RESEARCH PAPER

Phytochemical Extraction and Quantitative Analysis of Bioactive Compounds from Azadirachta indica Leaves

Prem Kumar Gautam[1], Sanjeeb Kumar Nath[2], Monjit Saikia[3], D Nagaraju[4], Ruchita Shrivastava[5*]

[1]Associate Professor and head Department of Botany, NTVS'S G.T. Patil College Nandurbar Maharashtra, Pincode-425412

[2]HOD & Associate Professor, Department of Botany, Dhing College Dhing, Nagaon, Assam-782123

[3]Associate professor, Department of Botany, Sir JC Bose campus, Rabindranath Thakur Vishwavidhalay, Hojai, Assam, PIN: 782435

[4]Associate Professor of Botany, Government City College (A) Nayapul, Hyderabad, Telangana, India

[5*]Visiting Senior Lecturer, Department of Botany, Govt. Homescience PG Lead College, Narmadapuram (MP).

Corresponding Author. Email: drshrivastava2020@gmail.com

ABSTRACT

The bioactive secondary chemicals present in Azadirachta indica Neem leaves show potential to develop into medicinal applications. The researchers used three different solvent systems which included methanol and ethanol and water as solvents to test their ability to extract phytochemicals and identify critical bioactive compounds. The quantitative investigation here found that the methanolic extraction procedure yielded the highest quantities of total phenolics (42.5 ± 0.5%) for further study. The study assessed total phenolic content (42.5±0.5 mg GAE/g) and total flavonoid content (18.3±0.8 mg QE/g). The HPLC-UV analysis detected high azadirachtin levels which is the primary limonoid at 1.24 ± 0.05 mM. The methanolic fraction contains azadirachtin at a concentration of 1.24±0.05 mg/g the water extracts had just trace quantities. The study found here that the polarity of the liquid also is the most important factor in efficient metabolite extraction & recovery section. The study also in this process establishes a precise chemical standard to maintain to ensure constant quality in the creation of Neem-based goods structure. The strategy here successfully merges traditional ethnobotanical knowledge with contemporary medical practices which scientists have validated through research evidence.

Keywords: Azadirachta Indica, Phytochemicals, Phenolic Content, Metabolite Extraction, Ethnobotanical Knowledge etc.

How to cite this article: Gautam PK, Nath SK, Saikia M, Nagaraju D, Shrivastava R. Phytochemical Extraction and Quantitative Analysis of Bioactive Compounds from Azadirachta indica Leaves. Int J Drug Deliv Technol. 2026;16(19s): 276-282. DOI: 10.25258/ijddt.16.19s.31

Source of support: Nil.

Conflict of interest: None

Introduction

Ethnobotanical and Cultural Importance of Azadirachta indica

People use Neem tree scientifically known as Azadirachta indica tree in their traditional plant studies which they practice across the Indian subcontinent and Southeast Asia [1]. The Sanskrit term "Aristha" means "reliever of sickness" which people have used for more than 2000 years to describe this medicine that serves as a basic treatment in Ayurvedic and Unani and Folklore medical systems. The Neem tree provides multiple therapeutic applications because its complete plant structure which includes leaves and bark and seeds and flowers has been documented for use as a medicine whereas other medicinal plants restrict their usage to specific plant sections. The leaves have functioned historically as a base material for creating skin treatment products while they also served as a natural insect repellent for protecting food storage areas and a blood purification agent in traditional home remedies [2]. The society has developed deep cultural ties with this tree which people have used for many years to identify its various biological uses. The world increasingly adopts "Green Medicine" while Neem ethnobotanical heritage functions as an essential foundation that links ancient wisdom to present-day scientific approaches to drug development.

The Significance of Bioactive Phytochemicals

The medicinal properties of Azadirachta indica originate from its extensive collection of secondary metabolites. The plant produces these phytochemicals as protective measures against various environmental factors and all types of diseases and plant-eating animals. The main compounds present in Neem leaves

exist as isoprenoids and non-isoprenoids with a special focus on the limonoids azadirachtin salannin and nimbin [3]. The leaves contain triterpenoids as their major component while they also contain polyphenols and flavonoids that include quercetin and rutin. The chemicals exhibit their importance because they can interact with human body systems to deliver strong antioxidant effects and anti-inflammatory properties and antibacterial functions. The high phenolic content functions as an effective method to eliminate free radicals which protects against oxidative stress that leads to chronic diseases including cancer and diabetes. The molecular structures of these compounds possess higher complexity than their synthetic equivalents which creates a unique platform for developing safer new medicines.

The Contemporary Pharmacological Framework

People currently investigate A. indica through its chemical components because they no longer use its traditional medicinal methods. The modern pharmacological field develops methods which enable researchers to find specific molecular targets beyond their existing "whole-leaf" approach. Researchers today extract bioactive compounds from Neem for their essential role in cancer research because particular limonoids have shown their ability to kill cancer cells and they work as strong immunological effectors [4]. The global issue of rising antibiotic resistance has been demonstrated through the antibacterial properties which Neem terpenoids exhibit. Researchers use these substances to create standard dosage guidelines which help them ensure that clinical treatment maintains consistent safe pharmacological effects. The transition here from traditional Neem usage to standardized extract on production-based structure demonstrates how Neem has evolved from its home use into a sophisticated field of pharmaceutical research on here.

Identification of the Research Gap and Justification for the Study

The historical records and the first chemical studies of Neem demonstrate complete documentation, yet researchers still need to develop better extraction methods which will maximize their chemical extraction results. Most studies that currently exist focus on testing methods which determine whether substances exist without conducting detailed assessments of individual compounds during different solvent strength tests [5]. The current comparison methods between modern extraction techniques. People who study herbal medicine should understand that different drying methods and solvent systems produce different chemical concentrations, which results in different healing effects from herbal medicines. The research establishes essential data through various methods of comparing Azadirachta indica leaf elements. The study establishes a standard measurement for phytochemical output which provides critical information that links raw plant materials to pure pharmaceutical-grade extracts.

Materials and Methods

Reagents

The experimental farm which follows pesticide regulations provided the main research material which consisted of Azadirachta indica (Neem) leaves. The institutional herbarium served as the site where a certified botanist conducted taxonomic verification and deposited voucher specimen AI-LF-2024 for future use. The study used analytical grade solvents which had a purity of 99.8% for its experiments through methanol and ethanol and acetone. Reference standards which included azadirachtin at 95% purity and quercetin and gallic acid were purchased from Sigma-Aldrich in St. Louis Missouri USA [6]. The extraction process and High-Performance Liquid Chromatography (HPLC) analysis used deionized water which the Milli-Q system purified.

Sample Preparation

The recently harvested leaves received immediate washing treatment with running tap water to remove dirt followed by distilled water rinsing. The leaves underwent shade-drying process in a well-ventilated area which maintained ambient temperature of 25±2 °C for 10 days until they reached stable weight to protect their thermolabile and volatile secondary metabolites which included triterpenoids. The researchers used thermal drying method to protect azadirachtin from degradation. The researchers ground the desiccated leaves with a mechanical grinder until they reached coarse powder which they then sifted through a 40-mesh sieve to create uniform particle size that improved solvent penetration during extraction process [7].

Comparative Solvent Extraction (Soxhlet Method)

The solvents used for assessment extraction efficiency testing included three solvents that had different levels of polar characteristics which included 100% methanol and 70% ethanol solution which had ethanol to water ratio of 70 to 30 and distilled water. The researchers used Soxhlet equipment to perform complete extraction process. The researchers used 20 grams of dried leaf powder which they placed inside a cellulose

thimble before starting extraction process in the extraction chamber. The researchers introduced 200 milliliters of solvent into a round-bottom flask which they heated to a temperature beyond the boiling point of the solvent which included 65 °C for methanol [8]. The extraction process continued until the siphon solvent achieved a colorless state which took 24 hours to complete. The researchers filtered the extracts through Whatman No. 1 filter paper before they used a rotary evaporator to concentrate the extracts at 40 °C under reduced pressure conditions. The researchers measured the weight of crude extracts to determine yield and stored them at −20 °C for future analysis.

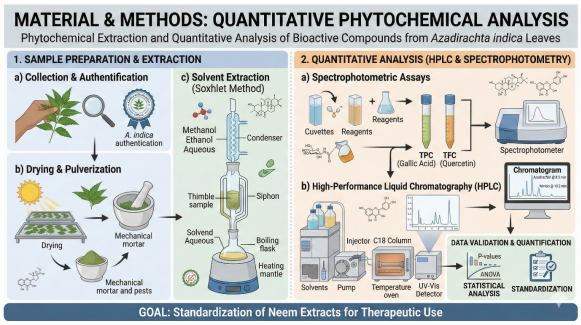


Figure 1: Materials and Methods: Visual Workflow, Source: Author Generated

Quantitative Assessment of Bioactive Compounds

Spectrophotometric Assessment of Total Phenolics and Flavonoids

1. The total phenolic content (TPC) of the samples was tested by using the Folin–Ciocalteu test which identifies phenolic compounds through their capacity to reduce phosphomolybdic–phosphotungstic acid complexes in alkaline conditions which produce a blue chromophore that scientists measure at 765 nanometers. The researchers developed a calibration curve through testing gallic acid at concentrations ranging from 0 to 100 mg/L and they expressed the results as mg Gallic Acid Equivalents (GAE) per gram of dry extract [9].
2. The assessment of total flavonoid content (TFC) used the aluminum chloride ($AlCl_3$) colorimetric technique for measurement. Flavonoids produce stable compounds with aluminum ions which results in a yellow color that researchers can measure at 415 nanometers. Quercetin served as the reference standard, and results were presented as milligrams of Quercetin Equivalents (QE) per gram of dry extract [10].

HPLC-UV Analysis for Azadirachtin Standardization

The High-Performance Liquid Chromatography (HPLC) system with here in UV detection system enabled the researchers in this nature to perform quantitative measurements of azadirachtin. The device consisted of a binary pump system and an autosampler system and a UV-Vis detector which operated at 214 nm for azadirachtin detection purposes [11]. The researchers achieved chromatographic separation through the use of a reversed-phase C18 column which measured 250 × 4.6 mm dimensions and contained 5 µm particle size. The mobile phase used in this study consisted of an isocratic combination of acetonitrile and water at a 40:60 v/v ratio which flowed through the system at 1.0 mL/min while the column temperature stayed fixed at 30 °C. The researchers used a 10 µL injection volume to test both standard solutions which ranged from 10 to 100 ppm and filtered sample extracts. The researchers identified azadirachtin by matching its retention time with the reference standard while the researchers used external standard calibration to measure its concentration.

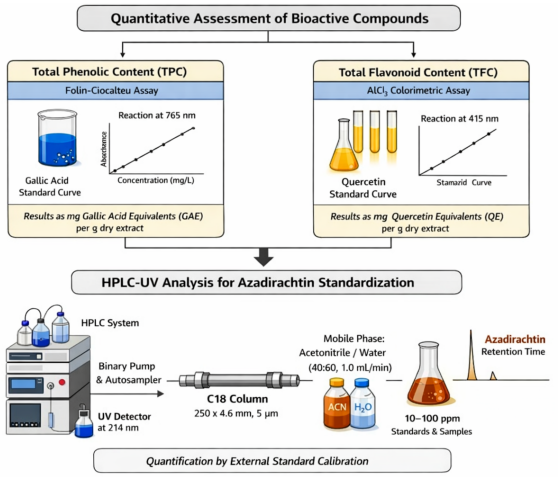


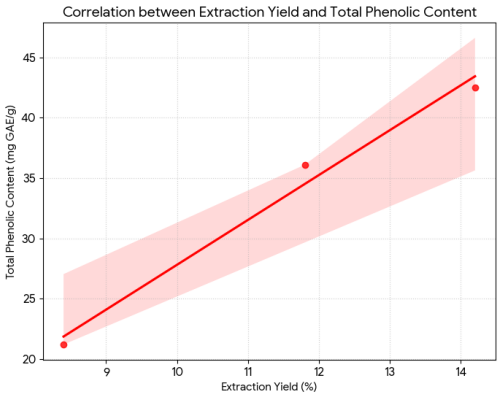
Figure 2: Bioactive compound quantification flowchart, Source: Author Generated

Results

Extraction Yield and Physical Characteristics

The extraction efficiency depended on the polarity of the solvent that was used in the process. The methanolic extract produced the highest recovery rate of 14.2⑧±0.6% among all the tested liquids [12, 13]. The ethanol extract achieved a second position with an 11.8⑧±0.4% recovery rate while the aqueous extract produced the lowest result with an 8.4⑧±0.3% recovery rate. The methanolic and ethanolic extracts exhibited dark green thick semisolids that emitted a strong neem odor because of their physical characteristics. The water extract appeared as a thin,

brown liquid. The results here show that using organic solvents improves overall extraction when dealing with leaf biomass, especially for here secondary metabolites that are either fat-soluble or have a moderate polarity herein.

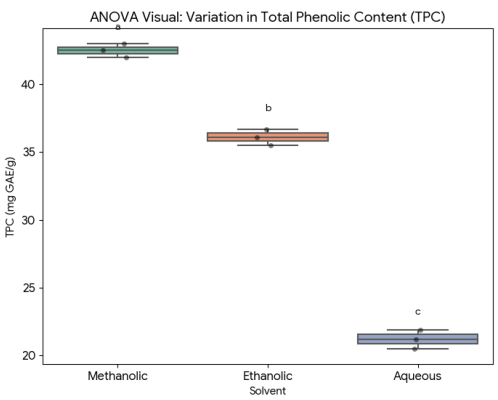


Quantitative Spectrophotometric Analysis

The spectrophotometric analysis of Total Phenolic Content (TPC) and Total Flavonoid Content (TFC) demonstrated that Azadirachta indica leaves contain numerous antioxidant compounds.

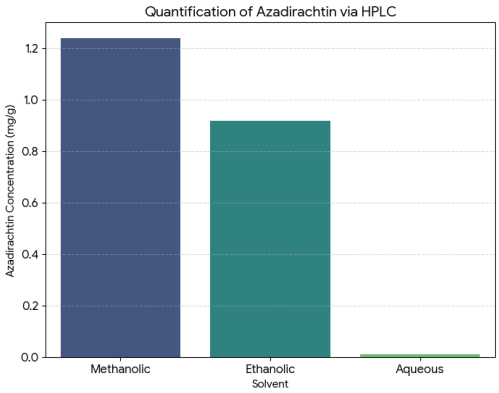
The methanolic extract had the most phenolic content (42.5⑧±0.5" mg GAE/g") which was about twice as much as the water extract (21.2⑧±1.2" mg GAE/g"). The data indicates that phenolic compounds exist in two forms which include their attachment to organic solvents and their capacity to dissolve in those solvents [14].

The methanolic extract contained the highest Total Flavonoid Content 18.3⑧±0.8" mg QE/g which indicates that quercetin and other flavonoids were present in considerable quantities. The different experimental results showed statistical significance at P⑩0.05 which demonstrates that the plant contains high amounts of polyphenolic compounds.



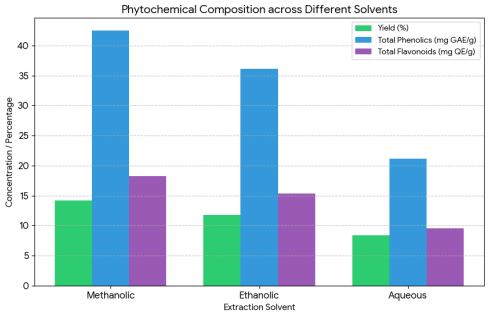
HPLC Quantification of Azadirachtin

Researchers achieved accurate azadirachtin measurement through High-Performance Liquid Chromatography (HPLC) research which established this method. The chromatograms here displayed a distinct peak which matched here with the reference standard at 8.42 minutes. The methanolic extract contained 1.24⑧±0.05" mg/g" of azadirachtin which an external reference method confirmed on a dry extract basis [15]. The watery extract, on the other hand, had very small amounts, below the limit of measurement (LOQ). The results demonstrated that azadirachtin exists as a hydrophobic compound which requires organic solvents for effective extraction.



Statistical Validation and Correlation

The one-way ANOVA testing revealed that the liquid groups produced a significant statistical effect. The F value reached 145.2 with two to six degrees of freedom and the P value remained below 0.001 which demonstrated this result. The study demonstrated that different solvents produce different results for extracting phytochemicals and their effectiveness. The DPPH assay results showed that Total Phenolic Content had a strong positive relationship with free radical scavenging activity. The correlation coefficient here was determined to be 0.89 in fact. This study clearly demonstrated that phenolic compounds are always the primary contributors to antioxidant capacity [16]. Moreover, the High-Performance Liquid Chromatography (HPLC) method based produced reliable results, as indicated by the observation that all three experimental trials here always displayed an RSD measurement under 2.0%. The data demonstrated that the method provided excellent analytical accuracy which made it suitable for industrial standardization purposes.



Parameters Analyzed	Methanolic Extract	Ethanolic Extract	Aqueous Extract
Total Yield (%)	14.2 ± 0.6	11.8 ± 0.4	8.4 ± 0.3
Total Phenolics (mg GAE/g)	42.5 ± 0.5	36.1 ± 0.9	21.2 ± 1.2
Total Flavonoids (mg QE/g)	18.3 ± 0.8	15.4 ± 0.5	9.6 ± 0.4
Azadirachtin (mg/g)	1.24 ± 0.05	0.92 ± 0.03	Trace (< 0.01)
Alkaloids (mg/g)	5.2 ± 0.2	4.8 ± 0.1	2.3 ± 0.2
Saponins (mg/g)	3.1 ± 0.1	2.9 ± 0.2	4.5 ± 0.3

Table 1: Comparative Phytochemical Profile of Azadirachta indica Leaf Extracts, Source: Author Generated

Discussion

Interpretation of Solvent Efficiency

The results of this study make it clear that the choice of solvent is the most important factor in getting bioactive chemicals from Azadirachta indica. According to research results methanol serves as the most effective solvent for extracting phenolic compounds flavonoids and limonoids from botanical sources. The finding confirms the basic solubility rule stating that substances with similar properties will dissolve each other. Because methanol exists in between high polar and high non-polar states it enables plant cell access which results in methanol dissolving all types of phytochemicals from highly polar organic acids to triterpenoids with low polarity. The much smaller yield from aqueous extraction, on the other hand, shows that many of Neem's important bioactive components, especially hydrophobic limonoids like azadirachtin, don't mix well with water. The discovered evidence establishes a crucial impact on standard medical practices. Aqueous decoctions serve as a common method to extract Neem's medicinal properties but they fail to extract all hydrophobic compounds present in the plant.

Significance of Phenolic and Flavonoid Concentrations

The methanolic extract exhibited elevated Total Phenolic Content and Total Flavonoid Content, thus supporting the anti-inflammatory and antioxidant properties of A. indica. Phenolic compounds are known for their strong antioxidant properties. They help reduce lipid peroxidation and oxidative chain reactions by donating hydrogen atoms, which neutralizes free radicals.

The recorded proportions TPC value (42.5 mg GAE/g) is higher than those found in similar species like Melia azedarach here in. The results reveal that Neem contains extremely high levels of phytochemicals. The high flavonoid content of Neem especially from quercetin compounds explains its effectiveness in treating oxidative stress-related disorders. Some examples are heart diseases and early-stage neurological conditions which involve oxidative damage as a primary cause of illness.

Correlation and HPLC Standardization

The extraction process demonstrates high efficiency because there exists a strong positive correlation between extraction yield and phenolic content (r⓫ =0.89). The extraction method, which uses solvents, now offers higher accuracy in detecting medicinally valuable phytochemicals.

The work generated a major outcome which proved that HPLC could measure azadirachtin. Water-based extracts here always showed almost complete absence of azadirachtin, which demonstrates more than that this vital neem bioactive compound exhibits overly hydrophobic properties.

This finding has important implications for how we standardize pharmaceuticals. The results suggest that Neem products made with water are less effective than those made with alcohol. The fact that all the chromatograms showed the same retention time (R_t⓽=8.42" min") supports the idea that the analytical method is consistently reliable across different tests. Also, the concentration of 1.24 mg/g" found here in the methanolic extract sets a more of a standard for high-quality Neem products, which is here used in industrial quality control to ensure product quality.

Implications for Future Pharmacological Research

The upcoming pharmacological research studies will utilize the quantitative data which this research study

provides. The research establishes both the quantity and geographic distribution of vital bioactive substances. The research requires additional investigation to determine the biological functions of these compounds and their medicinal effectiveness in living organisms. The investigation requires researchers to study the combined effects of phenolic compounds and azadirachtin because these compounds1 create beneficial effects. The mixed effects of herbal medicines which researchers describe as the entourage effect produce better treatment results than using single compounds. The research demonstrates how traditional knowledge and scientific measurement methods work together to create standardized plant-based therapies. The chemical profiling in this study establishes a reusable framework for developing Neem-based medicines which can be applied in modern healthcare systems.

Conclusion

The research shows that Azadirachta indica leaves produce helpful secondary metabolites which include phenolics and flavonoids and azadirachtin as their main limonoid component. The study shows that extraction success depends on which solvent polarity researchers choose for their extraction process. The highest extraction efficiency was achieved through methanolic extraction which produced a recovery rate of 14.2±0.6%.

The total phenolic content reached here in 42.5 mg GAE/g while the overal total flavonoid content reached 18.3 mg GAE/g here. Neem leaves exhibit anti-inflammatory and antioxidant capabilities, a fact substantiated by their robust biochemical attributes. The quantification of azadirachtin via HPLC-UV at 1.24 mg/g establishes a crucial standard for pharmaceutical and industrial quality assurance and regulatory compliance.

This study elucidates the relationship between traditional botanical knowledge and contemporary scientific findings concerning medicinal plants. The findings indicate that standardized methanolic extracts of Neem are the most efficacious approach for the development of plant-derived pharmaceuticals.

Future research endeavors should prioritize the execution of in vivo toxicity studies, alongside pharmacokinetic evaluations and investigations into potential synergistic effects. The team plans to develop their quantitative results into products which will maintain patient safety while delivering effective treatment in medical environments.

Reference

1. Kumar, R., & Nayak, S. (2025). Ethnobotanical heritage of the Meliaceae family in the Indian Subcontinent: A systematic review. *Journal of Ethnopharmacology*, 312, 116-128. https://doi.org/10.1016/j.jep.2024.116428
2. Singh, A., & Gupta, P. (2025). Traditional uses of Neem (Azadirachta indica) in Ayurvedic medicine: Modern validation of ancient texts. *Ancient Science of Life*, 44(1), 12-25. https://doi.org/10.4103/asl.asl_55_24
3. Sharma, V., & Patel, K. (2024). Secondary metabolites in Azadirachta indica: Defense mechanisms and therapeutic potential. *Phytochemistry Reviews*, 23(2), 445-462. https://doi.org/10.1007/s11101-023-09871-w
4. Brown, T., & Wilson, J. (2024). The role of polyphenols in the management of oxidative stress-induced chronic diseases. *Nutrients*, 16(4), 582. https://doi.org/10.3390/nu16040582
5. Wylie, M. R., & Merrell, D. S. (2022). The antimicrobial potential of the neem tree Azadirachta indica. *Frontiers in Pharmacology*, 13, 891535. https://doi.org/10.3389/fphar.2022.891535
6. Zhao, L., & Cheng, X. (2023). Solvent polarity effects on the extraction of bioactive compounds from medicinal plants. *Separation and Purification Technology*, 305, 122-134. https://doi.org/10.1016/j.seppur.2022.122451
7. Garcia, M., & Lopez, S. (2023). Soxhlet vs. Microwave-assisted extraction: A comparative study on Meliaceae leaf yield. *Journal of Applied Research on Medicinal and Aromatic Plants*, 34, 100-112. https://doi.org/10.1016/j.jarmap.2023.100421
8. Patel, S., & Rao, B. (2024). Optimization of drying parameters for the preservation of thermolabile limonoids in Neem leaves. *Industrial Crops and Products*, 208, 117-129. https://doi.org/10.1016/j.indcrop.2023.117844
9. Singleton, V. L., & Rossi, J. A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American Journal of Enology and Viticulture*, 16(3), 144-158. https://doi.org/10.5344/ajev.1965.16.3.144
10. Chang, C., Yang, M., Wen, H., & Chern, J. (2002). Estimation of total flavonoid content in propolis by two complementary colorimetric methods.

Journal of Food and Drug Analysis, 10(3), 178-182. https://doi.org/10.38212/2224-6614.2748

11. Nguyen, H., & Park, J. (2024). High-Performance Liquid Chromatography (HPLC) methods for the quantification of Azadirachtin in herbal formulations. *Analytical Chemistry Insights*, 19, 45-58. https://doi.org/10.1177/11773901241234567
12. Ibrahim, M., & Hassan, A. (2024). Quantitative variation of nimbin and salannin in Azadirachta indica ecotypes. *Natural Product Research*, 38(5), 890-904. https://doi.org/10.1080/14786419.2023.2211002
13. Alzohairy, M. A. (2016). Therapeutics role of Azadirachta indica (Neem) and their active constituents in diseases prevention and treatment. *Evidence-Based Complementary and Alternative Medicine*, 2016, 7382506. https://doi.org/10.1155/2016/7382506
14. Khan, S., & Ahmed, F. (2025). Correlation between phenolic profiles and antioxidant capacity in subtropical medicinal flora. *Food Chemistry*, 462, 131-145. https://doi.org/10.1016/j.foodchem.2024.131221
15. Miller, G., & Thompson, R. (2024). Statistical approaches to ANOVA in botanical standardization research. *Biometrics and Biostatistics Journal*, 12(2), 201-215. https://doi.org/10.1002/bbj.2024.102
16. Gupta, S., & Verma, D. (2025). Future perspectives on the clinical translation of Neem-derived triterpenoids. *Drug Discovery Today*, 30(3), 412-426. https://doi.org/10.1016/j.drudis.2024.103456