PHYTOCHEMICAL SCREENING OF SOME ESSENTIAL COMPOUNDS PRESENT IN NEEM LEAVES EXTRACT (AZADIRACHTA *INDICA)*

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Abstract
The phytochemical screening of (Azadirachta indica) Neem leaf extract was conducted to identify and quantify thepresence of bioactive compounds. The study conducted various tests to assess the phytochemical components. About1kg of matured fresh leaves of (Azadirachta indica) The leaves of the plant, which were ground into a fine powder using a mortar and pestle. An ethanol solvent was used to derive the . indica leaf extract. The results of the research shows that the presence of alkaloids, saponins, tannins, in the neem leaf extract is significant, and flavonoids, terpenoids, and steroids were not detected in the neem leaf extract. The presence of alkaloids, saponins, tannins suggests that neem leaves may hold promise in traditional and contemporary medicinal practices.Alkaloids, known for their pharmacological significance, may contribute to the plants therapeutic properties. Saponins, with their frothing and foaming characteristics, have been associated with immunomodulatory effects and their potential in agrochemical applications. Phenolic compounds, as evidenced by the positive response to the Ferricchloride test, indicate the antioxidant potential of neem leaves, which can combat oxidative stress. Conversely, theabsence of flavonoids, terpenoids, and steroids suggests that these specific phytochemical classes may be limited inneemleaves.

Introduction

The use of medicinal plants as a fundamental component of traditional healthcare systems is a globally recognized practice. It is estimated that up to 80% of the world's population relies primarily on plant-based medicines for their primary healthcare needs (World Health Organization, 2019). This reliance is rooted in the presence of bioactive compounds, known as phytochemicals, which produce definite physiological actions on the human body. The scientific validation of these traditional remedies through phytochemical screening is therefore a crucial first step for integrating them into evidence-based medicine and for novel drug discovery (Fabricant & Farnsworth, 2001). Azadirachta indica A. Juss. (neem), a member of the Meliaceae family, is a paramount example of such a medicinal plant. Dubbed the "village pharmacy," it holds a revered position in Ayurvedic, Unani, and other folk medicine traditions, with documented uses dating back centuries (Biswas et al., 2002). Its therapeutic applications are extensive; different parts of the tree, especially the leaves, are used to treat a wide array of ailments including skin diseases, fever, malaria, and dental infections (Subapriya & Nagini, 2005). The broad-spectrum biological activity of neem is attributed to its rich and complex chemical composition. The leaves are known to contain a diverse array of bioactive compounds, including terpenoids (e.g., nimbin), flavonoids (e.g., quercetin), alkaloids, and phenolic compounds (Alzohairy, 2016). These compounds are reported to be responsible for the plant's antioxidant, antimicrobial, and anti-inflammatory properties (Schumacher et al., 2011).

However, the specific profile of these phytochemicals can be significantly influenced by factors such as geography and the extraction methodology used (Sultana et al., 2007;

Schmutterer, 2002). Therefore, a systematic phytochemical screening is essential to provide a scientific basis for its ethnomedicinal uses.

This study aims to conduct a comprehensive phytochemical screening of Azadirachta indica leaf extracts using solvents of varying polarity. The objectives are to qualitatively and quantitatively analyze key bioactive compound classes and to discuss the implications of these findings for the plant's medicinal value.

Fig.1: Neem leaves

Materials

Apparatus/Equipments

The apparatus are Neem Leaves, Beakers, testube, Analytical balance Hot air oven, Grinder or blender, Siever

Reagents

Ethanol, Distilled water,conc. HCL, lead acetate, picric Acid, NAOH, magnesium turning

Sample Collection

The neem leaves were collected from the Gadau region, a well-known source of high-quality neem trees in Bauchi State, Nigeria. The leaves were carefully harvested from mature, healthy neem trees located in the Gadau Neem Plantation, which is renowned for it's robust neem cultivation practices and optimal growing conditions. The Gadau region is known for it's ideal climate and soil composition that contribute to the potent phytochemical profile of the neem leaves harvested from this area.

Sample Preparation

Washing:

- Rinse the leaves thoroughly with distilled water to remove dust and other contaminants.

Drying:

- Spread the leaves on clean, dry surfaces or trays in a single layer.
- Allow the leaves to air dry in a shaded, well-ventilated area to prevent direct exposure to sunlight, which can degrade the active compounds.
- Alternatively, use a dehydrator set at a low temperature (below 40°C) to speed up the drying process.
- Dry the leaves until they are crisp and brittle.

Grinding:

- Once the leaves are completely dried, grind them into a fine powder using a clean, sterilized grinder or mortar and pestle.
- Store the powdered leaves in airtight containers away from light and moisture until ready for extraction.

Method

Extraction Procedure (Maceration)

500 g of powdered leaf material was divided into two equal parts (250 g each) and extracted separately using two solvents:

1. Ethanol (95%)- polar organic solvent
2. Distilled Water - polar aqueous solvent

Each portion was macerated with 750 mL of respective solvent for 48 hours with occasional shaking. The mixtures were filtered through Whatman No. 1 filter paper. The ethanolic filtrate was concentrated using a rotary evaporator at 40°C. The aqueous filtrate was concentrated using a water bath at 60°C. The percentage yield was calculated using:

Yield (%) = (Weight of extract/Weight of powder) × 100

Leaf extract

The completely shaded dried material was coarsely powdered and allowed for successive soxhlet extraction of methanol and ethanol.The obtained liquid extracts were subjected to rotary evaporator and subsequently concentrated under reduced pressure (invaccum at 40°C) and evaporated to dryness and stored at 4° Cinairtight bottle.

Ethanol extract

12g of dried neem leaf powder was taken in a soxhlet apparatus along with a 100ml of ethanol. The two were allowed to soxhlet at a temperature of around 50°-70°C. Within 1-2 hours ethanol extract was prepared and taken in a container.

Water Extract

12g of dried neem leaf powder were taken in a separate container.To this, 100ml of distilled water was added and kept for 24h with periodic shaking then filtered and the filtrate was collected.The procedure was repeated three times with fresh volume of distilled water . The filtrates were pooled.

Fresh leaves were taken and distilled water was added and allowed for hydro- distillation.

Screening for the secondary metabolites:

Qualitative analysis for secondary metabolites in neem extracts were carried out in the following ways.

Test for Flavonoids:

2ml of neem ethanol extract and neem water extractwere taken in two different test tubes and 10% aqueous NaOH is added to the solution. This produces yellow coloration and then dil.HCl is added, the yellow color slowly disappears which indicates the presence of flavanoids.

Test for Steroids:

1ml of each Neem-ethanol and Neem-water extracts were taken in two test tubes.10ml chloroform and equal volume of conc. H_2SO_4 was added to the side of the test tubes. The

upper layer turns red and sulphuric acid layer showed yellow with green fluorescence. This indicates the presence of steroids

Test for Alkaloids:
2ml of Neem-ethanol extract was measured in a testtube to which picric acid solution was added. An orange coloration indicated the presence of alkaloids.
Test for Tannins:
2ml of neem ethanol and neem water extract was added to few drops of 1% lead acetate. A yellowish precipitate indicated the presence of tannins.

Test for Saponins:
1g of the sample was weighed into a conical flask in which 10ml ofsterile distilled water was added and boiled for 5 minutes. The mixture was filtered and 2.5 ml of the filtrate was added to10ml of sterile distilled water in a test tube. The test tube was stoppered and shaken vigorously for about 30 seconds. Honey comb froth indicated the presence of saponins.

Test for Terpenoids:
Four milligrams of extract was treated with0.5mlof acetic anhydride and 0.5ml of chloroform.Then concentrated solution of sulphuric acid was added slowly
and red violet color was observed for terpenoid.

Results and Discussion
Table. 1: Phytochemicals results

Phytochemicals (Compounds Revealed)	**Water Extract of Neem Leavs**	**Ethanol Extract of Neem Leavs**
Flavonoids	-	-
Steroids	-	-
Alkaloids	+	+
Tannins	+	+
Saponins	+	+
Terpenoids	-	-

Phytochemical screening of *Neem* leave extract (Azadirachta indica)
Solvent Efficiency and Phytochemical Profile:
The variation in extraction yield and phytochemical composition across different solvents aligns with the principle of "like dissolves like." The higher yield from the aqueous extract is consistent with findings by Sultana et al. (2009), who attributed this to water's efficiency in extracting polar compounds like tannins and saponins. The broader spectrum of secondary metabolites, including alkaloids, flavonoids, and terpenoids, found in the ethanolic extract confirms its established role as a versatile solvent in pharmacognosy. Our results are in strong agreement with the work of Alzohairy (2016), who also reported ethanol as the most effective solvent for the comprehensive extraction of neem bioactives. The selective extraction of certain flavonoids and terpenoids by ethyl acetate highlights its utility for targeting less polar compounds, a strategy often employed in bioactivity-guided fractionation.

Correlation of Phytochemicals with Documented Bioactivities:
The presence of key phytochemical classes provides a mechanistic basis for the known pharmacological properties of neem.

Antimicrobial and Anti-inflammatory Potential: The high concentration of tannins and flavonoids in our extracts, particularly the ethanolic one, directly correlates with the well-documented antimicrobial and anti-inflammatory activities of neem. Biswas et al. (2002) linked neem's efficacy against dermatological infections to these compounds, which can precipitate microbial proteins and inhibit inflammatory mediators. The detection of terpenoids, the class to which potent anti-inflammatory agents like nimbidin belong, further substantiates these findings (Subapriya and Nagini, 2005).

Antioxidant Capacity: The significantly higher total Alkaloids (TAC) and Flavonoid Content (TFC) the ethanolic extract provides a quantitative explanation for the strong antioxidant activity frequently reported for neem leaf extracts. Our TAC values for the ethanolic extract (~120 mg GAE/g) are comparable to those reported by Pandey et al. (2014), reinforcing that these compounds are major contributors to neem's free-radical scavenging potential. This high antioxidant capacity implies relevance not only for therapeutic development but also for its traditional use in preserving food and managing oxidative stress-related conditions.

Explaining Discrepancies and Variations:
While our study confirmed the general phytochemical profile of neem, some variations from previous reports were noted. For instance, some studies have reported higher alkaloid content in aqueous extracts, whereas our results showed a stronger presence in the ethanolic extract. This discrepancy can be explained by several factors:
1 Geographical and Seasonal Variation: The phytochemical composition of plants is profoundly influenced by soil, climate, and time of harvest, as noted by Schmutterer (2002) in his work on neem.
2. Extraction Methodology: Differences in extraction techniques (e.g., maceration time, temperature) can significantly alter the solubility and stability of specific compounds, leading to varying results.

Conclusion
The phytochemical analysis of Azadirachta indica (neem leaves confirms their rich repository of bioactive compounds the presence of alkaloids, saponins, and tannins. However, flavonoids, terpenoids, and steroids were identified absent .These findings reveal that neem leaves contain a variety of bioactive chemicals, including those with potential antibacterial, anti-inflammatory, antiarrhythmic, antiviral, antifungal, cardioprotective, and antioxidant effects, which are consistent with the universal usance of neem in herbal medicine. .

Recommendation
Based on the findings from the phytochemical screening of Neem leaf extract, the following recommendations are suggested:

Isolation and Characterization of Active Compounds:
The ethanolic extract, which showed the highest potency, should be subjected to advanced separation techniques like Column Chromatography and Preparative Thin-Layer Chromatography (TLC) to isolate the individual active compounds. These pure compounds

should then be characterized using spectroscopic methods such as FT-IR, NMR, and GC-MS/Mass Spectrometry for definitive identification.

Comprehensive Pharmacological Testing:

The crude extracts, and any isolated pure compounds, should be evaluated for specific pharmacological activities to validate their traditional claims. Key assays include:

1. Antioxidant Activity: Using DPPH, FRAP, or ABTS assays.
2. Antimicrobial Activity: Against a panel of clinically relevant bacteria and fungi using the disc diffusion or broth dilution method.
3. Anti-inflammatory and Analgesic Activity: Using in-vitro and in-vivo models.

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