solvents (flavonoids ~8%) (Sultana et al., 2009). Such variations may be attributed to differences in geographical location, soil mineral composition, seasonal harvest period, and choice of extraction solvent.

Neem leaves are known to contain flavonoids such as quercetin and kaempferol (Andersa et al., 2024; Sarkar et al., 2021; Biswas et al., 2002), which exhibit strong antioxidant activity. The terpenoid fraction includes azadirachtin, nimbin, and salannin, compounds widely recognized for antimicrobial and insecticidal activity (Islas et al., 2020; Cesa et al., 2019). Alkaloids like nimbolide have demonstrated anticancer and anti-inflammatory properties through apoptosis induction and NF-κB pathway modulation (Rajendran et al., 2024; Wang et al., 2016; Koul et al., 2016). The presence of tannins (e.g., gallic acid, catechin derivatives) and polyphenols (e.g., ferulic acid, chlorogenic acid) may further supports neem's antioxidant and antimicrobial potential.

Table 3: Quantitative phytochemical analysis of neem leaf

Quantitative Phytochemicals	Neem leaf (%)
Alkaloids	10.67±0.46
Flavonoids	13.8±0.17
Saponins	2.43±0.32
Terpenoids	13.13±0.5

A limitation of this study is that only ethanol was employed as the extraction solvent. While ethanol is effective in extracting a wide spectrum of phytochemicals and is safe for potential human applications, comparative extraction using aqueous

and other organic solvents may yield different or more diverse phytochemical profiles. Future studies should therefore incorporate multiple solvent systems to provide a more comprehensive phytochemical characterization.

Conclusion

The present study confirmed that neem (*Azadirachta indica*) leaves are a rich source of bioactive phytochemicals, with flavonoids, terpenoids, and alkaloids occurring in high concentrations, alongside saponins, tannins, polyphenols, and steroids. Specific compounds such as quercetin, kaempferol, azadirachtin, nimbin, and nimbolide are well documented for their diverse pharmacological effects, including antioxidant, antimicrobial, anti-inflammatory, and anticancer properties. While these findings provide a scientific basis for neem's traditional uses, it is important to note that the present study did not include bioassays. Therefore, claims regarding therapeutic applications remain hypothesis-generating rather than conclusive, underscoring the need for bioactivity-guided fractionation and in vivo validation in future research.

Authors contributions

Conceptualization, Oluwaseye Olayemi and Olusoji Oyesola; methodology, Oluwaseye Olayemi and Olusoji Oyesola; investigation, Oluwaseye Olayemi, Olusoji Oyesola, Olaniyi Soetan, Eunice Ojo-Adebayo and Elizabeth Anthony; data curation, Elizabeth Anthony; writing—original draft preparation, Oluwaseye Olayemi and Eunice Ojo-Adebayo; writing—review and editing, Olaniyi Soetan and Elizabeth Anthony; supervision, Olusoji Oyesola.; project administration, Oluwaseye Olayemi and Olusoji Oyesola. All authors have read and agreed to the published version of the manuscript."

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Conflict of interest

Authors declared no conflict of interest.

Declaration of generative AI and AI-assisted technologies in the writing process

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