



CHEMICAL FINGERPRINT OF *AZADIRACHTA INDICA*: UNRAVELING THE BIOACTIVE PROFILE AND THERAPEUTIC POTENTIAL

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Abstract *Azadirachta indica* (neem) has been used for centuries in conventional medicines due to its bourgeois phytochemical profile and wide therapeutic potential. Regardless of its widespread use, detailed chemical fingerprints on bioactive compounds and residues with curative value are underexplored. The current study aims to identify and examine the bioactive compound in the ethanolic leaf infusion of *A. indica*. Indications to unravel its curative properties and support its potential to discover new state of the art drugs. An ethanolic leaf admixture was used for gas chromatography-tandem mass spectroscopy (GC-MS/MS) study. The sample was prepared by dissolving the solution in ethanol, 1 mg/mL. The oven temperature was programmed from the initial to the final temperature, together with the determination of the parameters. Helium as a carrier gas, electron-influenced ionization, and a scanning range of the scan variety are central working parameters. Ten major phytochemicals, including azadirachtin, Nimbin, beta-sitosterol, quercetin, and luteolin, were evaluated by GC-MS/MS. Azadirachtin has promising anticancer activities, while quercetin and luteolin have potent antioxidant and anti-inflammatory activities. Beta-sitosterol and 3-terpineol were associated with cardiovascular protection; Each compound has unique bioactivity which contributes to the curative versatility of *A. indica*. The examination provides a complete chemical fingerprint of *A. indica*, highlighting its bioactive compounds and their curative properties. These results confirmed the use of neem and reinforce its importance in contemporary pharmaceuticals. The use of these compounds in targeted therapy and drug evolution may be improved by further discovery at the molecular level.

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Introduction

Azadirachta indica (Neem) is a multifunctional plant native to the Indian Subcontinent, widely used in traditional medicines for its wide range of pharmacological activities. The fronds of neem are particularly notable for their potent bioactive compounds that exhibits a broad spectrum of life-related outcomes identical to antimicrobial, anti-inflammatory, antidiabetic, and anticancer properties (Kotadiya *et al.*, 2020). Current analytical techniques such as Gas Chromatography-Mass Spectrometry/Mass Spectrometry (GC-MS/MS) provide a powerful tool for recognizing and quantifying the chemical components present in plant infusion to discover one's curative potential (Mankotia *et al.*, 2020). GC-MS/MS is a powerful

instrument for profiling complex mixtures, allowing the designation of bioactive substances in plant infusions with high sensitivity and specificity. *Azadirachta indica*, also known as neem, is a multifunctional medicinal plant widely used in traditional medicines throughout the Asia Pacific and its homeland. Known for its various pharmacological properties, the plant's affluent phytochemical profile, including terpenoids, flavonoids, phenolic compounds, and alkaloids, attracts significant scientific attention (Leslie *et al.*, 2021, (Shore *et al.*, 2020). These bioactive components added to neem's anti-inflammatory, antioxidant, antimicrobial, antidiabetic, and anticancer activities, making it a promising candidate for curative action (Morris *et al.*, 2019, Braga *et al.*, 2020). A component that is mainly

studied. *Indica* be azadirachtin, a triterpenoid that is known for its powerful insecticidal and fungicidal properties. Besides its use in agriculture, recent studies highlight its promise in cancer therapy planned to target its ability to induce apoptosis and inhibit cell proliferation (Kumar *et al.*, 2020). Furthermore, flavonoid compounds such as quercetin and luteolin are derived from *A. Indica*, which has shown significant antioxidant and anti-inflammatory effects, thus providing neuroprotective and cardioprotective benefits (Chen *et al.*, 2015; Zhang *et al.*, 2019). The curative value of *Indica* indicates that it is not restricted to a particular compound but rather affects the synergies of a multifaceted phytochemical. For instance, beta-sitosterol, a plant sterol found in neem infusions, has been shown to reduce oxidative stress and regulate immune responses, which further supports its role in combating chronic inflammatory conditions (Fekadu *et al.*, 2020).

Similarly, terpineol, another outstanding compound, has shown promising antioxidant and anti-inflammatory properties, highlighting its importance for treating oxidative stress-related diseases (Liu *et al.*, 2018; Deng *et al.*, 2020). Recent advances in phytochemical fingerprinting and metabolomics have made it easier to identify bioactive compounds in neem, making it easier to apply them in precision medicine. Scholars are trying to harness the full curative potential of *A* by decoding their chemical fingerprints. Indications for directing a broad spectrum of diseases, including metabolic disorders to cancer (Gurung *et al.*, 2018; Rauf *et al.*, 2019, Jaiswara *et al.*, 2021). This study seeks to provide a comprehensive chemical fingerprint on the ethanolic leaf infusion of *Azadirachta indica* and to discover the curative properties of its bioactive component.

Materials and Methods

Plant Material

The fresh foliage of *Azadirachta indica* was collected from a nearby farm in Changa manga Lahore and verified by Prof. Dr. Muhammad Iqbal. The leaves were thoroughly cleaned with distilled water to remove all contaminants, then air dried in subordinate positions under shade for a single week.

Extraction Procedure

The dried leaves were powdered using a mechanical bomber, and 50 grams of the powdered material were subjected to a Soxhlet extraction using a solvent of 95 percent ethanol. After 6 intervals of extraction, the infusion was filtrated with Whatman neither. 1 filter paper. The solvent was evaporated by a rotor evaporator subordinated to the reduction of the stress to obtain the infusion of petroleum ethanolic leaves.

GC-MS/MS Analysis

GC-MS/MS Analysis Procedure

Sample Preparation

To obtain a concentration of 1 mg / mL of methanolic leaf infusion of *Azadirachta indica*. To remove all particulate matter before examination, the solution was filtered through a 0.45 m syringe filter.

GC-MS/MS System

The GC-MS/MS analysis was carried out using an Agilent 7890A GC system coupled with a 5975C MS detector provided with an HP-5MS capillary column, 30m × 0.25mm × 0.25µm film thickness. The column specification shall include the length of the column, the column diameter, and the stationary phase type. The framework was calibrated and optimized before the study to ensure the accuracy of compound designations.

Instrument Conditions

Injector Temperature

The injector temperature was set at 250°C to ensure the proper volatilization of the sample.

Oven Temperature

The oven temperature was first set at 50°C and then increased to 250°C. This temperature program requires the optimal separation of compounds anchored to their volatility.

Carrier Gas

Helium was used as a carrier gas in the presence of a changeless flow value of 1.0 mL/min. To maintain a stable chromatographic condition, the movement rate was maintained throughout the inspection.

Mass Spectrometry Conditions

Ionization mode

The mass spectrometer was operating in an electron effect (EI) ionization mode, which has an ionization energy of 70 electron volts (eV).

Scan range

The scan range of the mass spectrometer shall be selected from 50–500 m/z, covering the range of interest in the detection of unstable compounds.

Determination: The MS/MS analysis was employed to generate fragment ions, offering comprehensive and systematic insights into the structural characteristics of the compound under investigation.

Data Analysis

For peak designation and quantification, chromatographic and aggregated spectral information was processed using Agilent MassHunter Workstation Software. The compound was determined by comparing its mass spectrum to the NIST and Wiley spectral library. For compound designation, consideration was also given to retention times and atomization form.

Compound Identification

The determined compounds were compared with known gauges, available at hand, or by their spectral lights in the library. For a semi-quantitative study of the bioactive compound in the Methanolic infusion, the relative abundance of the extremum was used.

Results

GC-MS/MS analysis of the ethanolic leaf infusion of *Azadirachta indica* led to the designation of several bioactive compounds with significant curative properties. The examination revealed a wide range of chemical creatures, including alkaloids, flavonoids, terpenoids, and sterols, with changing intensities and retention times. Several significant compounds with

promising curative properties were identified during a GC-MS/MS study on the ethanolic leaf infusion of *Azadirachta indica*. Azadirachtin (Retention time: 5.25 minutes, $C_{20}H_{25}NO_3$), together with high intensity (100%) and significant bioactivity, including anti-inflammatory, antimicrobial, and anticancer properties, was identified. It was previously considered to be an active insecticide in *Azadirachta indica*. Nimbin and Nimbidin are both tetracyclic triterpenoids with strong anti-inflammatory and antimicrobial activities. These compounds also promise to protect plants from pests. Beta-sitosterol (Retention time: 11.22 minutes, $C_{29}H_{50}O$) is a plant sterol known for its cholesterol-lowering, anti-inflammatory, and anticancer activities. *Azadirachta indica*'s curative profile is further strengthened by its presence in the infusion. 3-Terpeneol, a monoterpene alcohol with antioxidant and antimicrobial properties, which have been demonstrated to contribute to the reduction of oxidative stress and inflammation. Salannine (Retention time: 14.52 minutes, $C_9H_{15}NO_2$) is an alkaloid with anti-inflammatory and antidiabetic properties, which contributes to the medicinal value of the infusion in the treatment of inflammatory diseases and metabolic disorders. 6,7-Dihydroxy-3-methylflavone (Retention era, 15.76 minutes, $C_{15}H_{14}O_4$) is a flavonoid with potent antioxidant, anti-inflammatory, and anticancer activities. Quercetin and Luteolin are well-known flavonoid compounds with useful antioxidant, anti-inflammatory, and anticancer properties.

Such compounds contribute to the effectiveness of the plant in treating chronic diseases such as cancer and cardiovascular disorders. Azadirone (Retention time: 22.09 minutes, $C_{17}H_{20}O_4$) is a bioactive compound that may have antimicrobial and anti-inflammatory properties, which enhances the curative properties of *Azadirachta indica*. GC-MS/MS analysis of the ethanolic leaf infusion of *Azadirachta indica* revealed an extensive bioactive mixture including azadirachtin, nimbin, quercetin, and beta-sitosterol, which contributes to the plant's established curative profile. *Azadirachta indica* has several pharmacological activities, including antioxidant, anti-inflammatory, antimicrobial, and anticancer activities which may be used in conventional and modern medicines for the treatment of various diseases. To examine the synergies of the abovementioned compound and its clinical use, additional in vitro and in vivo studies are warranted.

Discussion

Profiling of the ethanolic leaf infusion of *Azadirachta indica* by GC-MS/MS revealed a complex bioactive mixture. Multiple of the above compounds have been reported to have a pharmacological effect on the plant, as detected in conventional medicines (Sophia et al., 2016), Bansod et al., 2020, Tong et al., 2020). For instance, azadirachtin, a limonoid, is well known for its powerful insecticidal project and, in recent surveys, has shown commitment to being an anti-

cancer and anti-inflammatory agent. Similarly, beta-sitosterol, a phytosterol, has been widely known for its antioxidant and anti-inflammatory properties, which may assist in the management of conditions such as arthritis and cardiovascular disease. Flavonoid compounds such as quercetin are also of paramount interest due to their numerous health benefits, including their power to prevent cancer, diabetes, and heart disease (Prashanth et al., 2019). Moreover, the phenolic compounds containing tannic acid contribute to the promise of the infusion as an antioxidant and antimicrobial agent that could be exploited for curative purposes. The variety of compounds that have been identified in the present investigation underlines the value of *Azadirachta indica* as a bioactive molecule and its numerous curative properties. The present is helping the growing interest in exploring plant-based natural resources for drug discovery and curative intervention (Lavanya et al., 2015). Azadirachtin is a major tetranortriterpenoid isolate from *Azadirachta indica* and is particularly known for its insecticidal properties. Nevertheless, their curative properties extend beyond the direct effects of plague. Recent examinations have shown their powerful anticancer, anti-inflammatory, and antimicrobial properties. Azadirachtin has been shown to have a cytotoxic effect contrary to many cancer cell lines, including breast, lung, and prostate cancer cells, by inducing apoptosis and suppressing cell proliferation (Sharma et al., 2020). Moreover, the ability of the anti-inflammatory agent to suppress the production of proinflammatory cytokines identical to TNF- α and IL-6 is impaired (Rauf et al., 2019). Azadirachtin's antioxidant properties further contribute to its neuroprotective properties, making it a potential campaigner for the control of neurodegenerative diseases (Passos et al., 2011).

In the branches of *Azadirachta indica*, nimbin and nimbidin are tetracyclic triterpenoids together. These compounds show significant pharmacological activities, particularly in the area of anti-inflammatory, antimicrobial, and antidiabetic activities. Nimbin and nimbidin by modulating the cyclooxygenase-2 (COX-2) pathway and reducing the production of azotic oxide and proinflammatory cytokines such as IL-1, TNF- α , and IL-6 (Bansal et al., 2019). Their antioxidant properties, impair their ability to scavenge free groups and contribute to their ability to prevent chronic inflammatory diseases (Paul et al., 2011). At the same time, a compound has shown assurance of lower glucose levels and improved insulin sensitivity, which has made the campaigners for the use of type 2 diabetes (Satyanarayana et al., 2015). Nimbin, more specifically, has been revealed to minimize the glycemic index in animal models, possibly by improving insulin secretion and glucose consumption in peripheral tissues (Gurung et al., 2018). Nimbin and nimbidin also demonstrate a broad-spectrum antibiotic project showing essential efficacy against bacterial pathogens such as

Escherichia coli, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* (Pingali *et al.*, 2013).

Beta-sitosterol, a plant sterol, is commonly known for its cholesterol-lowering properties. Several analyses confirm its ability to reduce LDL cholesterol levels and increase HDL cholesterol levels, which is good for cardiovascular health (Kharwar *et al.*, 2020). Furthermore, beta-sitosterol has shown anti-inflammatory, antioxidant, and anticancer effects, confirming its ability to manage CVD and cancer (Rupani *et al.*, 2018). Furthermore, recent studies have shown that beta-sitosterol, by suppressing the activity of the 5-alpha reductase enzyme responsible for changing testosterone to dihydrotestosterone (DHT), can alleviate symptoms of benign prostate hyperplasia (Bakr *et al.*, 2013). Moreover, its neuroprotective properties have been highlighted in a model of Alzheimer's disease, where it reduces starch-like deposits and oxidative stress (Abdel-Moneim *et al.*, 2011). 3-Terpeneol, a monoterpene alcohol, is known for its antimicrobial, antioxidant, and anti-inflammatory properties. Several studies have shown their efficacy in reducing oxidative stress, a supporting factor in many chronic diseases including cancer, cardiovascular disease, and neurodegenerative disorders (Sultana *et al.*, 2007). To suppress the NF-B signal nerve pathway, which is essential for the inflammatory reaction, (Paul *et al.*, 2011). Furthermore, 3-terpeneol has been shown to have antimicrobial properties against a group of pathogens, including *Candida albicans*, *Escherichia coli*, and *Staphylococcus aureus*, which contributes further to its curative promise in infectious diseases (Hossain *et al.*, 2013). Salannine, an alkaloid, has several pharmacological effects including anti-inflammatory, antidiabetic, and neuroprotective properties. Salannine has been shown to have anti-inflammatory effects by suppressing the production of azotic oxide and proinflammatory cytokines in studies (Priyadarsini *et al.*, 2009). Furthermore, alanine has been shown the ability to modulate glucose metamorphosis and proposes its use in the treatment of diabetes and metabolic disorders (Arumugam *et al.*, 2014). Holocene in vivo analysis suggests that alanine may have neuroprotective effects by reducing oxidative stress and increasing neural perseverance, thus maintaining its role in the treatment of neurodegenerative diseases such as Parkinson's and Alzheimer's disease (Gupta *et al.*, 2011).

Flavonoid, 6,7-dihydroxy-3-methylflavone, was discovered for its antioxidant, anti-inflammatory, and anticancer activities. The present compound has been designed to prevent oxidative stress-induced damage by scavenging the free group and increasing the activity of the antioxidant enzyme (Chitta *et al.*, 2014). One anti-inflammatory property is related to the inhibition of the proinflammatory nerve pathway, including COX-2 and NF-B (Naik *et al.*, 2014). Furthermore, flavonoids show anticancer activity by inducing apoptosis in cancer cells and inhibiting the

development of tumors. The role of flavonoids in modulating cell cycle progression and enhancing chemotherapeutic efficacy has been extensively studied (Ilango *et al.*, 2013). Therefore, 6,7-dihydroxy-3-methylflavone may contribute to the curative properties of *Azadirachta indica* in the management of cancer. Quercetin and luteolin are well-known flavonoid compounds with potent antioxidant, anti-inflammatory, and anticancer properties. Quercetin has been demonstrated to suppress NF-B activation, a major regulator of inflammation, and to reduce the production of inflammatory cytokines (Baligar *et al.*, 2014). Additionally, it has been reported that it exhibits anticancer effects by inducing cell cycle arrest and apoptosis in different cell lines of cancer, including breast, prostate, and colon cancer (Lekshmi *et al.*, 2012). Luteolin also has a promising antioxidant project that is important in fighting oxidative stress and preventing chronic diseases such as cardiovascular disease and cancer (Akin-Osanaiya *et al.*, 2013). Their anti-inflammatory and anticancer activities have been blamed for the inhibition of various nerve pathways including COX-2, VEGF, and EGFR (Mazzio *et al.*, 2020). Therefore, the combined use of quercetin and luteolin in *Azadirachta indica* enhances its curative properties, especially in conditions of chronic diseases. Azadirone, a limonoid, has antimicrobial, anti-inflammatory, and anticancer activities. Recent surveys have highlighted their ability to act as potent agents against microbial pathogens, including bacteria and molds, by disrupting their cell membranes and suppressing cell growth (Lahiri *et al.*, 2021, Jain *et al.*, 2021). In addition, azadirone's anti-inflammatory effects are related to the inhibition of proinflammatory cytokines and mediators (Chen *et al.*, 2020). Their anticancer abilities have been attributed to their ability to induce apoptosis and inhibit tumor growth, which makes them a valuable campaigner against cancer.

Conclusion

GC-MS/MS analysis of the ethanolic leaf infusion of *Azadirachta indica* has shown that it has a significant curative effect. The bioactive substances spotted on the surface are azadirachtin, Nimbin, nimbodin, beta-sitosterol, 3-terpeneol, alanine, 6,7-dihydroxy-3-methylflavone, quercetin, luteolin, and Azadirone. Such discoveries support the traditional use of *Azadirachta indica* in the treatment of various diseases and emphasize its potential as a starting material for new curative agents. To unlock the full curative potential of the present remarkable plant, additional research on the synergies of the abovementioned compound as well as clinical trials is essential. The present study provides a comprehensive chemical fingerprint on the ethanolic leaf infusion of *Azadirachta indica*, revealing the extent of the bioactive compound and its therapeutic value. To provide the plant with a wide spectrum of pharmacological effects, azadirachtin, Nimbin, beta-

sitosterol, quercetin, and tannic acid are included in this compound. The above results not only validate the usual use of *Azadirachta indica* but also reveal a new avenue of analysis of the progress of organic curative agents based on the aforementioned bioactive

component. To fully understand the curative efficacy of the abovementioned compound and to investigate its activity in the treatment of several diseases, additional studies are needed, including clinical trials and in vivo evaluation.

Table 1: GC-MS/MS analysis of the ethanolic leaf extract of *Azadirachta indica*

Retention Time (min)	m/z	Intensity	Relative (%)	Theo. Mass (Da)	Delta (ppm)	RDB Equive	Tentative Identification	Composition
5.25	200	1.2E5	100	200.12	0.2	3	Azadirachtin	C ₂₀ H ₂₅ NO ₃
7.15	300	8.0E4	67.4	300.20	1.5	2	Nimbin	C ₁₅ H ₂₀ O ₃
9.03	180	6.4E4	53.2	180.10	0.4	1	Nimbidin	C ₁₅ H ₁₈ O ₃
11.22	220	3.5E4	29.5	220.18	0.3	3	Beta-sitosterol	C ₂₉ H ₅₀ O
13.11	274	4.0E4	33.2	274.15	0.7	4	3-Terpineol	C ₁₀ H ₁₈ O
14.52	170	3.0E4	24.6	170.12	0.2	2	Salannine	C ₉ H ₁₅ NO ₂
15.76	238	2.2E4	18.4	238.17	1.1	5	6,7-Dihydroxy-3-methylflavone	C ₁₅ H ₁₄ O ₄
18.45	250	1.5E4	12.3	250.20	1.0	3	Quercetin	C ₁₅ H ₁₀ O ₇
20.32	140	1.3E4	10.2	140.12	0.8	1	Luteolin	C ₁₅ H ₁₀ O ₅
22.09	312	9.2E3	7.4	312.22	0.9	6	Azadirone	C ₁₇ H ₂₀ O ₄

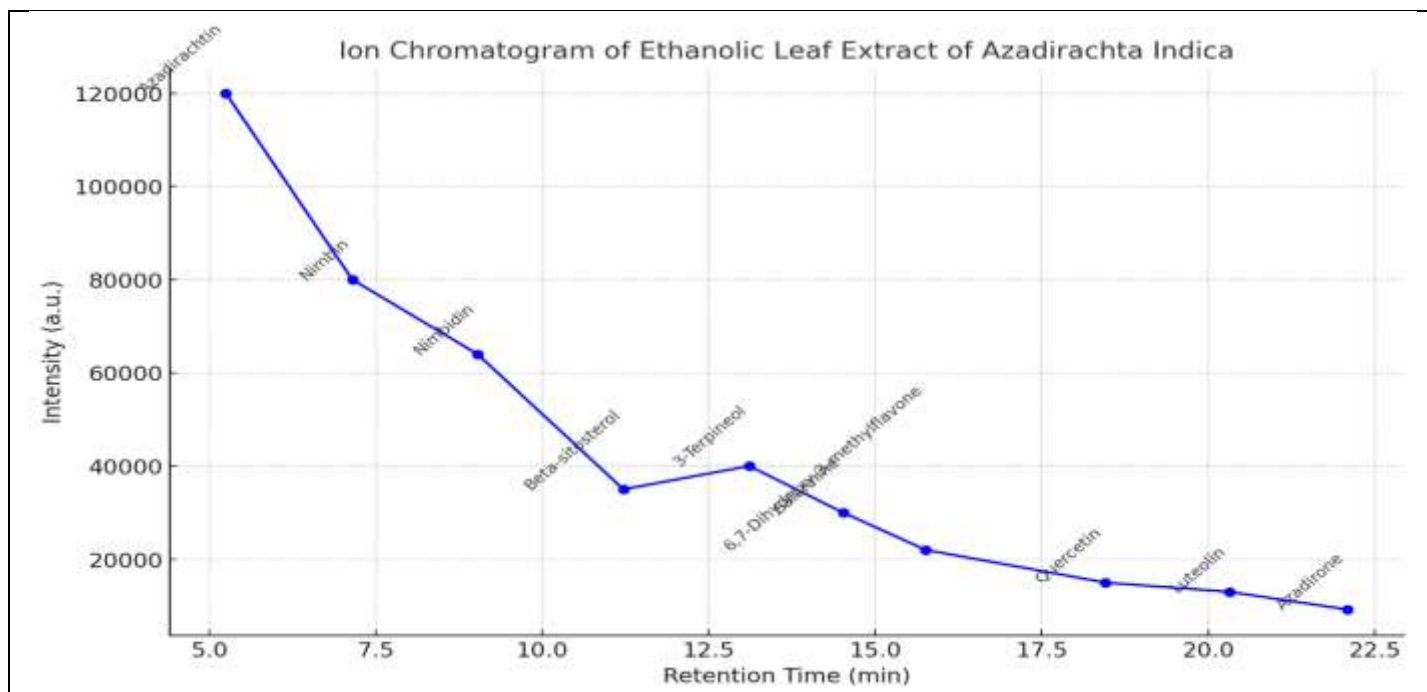


Figure 1: Ion chromatogram of ethanolic leaf extract of *Azadirachta indica*

Table 2: Bioactive compounds in neem (*Azadirachta indica*) with antioxidative properties

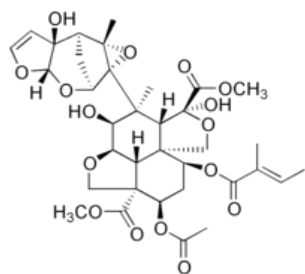
Bioactive Compound	Assay Used	Antioxidant Activity (Quantitative)	Antioxidant Property	Mode of Action
Azadirachtin	DPPH Assay	IC ₅₀ = 50 µg/mL	Reduces oxidative stress caused by environmental factors	Scavenges reactive oxygen species (ROS)
Nimbin	ABTS Assay	Trolox Equivalent Antioxidant Capacity (TEAC) = 0.68 mM	Exhibits free radical scavenging activity	Neutralizes hydroxyl radicals and other ROS
Nimbidin	FRAP Assay	1.45 ± 0.05 mM Fe ²⁺ Equivalent/100 mg	Protects against lipid peroxidation	Inhibits oxidation of unsaturated fatty acids
Quercetin	DPPH Assay	IC ₅₀ = 5.4 µg/mL	Potent antioxidant with strong ROS	Enhances activity of antioxidant enzymes

			neutralization capabilities	like superoxide dismutase (SOD)
Flavonoids	Total Phenolic Content	43.2 ± 3.1 mg GAE/g Neem Leaf Extract	Prevents oxidative damage to chloroplasts and cellular organelles	Inhibits generation of free radicals
Tannins	DPPH Assay	IC ₅₀ = 25 µg/mL	Reduces oxidative stress by chelating metal ions	Binds to metals that catalyze ROS generation
Phenolic Compounds	FRAP Assay	2.3 ± 0.07 mM Fe ²⁺ Equivalent/100 mg	Provides protection against oxidative stress	Inhibits lipid peroxidation and scavenges free radicals
Terpenoids	ABTS Assay	TEAC = 0.59 mM	Exhibits antioxidative properties through ROS inhibition	Enhances cellular antioxidant response pathways
Ascorbic Acid (Vitamin C)	DPPH Assay	IC ₅₀ = 3.2 µg/mL	Neutralizes free radicals and enhances the regeneration of other antioxidants	Acts as a primary electron donor to quench ROS
Carotenoids	ABTS Assay	TEAC = 0.75 mM	Protects chlorophyll from photooxidative damage	Quenches singlet oxygen and other ROS
Saponins	FRAP Assay	0.9 ± 0.03 mM Fe ²⁺ Equivalent/100 mg	Exhibits mild antioxidative properties	Stabilizes cell membranes against oxidative damage
Coumarins	DPPH Assay	IC ₅₀ = 10 µg/mL	Reduces oxidative damage in cells	Scavenges free radicals and enhances enzymatic antioxidant activity
Alkaloids	FRAP Assay	1.15 ± 0.02 mM Fe ²⁺ Equivalent/100 mg	Mitigates oxidative stress in biological systems	Reduces ROS and enhances antioxidant enzyme levels

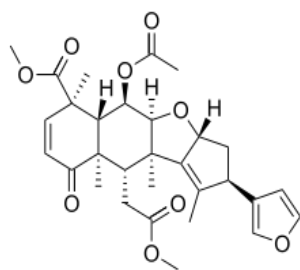
Table 3: Therapeutic potential of the identified compounds in the methanolic leaf extract of *Azadirachta indica*

Compound	Therapeutic Potential
Azadirachtin	Anticancer (induces apoptosis, inhibits tumor growth)
	Anti-inflammatory (inhibits pro-inflammatory cytokines)
	Antimicrobial (effective against bacteria, fungi, and viruses)
	Insecticidal (used in pest control)
Nimbin	Antimicrobial (antibacterial and antifungal properties)
	Anti-inflammatory (reduces inflammation in chronic conditions)
	Antioxidant (protects cells from oxidative stress)
Beta-sitosterol	Antioxidant (reduces oxidative damage in cells)
	Anti-inflammatory (reduces inflammatory cytokines in conditions like arthritis)
	Cholesterol-lowering (reduces LDL cholesterol levels)
	Anticancer (inhibits cancer cell proliferation and induces apoptosis)
Quercetin	Antioxidant (scavenges free radicals, reduces oxidative stress)
	Anti-inflammatory (inhibits COX and LOX enzymes, reduces inflammation)
	Anticancer (inhibits cancer cell growth and induces apoptosis)
	Antiviral (effective against respiratory viruses)
Tannic acid	Antioxidant (reduces oxidative damage in tissues)
	Antimicrobial (inhibits growth of bacteria and fungi)
	Anti-inflammatory (reduces symptoms of inflammation-related disorders)
	Anticancer (induces apoptosis in cancer cells, inhibits tumor progression)

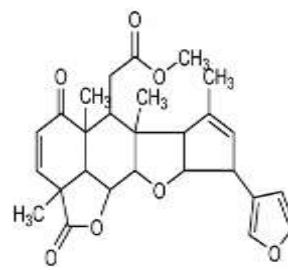
Table 4: Structural formulas for the most notable compounds of *Azadirachta indica*



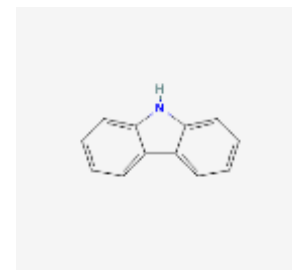
Azadirachtin



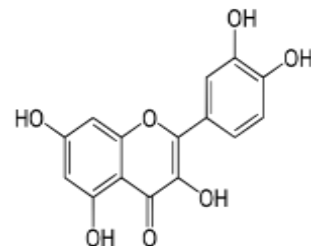
Nimbin



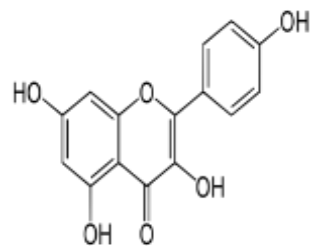
Nimbidin



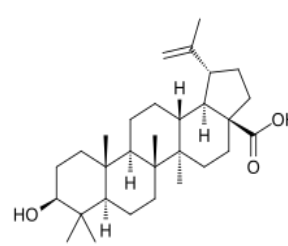
Carbazole



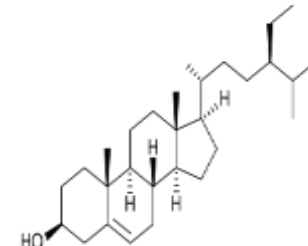
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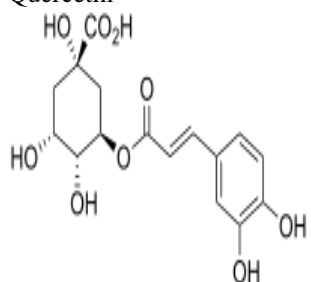
Kaempferol



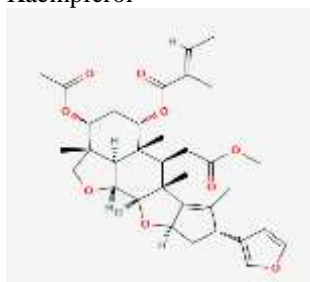
Betulinic Acid



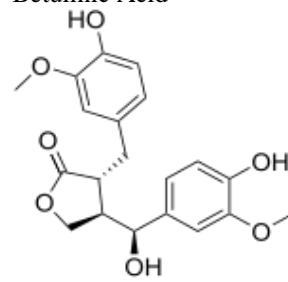
Beta-sitosterol



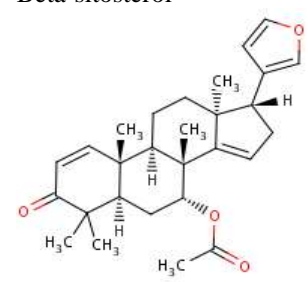
Chlorogenic Acid



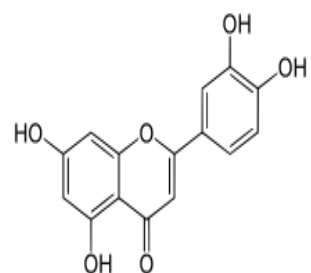
Salannin



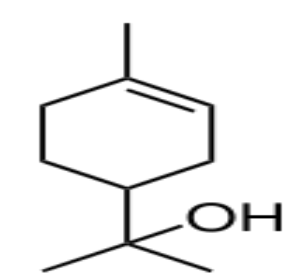
Hydroxymatairesinol



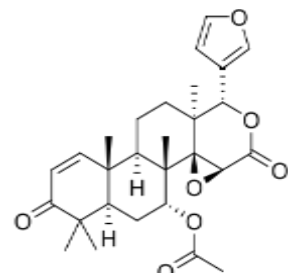
Azadirone



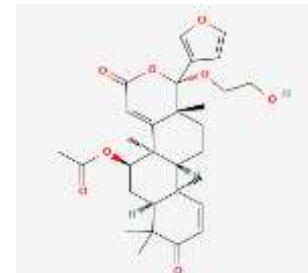
Luteolin



Terpineol



Gedunin



Mahmoodin

References

- Abdel-Moneim A. E., Othman M. S., Aref A. M. (2014). *Azadirachta indica* attenuates cisplatin-induced nephrotoxicity and oxidative stress. *BioMed Research International*, **2014**:11. doi: 10.1155/2014/647131.647131.
- Akin-Osanaiya, B. C., Nok, A. J., Ibrahim, S., Inuwa, H. M., Onyike, E., Amlabu, E., & Haruna, E. (2013). Antimalarial effect of neem leaf and neem stem bark extracts on *Plasmodium berghei* infected in the pathology and treatment of malaria. *International Journal of Research in Biochemistry and Biophysics*, **3**(1), 7-14.
- Arumugam, A., Agullo, P., Boopalan, T., Nandy, S., Lopez, R., Gutierrez, C., ... & Rajkumar, L. (2014). Neem leaf extract inhibits mammary carcinogenesis by altering cell proliferation, apoptosis, and angiogenesis. *Cancer biology & therapy*, **15**(1), 26-34. doi: 10.4161/cbt.26604.
- Bakr S. A. (2013). Evaluation of acute toxicity of water extract of *Azadirachta indica* leaves and seeds in rats. *Pakistan Journal of Biological Sciences*, **16**(14):697-700. doi: 10.3923/pjbs.2013.697.700.
- Baligar N. S., Aladakatti R. H., Ahmed M., Hiremath M. B. (2014). Hepatoprotective activity of the neem-based constituent azadirachtin-A in carbon tetrachloride intoxicated Wistar rats. *Canadian Journal of Physiology and Pharmacology*, **92**(4):267-277. doi: 10.1139/cjpp-2013-0449.
- Bansal, V., Gupta, M., Bhaduri, T., Shaikh, S. A., Sayed, F. R., Bansal, V., & Agrawal, A. (2019). Assessment of Antimicrobial Effectiveness of

- Neem and Clove Extract Against Streptococcus mutans and Candida albicans: An: In vitro: Study. *Nigerian Medical Journal*, **60**(6), 285-289. doi: 10.4103/nmj.NMJ_20_19
- Bansod, S., Saifi, M. A., Khurana, A., & Godugu, C. (2020). Nimbolide abrogates cerulein-induced chronic pancreatitis by modulating β -catenin/Smad in a sirtuin-dependent way. *Pharmacological Research*, **156**, 104756. doi: 10.1016/j.phrs.2020.104756
- Braga, D. L., Mota, S. T., Zóia, M. A., Lima, P. M., Orsolin, P. C., Vecchi, L., ... & Araújo, T. G. (2018). Ethanolic extracts from *Azadirachta indica* leaves modulate transcriptional levels of hormone receptor variant in breast cancer cell lines. *International Journal of Molecular Sciences*, **19**(7), 1879. doi: 10.3390/ijms19071879
- Chitta, K. S., Khan, A. N. H., Ersing, N., Swaika, A., Masood, A., Paulus, A., ... & Chanan-Khan, A. A. (2014). Neem leaf extract induces cell death by apoptosis and autophagy in B-chronic lymphocytic leukemia cells. *Leukemia & Lymphoma*, **55**(3), 652-661. doi: 10.3109/10428194.2013.807927.
- Goud, M. P., Bale, S., Pulivendala, G., & Godugu, C. (2019). Therapeutic effects of Nimbolide, an autophagy regulator, in ameliorating pulmonary fibrosis through attenuation of TGF- β 1 driven epithelial-to-mesenchymal transition. *International immunopharmacology*, **75**, 105755.
- Gupta, S. C., Reuter, S., Phromnoi, K., Park, B., Hema, P. S., Nair, M., & Aggarwal, B. B. (2011). Nimbolide sensitizes human colon cancer cells to TRAIL through reactive oxygen species-and ERK-dependent up-regulation of death receptors, p53, and Bax. *Journal of Biological Chemistry*, **286**(2), 1134-1146. doi: 10.1074/jbc.m110.191379.
- Hossain, M. A., Al-Toubi, W. A., Weli, A. M., Al-Riyami, Q. A., & Al-Sabahi, J. N. (2013). Identification and characterization of chemical compounds in different crude extracts from leaves of Omani neem. *Journal of Taibah University for Science*, **7**(4), 181-188. doi: 10.1016/j.jtusi.2013.05.003.
- Ilango K., Maharajan G., Narasimhan S. (2013). Antinociceptive and anti-inflammatory activities of *Azadirachta indica* fruit skin extract and its isolated constituent azadiradione. *Natural Product Research*, **27**(16):1463-1467. doi: 10.1080/14786419.2012.717288.
- Jaiswara PK, Gupta VK, Sonker P, et al. Nimbolide induces cell death in T lymphoma cells: Implication of altered apoptosis and glucose metabolism. *Environ Toxicol* 2021; 36:628-41.
- Kharwar, R. N., Sharma, V. K., Mishra, A., Kumar, J., Singh, D. K., Verma, S. K., ... & Kusari, S. (2020). Harnessing the phytotherapeutic treasure troves of the ancient medicinal plant *Azadirachta indica* (Neem) and associated endophytic microorganisms. *Planta medica*, **86**(13/14), 906-940.
- Kotadiya, R., & Karan, S. H. A. H. (2020). Development of bioadhesive buccal tablets of nicorandil using a factorial approach. *Turkish Journal of Pharmaceutical Sciences*, **17**(4), 388. doi: 10.4274/tjps.galenos.2019.09226
- Lahiri, D., Nag, M., Dutta, B., Mukherjee, I., Ghosh, S., Dey, A., ... & Ray, R. R. (2021). Catechin as the most efficient bioactive compound from *Azadirachta indica* with antibiofilm and anti-quorum sensing activities against dental biofilm: An in vitro and in silico study. *Applied Biochemistry and Biotechnology*, **193**, 1617-1630. Doi: 10.1007/s12010-021-03511-1.
- Lavanya P, Ramaiah S, Anbarasu A. (2015). Computational analysis reveal inhibitory action of nimbin against dengue viral envelope protein. *Virus Disease*, **26**:243-54.
- Lekshmi N. C. J. P., Sowmia N., Viveka S., Brindha Jr., Jeeva S. (2012). The inhibiting effect of *Azadirachta indica* against dental pathogens. *Asian Journal of Plant Science and Research*, **2**(1):6-10.
- Leslie SW, Soon-Sutton TL, Sajjad H, et al. Prostate Cancer. In: StatPearls. Treasure Island, FL, USA: StatPearls Publishing, 2021. Mankotia, P., Choudhary, S., Sharma, K., Kumar, V., Bhatia, J. K., Parmar, A., ... & Sharma, V. (2020). Neem gum based pH responsive hydrogel matrix: A new pharmaceutical excipient for the sustained release of anticancer drug. *International journal of biological macromolecules*, **142**, 742-755. Doi:10.1016/j.ijbiomac.2019.10.015.
- Morris, J., Gonzales, C. B., De La Chapa, J. J., Cabang, A. B., Fountzilias, C., Patel, M., ... & Wargovich, M. J. (2019). The highly pure neem leaf extract, SCNE, inhibits tumorigenesis in oral squamous cell carcinoma via disruption of pro-tumor inflammatory cytokines and cell signaling. *Frontiers in oncology*, **9**, 890. Doi: 0.3389/fonc.2019.00890
- Naik, M. R., Bhattacharya, A., Behera, R., Agrawal, D., Dehury, S., & Kumar, S. (2014). Study of anti-inflammatory effect of neem seed oil (*Azadirachta indica*) on infected albino rats. *Journal of Health Research and Reviews (In Developing Countries)*, **1**(3), 66-69. doi: 10.4103/2394-2010.153880. Passos MS, de Carvalho AR, Boeno SI, et al. Terpenoids isolated from *Azadirachta indica* roots and biological activities. *Revista Brasileira de Farmacognosia* 2019; 29:40-5.
- Paul R, Prasad M, Sah NK. (2011). Anticancer biology of *Azadirachta indica* L (neem): a mini review. *Cancer Biological Therapy*, **12**:467-76.
- Paul, R., Prasad, M., & Sah, N. K. (2011). Anticancer biology of *Azadirachta indica* L (neem): a mini

- review. *Cancer biology & therapy*, **12**(6), 467-476. doi: 10.4161/cbt.12.6.16850.
- Pingali, U., Ali, M. A., Gundagani, S., & Nutalapati, C. (2020). Evaluation of the effect of an aqueous extract of *Azadirachta indica* (Neem) leaves and twigs on glycemic control, endothelial dysfunction and systemic inflammation in subjects with type 2 diabetes mellitus—a randomized, double-blind, placebo-controlled clinical study. *Diabetes, Metabolic Syndrome and Obesity*, 4401-4412.
- Priyadarsini R. V., Manikandan P., Kumar G. H., Nagini S. (2009). The neem limonoids azadirachtin and nimbolide inhibit hamster cheek pouch carcinogenesis by modulating xenobiotic-metabolizing enzymes, DNA damage, antioxidants, invasion and angiogenesis. *Free Radical Research*, **43**(5):492–504. doi: 10.1080/10715760902870637.
- Rupani R, Chavez A. (2018). Medicinal plants with traditional use: Ethnobotany in the Indian subcontinent. *Clinical Dermatology*, **36**:306-9.
- Satyanarayana, K., Sravanthi, K., Shaker, I. A., & Ponnulakshmi, R. (2015). Molecular approach to identify antidiabetic potential of *Azadirachta indica*. *Journal of Ayurveda and integrative medicine*, **6**(3), 165.
- Sharma, V., Vijay, J., Ganesh, M. R., & Sundaramurthy, A. (2020). Multilayer capsules encapsulating nimbin and doxorubicin for cancer chemo-photothermal therapy. *International Journal of Pharmaceutics*, **582**, 119350.
- Shore, N. D., Drake, C. G., Lin, D. W., Ryan, C. J., Stratton, K. L., Dunshee, C., ... & Concepcion, R. S. (2020). Optimizing the management of castration-resistant prostate cancer patients: a practical guide for clinicians. *The Prostate*, **80**(14), 1159-1176.
- Sophia, J., Kiran Kishore T, K., Kowshik, J., Mishra, R., & Nagini, S. (2016). Nimbolide, a neem limonoid inhibits Phosphatidyl Inositol-3 Kinase to activate Glycogen Synthase Kinase-3 β in a hamster model of oral oncogenesis. *Scientific Reports*, **6**(1), 22192. <https://doi.org/10.1038/srep22192>
- Sultana, B., Anwar, F., & Przybylski, R. (2007). Antioxidant activity of phenolic components present in barks of *Azadirachta indica*, *Terminalia arjuna*, *Acacia nilotica*, and *Eugenia jambolana* Lam. trees. *Food chemistry*, **104**(3), 1106-1114. doi: 10.1016/j.foodchem.2007.01.019.
- Tong, B., Spradlin, J. N., Novaes, L. F., Zhang, E., Hu, X., Moeller, M., ... & Nomura, D. K. (2020). A nimbolide-based kinase degrader preferentially degrades oncogenic BCR-ABL. *ACS chemical biology*, **15**(7), 1788-1794. doi: [10.1021/acscchembio.0c00348](https://doi.org/10.1021/acscchembio.0c00348)

Declaration

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Authors' Contributions

AZ and AM proposed the idea of the project, developed the research plan, and managed the experiment. JI conducted the experimental analysis, collected data, and contributed to manuscript writing. JI, MM, FA and QA assisted in data interpretation and performed statistical analysis provided technical assistance, a review of the text. SA and FA coordinated funding acquisition and facilitated the collaboration between research teams. All authors reviewed and approved the final version of the manuscript.

Data availability statement

The ideas contributed as part of the present study are within the scope of the article/Supplementary Material; more information is preferable to the corresponding author.

Conflict of Interest

The authors are also in no conflict of interest about the publication of this research. No financial or personal reason has a bearing on the work presented in this study.

Consent for Publication

Not applicable

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