



Phytochemical Profiling and *In vitro* Antioxidant and Antimicrobial Evaluation of *Azadirachta indica* and *Melia azedarach*

¹Rani Yadav, ¹Jyoti Yadav, ²Deepu Yadav, ²Neelam Yadav, ³Neha Yadav, ¹JayPrakash Gupta, ⁴Shalini Jain, ⁵Gauri Shankar Rajpoot, ⁵K. S. Dadoriya, ⁵Arvind Prakash, ⁵Mukesh Yadav*

¹Department of Pharmacy, PK university, Shivpuri, (M.P.), India

²Advance college of Pharmacy, Datia, (M.P.), India

³Department of pharmacy, Vikrant University, Gwalior, (M.P.), India

⁴USF Center for Microbiome Research, Institute for Microbiomes, Department of Neurosurgery and Brain Repair, 4001 E. Fletcher Avenue Tampa, FL 33613, USA

⁵Department of Chemistry, PMCoE Govt. P. G. (Auto.) College, Datia, (M.P.). India

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Abstract

The present study investigated the phytochemical composition, antioxidant potential, and antibacterial activity of *Azadirachta indica* and *Melia azedarach* leaf methanolic extracts. Preliminary phytochemical screening detected steroids, alkaloids, phenols, flavonoids, saponins, tannins, anthraquinones, and amino acids in both extracts. Phytochemical fingerprinting was performed using high-performance liquid chromatography (HPLC) and thin-layer chromatography (TLC). HPLC analysis revealed seven major chromatographic peaks in *M. azedarach* and three in *A. indica*. TLC profiling identified eight phenolic and three steroidal bands in *A. indica*, while *M. azedarach* exhibited sixteen phenolic and three steroidal bands. Antioxidant activity was evaluated using DPPH, ABTS, CUPRAC, and FRAP assays, while total phenolic content was determined using the Folin–Ciocalteu method. Antibacterial activity was assessed against *Escherichia coli*, *Bacillus cereus*, *Staphylococcus epidermidis*, and *Enterobacter aerogenes* using disc- and well-diffusion methods. *A. indica* exhibited the highest activity against *E. coli*, with inhibition zones of 18 ± 0.18 and 21 ± 0.18 mm, respectively, and against *E. aerogenes*, with zones of 16 ± 0.11 and 18 ± 0.27 mm. MIC analysis further demonstrated concentration- and exposure-time-dependent antibacterial effects. Overall, both extracts demonstrated diverse phytochemical profiles, antioxidant activity, and antibacterial potential, supporting their further investigation as natural sources of bioactive compounds.

*Corresponding Author:

Dr. Mukesh Yadav (mukeshyadav2009@gmail.com)

ORCID ID: 0009-0008-2526-245X

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1. Introduction

Medicinal plants have been widely utilized for the preparation of traditional remedies and have served as important sources for the development of modern pharmaceuticals. In recent decades, increasing attention has been directed toward plant-derived medicines because of their therapeutic efficacy and comparatively lower adverse effects [1-2]. The emergence and widespread use of antibiotics have resulted in the development of antimicrobial resistance among several human pathogens, thereby creating a significant challenge in the management of infectious diseases. Multidrug-resistant microorganisms, including *Staphylococcus aureus*, vancomycin-resistant enterococci, *Mycobacterium tuberculosis*, and *Klebsiella pneumoniae*, have become major causes of hospital-acquired infections and have reduced the effectiveness of conventional antimicrobial agents. Consequently, medicinal plants have been extensively investigated as alternative sources of bioactive compounds with potent antimicrobial properties [3-5].

Among the medicinal plants belonging to the family Meliaceae, *Azadirachta indica* A. Juss. and *Melia azedarach* Linn. have received considerable scientific attention because of their broad spectrum of pharmacological activities. Antibacterial activity against both Gram-positive and Gram-negative bacteria has been reported for extracts of these species. Furthermore, numerous polyphenolic compounds isolated from members of the Meliaceae family have been demonstrated to possess significant antibacterial, antioxidant, anti-inflammatory, and anticancer properties. The phytochemicals present in *A. indica* have also been reported to exhibit antimicrobial activity against a wide range of pathogenic microorganisms, while the anticancer potential of this species has been demonstrated in several experimental studies.

Bioactive compounds present in *A. indica* and *Melia azedarach* possessing antioxidant, chemo preventive, and mood-enhancing properties, have been recognized for their significant contribution to human health through the prevention of chronic degenerative diseases. The study was undertaken to provide a comprehensive overview of the pharmacological properties and medicinal applications of *Azadirachta indica* (neem). Emphasis was placed on its anti-inflammatory, antimalarial, antiulcer, antidiabetic, antifungal, antibacterial, wound healing, and antioxidant activities, along with other reported pharmacological effects. The available scientific evidence regarding the therapeutic potential and medicinal uses of neem was critically reviewed and summarized [6]. The essential oil composition and antioxidant activity of *Melia azedarach* collected from southeastern Iran were reported for the first time. The

findings indicated that the essential oil of *M. azedarach* represents a rich natural source of trans-nerolidol, a bioactive compound with diverse biological activities [7]. Novel antibacterial activity against various Gram-positive and Gram-negative bacteria was reported for *Melia azedarach* Linn. and *Azadirachta indica* A. Juss. by [8-9].

A. indica extracts exhibit significant antibacterial, antidiabetic, anti-inflammatory, antioxidant, and antiproliferative properties, supporting their use in wound healing, disease management, and as natural larvicides. Agriculturally, Neem formulations prove effective as nematocides and insecticides, demonstrating systemic effects and persistence against pests like root-knot nematodes. Environmentally, Neem root extracts show promise in crude oil degradation in polluted soils, and innovative mechanical systems are being developed for efficient fruit collection, addressing economic and labour challenges [10].

Among the various methods available for the assessment of antioxidant activity, the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay has been most extensively employed for foods rich in phenolic compounds. In this assay, the ability of phenolic constituents to function as free radical scavengers or hydrogen donors is measured. A strong correlation between the antioxidant activity determined by the DPPH assay and the phenolic compound content of chocolate has been consistently reported by numerous researchers [11-14].

Azadirachta indica A. Juss., commonly known as neem, has been recognized as one of the most valuable medicinal plants because of its diverse biological activities. Extracts obtained from different parts of the plant have been reported to exhibit antibacterial, antifungal, antiviral, antimalarial, antidiabetic, anti-inflammatory, antioxidant, hepatoprotective, cardioprotective, antiulcer, antitumor, insecticidal, and immunomodulatory properties [15]. More than one hundred bioactive compounds with diverse chemical structures have been identified from different parts of the plant, although only a limited number have been comprehensively evaluated for their pharmacological potential. Among these compounds, azadirachtin, a limonoid triterpenoid, has been extensively studied for its insecticidal and therapeutic applications. In traditional medicine, *A. indica* has been employed for the treatment of various skin disorders, including eczema, ringworm, scabies, urticaria, alopecia, and lice infestation, as well as for the management of fungal, bacterial, and viral infections [16-20].

Similarly, *Melia azedarach* Linn., another important member of the family Meliaceae, has been widely

distributed throughout tropical and subtropical regions and has traditionally been used in herbal medicine. The plant has been reported to contain a wide variety of phytochemicals, particularly alkaloids, which are abundantly present in the inner bark. Extracts of *M. azedarach* have been demonstrated to possess antibacterial, antifungal, antimalarial, anticancer, analgesic, anthelmintic, antifertility, insecticidal, antifeedant, and antiviral activities. In addition, immunomodulatory effects, including the inhibition of phagocytosis and respiratory burst by leaf extracts, have been reported. Stem extracts have been shown to induce larval mortality and exhibit significant insecticidal activity. Furthermore, a novel antiviral peptide, meliacine, isolated from the leaves, has been reported to inhibit the multiplication of the foot-and-mouth disease virus, thereby highlighting the therapeutic potential of this medicinal plant [21-24].

Overall, the available scientific evidence indicates that *Azadirachta indica* and *Melia azedarach* are rich sources of pharmacologically active phytochemicals with significant antimicrobial and antioxidant potential. Therefore, in vitro phytochemical, antioxidant, and antibacterial findings about selected medicinal plants (*Azadirachta indica* and *Melia azedarach*) continue to be regarded as promising candidates for the discovery and development of future drugs for the management of infectious diseases and antioxidant.

2. Material and Methods

2.1. Collection and Processing the Plant Material

The leaves of *Azadirachta indica* and *Melia azedarach* were collected from Gwalior Market on 10 March 2024. The collected plant materials were authenticated by Dr Shivaraman Pandey Assistant Professor Govt. PG (Auto) College Datia (M.P.) India, and voucher specimens were deposited for future reference under Voucher No. 125, dated 10/03/2024. The leaves were thoroughly washed with tap water to remove adhering dust and impurities and then air-dried under shade at room temperature until a constant weight was obtained. The dried leaves were pulverized into a fine powder using a mechanical grinder and stored in airtight containers at room temperature in a cool, dry place until further phytochemical and biological analyses.

2.2. Preparation of the Neem Leaves Crude Extract

The powdered plant material was extracted using methanol at a solvent-to-sample ratio of 1:10 (w/v). Briefly, 10 g of powdered plant material was extracted with 100 mL of methanol. The extraction was carried out for 5 cycles/5 hours, with the extraction repeated 3 times. The combined extracts were filtered and

concentrated under reduced pressure using a rotary evaporator at 50°C. The concentrated extract was further dried to constant weight, and the extraction yield was calculated as a percentage of the initial dry plant material. The methanolic extract yielded approximately 20%.

2.3. Phytochemical Analysis

The phytochemicals extracted from the selected medicinal plants *Melia azedarach* and *Azadirachta indica*, using four different solvents (chloroform, methanol, hexane and water) were screened for the presence of steroids, alkaloids, phenols, flavonoids, saponins, tannins, anthraquinones, and amino acids, following the methods described [25-26].

2.4. Determination of Bioactive Components

High-performance liquid chromatography (HPLC) analysis was performed using a Waters Associates HPLC system equipped with a reversed-phase C18 analytical column (5 μ m, 4.6 mm $\times$ 250 mm). The mobile phase consisted of solvent A (0.1% trifluoroacetic acid) and solvent B (acetonitrile) in a ratio of 60:40 (v/v). The flow rate was maintained at 1.0 mL/min, the column temperature was set at 40°C, and the total run time was 25 min. The bioactive compounds were eluted using the filtered mobile phase. A 20 μ L volume of each sample was manually injected, and the bioactive constituents were detected using a UV-VIS detector at a wavelength of 220 nm, following the method described by [27].

2.5. Isolation of Phenols and Flavonoids Using Thin Layer Chromatography

The methanolic extracts were subjected to separation by thin-layer chromatography (TLC). The TLC analysis for phenolic compounds and alkaloids was performed according to the method described by [28].

2.6. Determination of the Antimicrobial Activities

The *Azadirachta indica* leaves and *Melia azedarach* leaves extract using 50 μ g/mL bactericidal potential was evaluated against selected bacterial strains, namely *Escherichia coli*, *Bacillus cereus*, *Staphylococcus epidermidis*, and *Enterobacter aerogenes* with the disc-diffusion assay based on inhibition-zone formation. Subsequently, the broth microdilution method was employed to determine the minimum inhibitory concentration (MIC) values [29].

2.7. Minimum Inhibitory Concentration Determination

Broth dilution assay and estimation of MIC values. The minimum inhibitory concentrations (MICs) of the leaf extracts of *A. indica* L. and *M. azedarach* L. against the above-mentioned microbial pathogens were

determined using the broth dilution method. Briefly, 100 µL of freshly grown microbial culture was inoculated into 10 mL of sterile distilled water (dH₂O) containing different concentrations (25, 50, 75, and 100 mg/mL) of the respective leaf extracts. Control tubes containing each tested microbial strain without the extracts were also maintained. All experiments were conducted at ambient temperature (25 ± 0.43 °C) with agitation at 250 rpm in a shaker. Samples were collected from each tube at four different time intervals (5, 10, 20, and 30 min). Bacterial cell densities were determined before exposure to the extracts (initial count) and after exposure. The viable bacterial counts were expressed as CFU/mL following incubation for 24 h at 37 °C using the pour-plate method according to APHA guidelines [30]. All experiments were performed in triplicate and repeated twice on different occasions [31].

2.8. Determination of Antioxidant Capacity

The antioxidant capacity (AC) was determined

$$\%DPPH_{scavenging} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100\%$$

Where: $A_{control}$ —absorbance of DPPH radical with methanol; A_{sample} —absorbance of DPPH radical with extract (or standard solution).

The calibration curve, $\%DPPH = (702.10 \pm 4.69) cTE + (3.98 \pm 0.42)$, was prepared using working solutions of TE in methanol between 0.02 and 0.10 mol/mL.

2.8.2. ABTS Assay

The antioxidant activity of the extracts was determined using the ABTS radical cation (ABTS^{•+}) assay. Aliquots of 0.1–0.3 mL of the test extracts were added to 2.4–2.2 mL of ABTS^{•+} solution (7 mmol/L), and the resulting

spectrophotometrically using DPPH, ABTS, CUPRAC, and FRAP assays according to previously described procedures with minor modifications [29]. The antioxidant capacity values were expressed as millimoles of Trolox equivalent (TE) per 100 g of the analysed sample.

2.8.1. DPPH Assay

The antioxidant capacity (AC) of the methanol extract of *A. indica* and *M. azedarach* was determined using the DPPH assay. Briefly, 0.2–0.5 mL of each extract was mixed with 1.8–1.5 mL of methanol and 0.5 mL of DPPH methanolic solution (304.0 µmol/L). The resulting mixtures were vigorously shaken and incubated in the dark for 15 min. The absorbance was then measured at 517 nm against a reagent blank consisting of 2 mL of methanol and 0.5 mL of DPPH methanolic solution using a Hitachi U-2900 UV–Vis spectrophotometer with a 1 cm glass cuvette. The DPPH radical scavenging activity was subsequently calculated using Equation (1).

mixtures were incubated at 30 °C for 5 min. The absorbance was measured at 734 nm against a reagent blank containing 2.5 mL of ABTS^{•+} solution. The ABTS radical scavenging activity was subsequently calculated using Equation (2).

where: $A_{control}$ —absorbance of ABTS radical cation with methanol; A_{sample} —absorbance of ABTS radical cation with extract (or standard solution).

The calibration curve, $\%ABTS = (407.40 \pm 3.40) cTE + (8.98 \pm 0.29)$, was prepared using working solutions of TE between 0.01 and 0.15 mol/mL.

$$\%ABTS_{scavenging} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100\%$$

2.8.3. FRAP Assay

The ferric reducing antioxidant power (FRAP) assay was performed by transferring 0.1–0.6 mL of the leaf powder and chocolate extracts, together with 2 mL of FRAP reagent (prepared by mixing 100 mL of 10 mmol/L TPTZ solution in 40 mmol/L HCl, 10 mL of 20 mmol/L FeCl₃, and 25 mL of 0.1 mol/L acetate buffer, pH 3.6), into 10 mL volumetric flasks. The volume was then adjusted to 10 mL with redistilled water. After incubation for 20 min, the absorbance was measured at 593 nm against a reagent blank consisting of 2 mL of FRAP reagent diluted to 10 mL with redistilled water. A calibration curve, $A_{593} = (50.49 \pm 0.42) cTE - (0.0022 \pm 0.0039)$, was prepared using Trolox

equivalent (TE) working solutions in methanol at concentrations ranging from 0.001 to 0.017 µmol/mL

2.8.4. CUPRAC Assay

The cupric ion reducing antioxidant capacity (CUPRAC) assay was performed by transferring 0.6 mL of each prepared extract, 2 mL of 0.01 mol/L Cu (II) solution, 2 mL of neocuproine solution (0.0075 mol/L), and 2 mL of ammonium acetate buffer (pH 7.0) into 10 mL volumetric flasks. The reaction mixtures were made up to the final volume with redistilled water and allowed to stand at room temperature for 30 min. The absorbance was then measured at 450 nm against a reagent blank containing 2 mL of CuCl₂ solution, 2 mL of

neocuproine solution, and 2 mL of ammonium acetate buffer, diluted to 10 mL with redistilled water.

A calibration curve, $A_{450} = (7.98 \pm 0.07) \text{ cTE} - (0.0099 \pm 0.0030)$, was prepared using working solutions of Trolox equivalent (TE) in methanol at concentrations ranging from 0.01 to 0.08 $\mu\text{mol/mL}$.

3. Results and Discussion

3.1. Phytochemical Components Analysis

In the present study, the preliminary phytochemical screening of *Azadirachta indica* and *Melia azedarach*

secondary metabolites, was carried out in four different solvents (chloroform, methanol, hexane and water). The presence of steroids, saponins, tannins, anthraquinones, amino acids, flavonoids, phenolic compounds, and alkaloids was detected. In *Azadirachta indica* methanolic extracts alkaloids, saponins, phenolic compounds, anthraquinones, flavonoids, and tannins were identified, whereas the aqueous extract was found to contain alkaloids, steroids, flavonoids, and saponins. In the methanolic extract of *Melia azedarach*, steroids, phenolic compounds, anthraquinones, flavonoids, and tannins were detected (Table 1).

Table 1: Preliminary phytochemical screening of different extracts of *Azadirachta indica* and *Melia azedarach* was performed.

Types of constituents	Chloroform		Methanol		Hexane		Aqueous	
	A. indica	M. azedarach	A. indica	M. azedarach	A. indica	M. azedarach	A. indica	M. azedarach
Steroids	–	–	–	+	–	+	+	–
Phenol	+	–	+	+	+	–	–	+
Flavonoids	+	–	+	+	–	+	+	+
Tannins	–	+	+	–	–	–	–	+
Saponins	–	+	+	–	+	–	+	+
Alkaloids	+	+	+	–	–	–	+	–
Anthraquinones	–	–	+	+	–	–	–	–
Amino acid	–	–	–	+	–	+	–	–

Qualitative phytochemical screening confirmed the presence of several classes of secondary metabolites, including phenolics, flavonoids, alkaloids, tannins, saponins, steroids, anthraquinones, and amino acids. However, the distribution of these metabolites differed according to the extraction solvent, indicating that solvent polarity markedly influences extraction efficiency. Methanol extracted the widest range of phytochemicals from both *A. indica* and *M. azedarach*, which is consistent with the high polarity of methanol and its ability to solubilize both polar and moderately polar compounds. Similar observations have been reported by [33], [34], who demonstrated that methanolic extracts of both species contain abundant phenolics, flavonoids, alkaloids, tannins, and saponins.

Phenolic compounds and flavonoids are among the major natural antioxidants because of their ability to donate hydrogen atoms and electrons, thereby

neutralizing reactive oxygen species. Likewise, alkaloids, tannins, saponins, and anthraquinones have been reported to possess antimicrobial, anti-inflammatory, anticancer, and immunomodulatory properties. Therefore, the presence of these metabolites provides a biochemical basis for the pharmacological activities observed in the present study.

3.2. Isolation of Compounds by HPLC

High-performance liquid chromatography (HPLC) analysis was performed to separate and characterize the phytochemical constituents present in the methanolic extracts of *Azadirachta indica* and *Melia azedarach* (Figures 1 and 2). In the HPLC chromatogram of *A. indica*, three major peaks were detected. Among these, the second peak, with a retention time of 9.56 min, exhibited the highest relative concentration (41.06%), whereas the third peak, observed at a retention time of 10.90 min, showed the lowest peak intensity (28.57%).

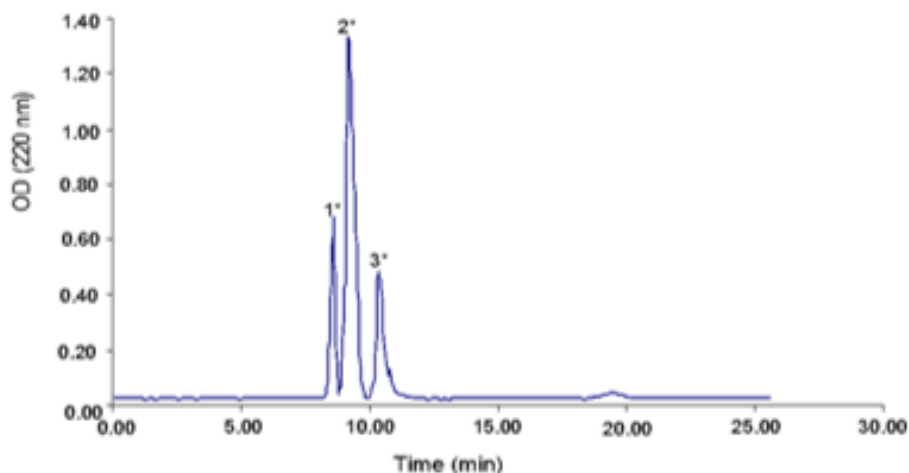


Figure 1: HPLC fingerprint profile of the methanolic extract of *Azadirachta indica*.

Similarly, the HPLC chromatogram of the methanolic extract of *M. azedarach* was recorded at a detection wavelength of 220 nm. A total of seven chromatographic peaks were detected. The fourth peak,

appearing at a retention time of 12.55 min, exhibited the highest relative concentration (27.99%), while the first peak, with a retention time of 3.23 min, displayed the lowest peak intensity (3.26%).

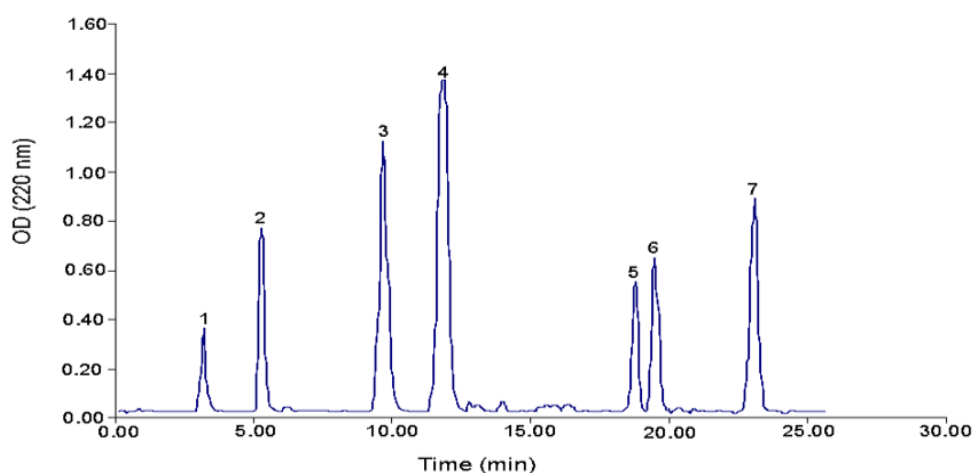


Figure 2: HPLC fingerprint profile of the methanolic extract of *Melia azedarach*.

HPLC chromatographic analysis revealed distinct phytochemical profiles for both plant species. The chromatograms of *A. indica* and *M. azedarach* exhibited seven and ten major peaks, respectively, indicating the presence of multiple phytochemical constituents. The predominance of one major peak in each chromatogram suggests that a few compounds are present at relatively higher concentrations than others.

Although the compounds were not identified by comparison with authentic standards or LC-MS analysis, the chromatographic fingerprints provide valuable information regarding the chemical complexity of the extracts and may serve as quality-control markers for future standardization. Previous investigations have similarly reported complex HPLC chromatograms for methanolic extracts of *A. indica* and *M. azedarach*, reflecting the presence of limonoids, flavonoids,

phenolic acids, and other secondary metabolites.

3.3 TLC Profiling of Phenolic Compounds and Flavonoids

Thin-layer chromatography (TLC) was performed to separate the phenolic compounds present in the methanolic extracts of *Azadirachta indica* and *Melia azedarach*. In the *A. indica* extract, eight distinct phenolic bands were detected with R_f values of 0.108, 0.142, 0.227, 0.244, 0.532, 0.671, 0.903, and 0.998. In the *M. azedarach* extract, sixteen distinct phenolic bands were observed with R_f values of 0.084, 0.122, 0.169, 0.216, 0.273, 0.348, 0.367, 0.442, 0.556, 0.650, 0.744, 0.782, 0.820, 0.839, 0.933, and 0.971. All chromatographic bands appeared golden yellow in colour and were visualized only after treatment with iodine spray (Table 2).

Table 2: Thin-Layer Chromatographic Profiling of phenols in *Azadirachta indica* and *Melia azedarach*.

Rf value	A. indica	M. azedarach
0.084	–	+
0.108	+	–
0.122	–	+
0.142	+	–
0.169	–	+
0.216	–	+
0.227	+	–
0.244	+	–
0.273	–	+
0.348	–	+
0.367	–	+
0.442	–	+
0.532	+	–
0.556	–	+
0.650	–	+
0.671	+	–
0.744	–	+
0.782	–	+
0.820	–	+
0.839	–	+
0.903	+	–
0.933	–	+
0.971	–	+
0.998	+	–
total	8	16

TLC analysis was also performed for the separation of flavonoid compounds present in the methanolic extracts of *A. indica* and *M. azedarach*. In the *A. indica* extract, three distinct flavonoids bands were detected with Rf values of 0.040, 0.456, and 0.650. Similarly,

three flavonoid bands were observed in the *M. azedarach* extract with Rf values of 0.026, 0.107, and 0.171. All steroidal bands exhibited a bluish-green colour and were visualized only after exposure to iodine vapour (Table 3).

Table 3: Thin-Layer Chromatographic Profiling of Flavonoids in *Azadirachta indica* and *Melia azedarach*.

Rf value	A. indica	M.
0.026	–	+
0.040	+	–
0.107	–	+
0.171	–	+
0.456	+	–
0.650	+	–
Total	3	3

TLC analysis further demonstrated the chemical diversity of the methanolic extracts. The higher number of phenolic bands observed in *M. azedarach* compared

with *A. indica* suggests greater qualitative diversity of phenolic constituents. Similarly, flavonoid compounds were successfully separated in both species, confirming

the presence of structurally distinct metabolites.

TLC fingerprinting remains an inexpensive and reliable technique for the preliminary identification and quality assessment of medicinal plants. The characteristic R_f values obtained in the present study may therefore be useful as reference fingerprints for authentication and routine quality control of these plant materials.

3.4. Correlation Between Antioxidant Capacity and Phytochemical Constituents

The Pearson correlation coefficient was used to evaluate the relationship between phytochemical constituents and antioxidant capacity. In the present (Table 4) data, phenolic compounds showed a very strong positive

correlation with antioxidant capacity, particularly with DPPH ($r = 0.99$), followed by ABTS ($r = 0.95$), FRAP ($r = 0.93$) and CUPRAC ($r = 0.95$). Alkaloids also showed relatively strong positive correlations with DPPH ($r = 0.82$), ABTS ($r = 0.78$), FRAP ($r = 0.69$) and CUPRAC ($r = 0.75$). Flavonoids showed moderate positive correlations ($r = 0.49$ – 0.56), whereas saponins showed weak-to-moderate positive correlations ($r = 0.29$ – 0.52).

Steroids, tannins, anthraquinones, and amino acids generally exhibited weak positive correlations with antioxidant capacity. Among these, amino acids showed the weakest associations, particularly with FRAP ($r = 0.03$) and CUPRAC ($r = 0.04$).

Table 4: Correlation coefficients (r) between antioxidant capacity determined by different analytical methods, total phenol, steroids, flavonoids, Tannins, saponins, alkaloids, anthraquinones and Amino acid in with standard.

Compounds	DPPH	ABST	FRAP	CUPRAC
Steroids	0.40	0.24	0.20	0.25
Phenol	0.99 *	0.95	0.93	0.95
Flavonoids	0.50	0.49	0.52	0.56
Tannins	0.29	0.11	0.08	0.11
Saponins	0.52	0.32	0.29	0.33
Alkaloids	0.82	0.78	0.69	0.75
Anthraquinones	0.18	0.33	0.39	0.41
Amino acid	0.27	0.09	0.03	0.04

* Statistically significant at $p \leq 0.05$.

Correlation analysis demonstrated that total phenolic content showed the strongest positive relationship with antioxidant activity in all four antioxidant assays, particularly DPPH ($r = 0.99$, $p < 0.05$). This finding indicates that phenolic compounds are the principal contributors to the antioxidant potential of the investigated plants.

Moderate correlations were observed for flavonoids and saponins, suggesting that these metabolites also contribute to antioxidant activity through complementary mechanisms. In contrast, steroids, alkaloids, tannins, anthraquinones, and amino acids exhibited comparatively weaker correlations, indicating a relatively smaller contribution to the overall antioxidant capacity.

These observations are consistent with numerous previous studies demonstrating that phenolic compounds are the major determinants of antioxidant activity in medicinal plants.

3.5. Antioxidant Capacity of Azadirachta indica and Melia azedarach Leaves extract

The antioxidant capacity of Azadirachta indica and Melia azedarach leaves extract was determined using in vitro antioxidant assays, namely DPPH, ABTS, CUPRAC, and FRAP. The results, expressed as mmol

Trolox equivalents (TE)/100 g, are presented in (Table 5). Among the two leaves extract, A. indica exhibited substantially higher antioxidant capacity than M. azedarach in all the assays performed. The DPPH radical scavenging assay showed the highest value 258 ± 9 mmol TE/100 g for A. indica, whereas M. azedarach exhibited a considerably lower value of 30.7 ± 1.2 mmol TE/100 g. Similarly, the ABTS assay revealed antioxidant capacities of 256.8 ± 1.3 mmol TE/100 g for A. indica and 31.9 ± 0.4 mmol TE/100 g for M. azedarach. The CUPRAC assay demonstrated a cupric ion reducing capacity of 151.8 ± 1.9 mmol TE/100 g for A. indica, which was markedly higher than the value of 42.8 ± 0.8 mmol TE/100 g recorded for M. azedarach. Likewise, the ferric reducing antioxidant power (FRAP) assay showed values of 50.9 ± 0.15 mmol TE/100 g and 8.99 ± 0.05 mmol TE/100 g for A. indica and M. azedarach, respectively.

Overall, A. indica leaves extract demonstrated significantly greater antioxidant capacity than M. azedarach leaf powder across all four antioxidant assays. The highest antioxidant capacities were observed in the DPPH and ABTS assays for A. indica, whereas the lowest values were recorded in the FRAP assay for M. azedarach. These findings indicate the superior free radical scavenging and reducing potential of A. indica leaves extract compared with M. azedarach

leaves extract.

Table 5: Antioxidant capacity of *A. indica* leaf powder and *M. azedarach* leaves extract.

Antioxidant capacity (mmol TE/100 g)		
Quality characteristics	A. Indica (leaves extract)	M. azedarach (leaves extract)
DPPH assay	258 ± 9	30.7 ± 1.2
ABTS assay	256.8 ± 1.3	31.9 ± 0.4
CUPRAC assay	151.8 ± 1.9	42.8 ± 0.8
FRAP assay	50.9 ± 0.15	8.99 ± 0.05

Values are expressed as mean ± SD. Statistical significance should be determined using the Tukey's test; $p < 0.05$.

Both plant species exhibited considerable antioxidant capacity; however, *A. indica* consistently demonstrated higher antioxidant activity than *M. azedarach* in the DPPH, ABTS, FRAP, and CUPRAC assays. These findings indicate that *A. indica* possesses greater free radical scavenging ability and reducing power.

The superior antioxidant activity of *A. indica* is likely attributable to its higher concentration of phenolic compounds and flavonoids. Phenolic hydroxyl groups readily donate hydrogen atoms or electrons to free radicals, thereby interrupting oxidative chain reactions. Similar findings have been reported by [35], and other researchers, who observed strong antioxidant activity in neem leaf extracts owing to their high phenolic and flavonoid contents.

The markedly higher values obtained in the DPPH and ABTS assays compared with FRAP and CUPRAC indicate that the extracts possess greater radical scavenging capacity than reducing ability. This difference reflects the distinct mechanisms measured by each antioxidant assay.

3.6. Antimicrobial Activity of *Azadirachta indica* and *Melia azedarach* Methanolic Extracts

The antimicrobial activity of *Azadirachta indica* leaves and *Melia azedarach* leaves extracts was evaluated against *E. coli*, *E. aerogenes*, *Staphylococcus E.* and *Bacillus C.* using disc- and well-diffusion assays. The zones of inhibition (ZOIs) obtained for both plant extracts varied according to the bacterial strain and assay method. Results are presented in (Table 6). These results indicated that the Gram negative (GN) bacterial species were more susceptible to all the tested plant extracts than the Gram Positive (GP) bacterial species. Conversely, smaller zones of inhibition (ZOIs) were observed around the discs than around the wells. These

findings agreed with those reported in a previous study [32].

For *A. indica*, the inhibition zones obtained by the disc-diffusion assay were 15 ± 0.11 , 11 ± 0.17 , 18 ± 0.23 , and 16 ± 0.11 mm against *Bacillus C.*, *Staphylococcus E.*, *E. coli*, and *E. aerogenes*, respectively. In the well-diffusion assay, the corresponding inhibition zones were 16 ± 0.28 , 12 ± 0.21 , 21 ± 0.18 , and 18 ± 0.27 mm, respectively. The maximum inhibition zone for *A. indica* was therefore recorded against *E. coli* (21 ± 0.18 mm) in the well-diffusion assay.

For *M. azedarach*, inhibition zones of 12 ± 0.15 , 9 ± 0.42 , 16 ± 0.28 , and 13 ± 0.15 mm were recorded against *Bacillus C.*, *Staphylococcus E.*, *E. coli*, and *E. aerogenes*, respectively, using the disc-diffusion assay. The corresponding values obtained by the well-diffusion assay were 12 ± 0.29 , 11 ± 0.16 , 18 ± 0.21 , and 12 ± 0.15 mm, respectively. The highest inhibition zone for *M. azedarach* was observed against *E. coli* (18 ± 0.21 mm) using the well-diffusion assay.

Among the reference antibiotics, vancomycin produced inhibition zones of 12 ± 0.22 , 9 ± 0.11 , 7 ± 0.21 , and 6 ± 0.21 mm in the disc-diffusion assay and 10 ± 0.23 , 9 ± 0.24 , 8 ± 0.24 , and 14 ± 0.27 mm in the well-diffusion assay against *Bacillus C.*, *Staphylococcus E.*, *E. coli*, and *E. aerogenes*, respectively.

Ciprofloxacin produced inhibition zones of 8 ± 0.24 , 9 ± 0.15 , 14 ± 0.28 , and 11 ± 0.15 mm in the disc-diffusion assay and 16 ± 0.19 , 12 ± 0.31 , 11 ± 0.31 , and 10 ± 0.29 mm in the well-diffusion assay against the respective bacterial strains. Overall, the maximum inhibition zone among all tested samples was recorded for *A. indica* against *E. coli* (21 ± 0.18 mm) using the well-diffusion assay.

Table 6: Antimicrobial activity and zone of inhibition (diameter in mm) of the tested plant leaves extracts of *A. indica* L. and *M. azedarach* L. against selected Gram negative and Gram-positive bacteria.

Tested Extracts	Diffusion assay	Targeted Pathogenic bacteria			
		Bacillus C.	Staphylococcus	E. Coli	E.

			E.		Aerogenes
A. indica	Disc	15 ±0.11	11 ±0.17	18 ±0.23	16 ±0.11
	Well	16 ±0.28	12 ±0.21	21 ±0.18	18 ±0.27
M. azedarach	Disc	12 ±0.15	9 ±0.42	16 ±0.28	13 ±0.15
	Well	12 ±0.29	11 ±0.16	18 ±0.21	12 ±0.15
Vanco.	Disc	12 ±0.22	9 ±0.11	7 ±0.21	6 ±0.21
	Well	10 ±0.23	9 ±0.24	8 ±0.24	14 ±0.27
Cipro.	Disc	8 ±0.24	9 ±0.15	14 ±0.28	11 ±0.15
	Well	16 ±0.19	12 ±0.31	11 ±0.31	10 ±0.29

The findings demonstrated that both *A. indica* and *M. azedarach* extracts possessed antibacterial activity against all the tested bacterial strains. However, the magnitude of inhibition varied according to the plant extract, bacterial strain, and diffusion method. In general, the well-diffusion assay produced larger inhibition zones than the disc-diffusion assay for the plant extracts, with the most pronounced activity being observed for *A. indica* against *E. coli*.

The comparatively greater activity of *A. indica* may be associated with the presence of bioactive phytochemicals capable of exerting antibacterial effects. The higher inhibition observed against *E. coli* suggests that the constituents of the extract may be particularly effective against this bacterial strain. In comparison, *M. azedarach* also exhibited its highest activity against *E. coli*, although the inhibition zone was lower than that observed for *A. indica*. These differences may reflect variations in the type, concentration, and antibacterial potency of phytoconstituents present in the two plant extracts.

The well-diffusion assay generally resulted in larger inhibition zones than the disc-diffusion assay. This difference may be related to the greater quantity of extract that can be introduced directly into the agar well and the subsequent diffusion of soluble phytoconstituents through the agar medium. In contrast, diffusion from a paper disc may be influenced by the adsorption of extract constituents onto the disc material and by the rate at which the compounds are released into the agar. Thus, the well-diffusion method may provide improved exposure of bacterial cells to certain extract constituents.

The reference antibiotics also exhibited variable inhibition zones between the two assay methods. Vancomycin showed comparatively greater activity

against *E. aerogenes* in the well-diffusion assay, whereas ciprofloxacin produced its highest inhibition against *Bacillus C.* in the well-diffusion assay. Such differences indicate that the diffusion characteristics of individual antimicrobial agents can vary according to the assay format and bacterial strain.

Notably, the inhibition zone produced by *A. indica* against *E. coli* (21 ± 0.18 mm) was greater than those produced by the tested reference antibiotics against the same organism under the corresponding assay conditions. This observation suggests a promising antibacterial potential of the *A. indica* extract. Nevertheless, direct comparison between crude plant extracts and standard antibiotics should be interpreted cautiously because their concentrations, diffusion characteristics, chemical composition, and mechanisms of action differ.

Overall, the results indicate that both *A. indica* and *M. azedarach* extracts possess promising antibacterial properties, with *A. indica* showing comparatively stronger activity under the present experimental conditions. The well-diffusion assay appeared to be more suitable for demonstrating the antibacterial activity of these extracts, particularly against *E. coli*. Further studies are warranted to identify the active phytoconstituents and elucidate their mechanisms of antibacterial action.

3.7. Minimum inhibition concentration

The antibacterial activity of different concentrations (10–100 mg/mL) of *Azadirachta indica* leaves and *Melia azedarach* leaves extracts were evaluated by determining the minimum inhibitory concentration (MIC) at different exposure times (5, 10, 20, and 30 min) against the selected bacterial strains as shown in Figure 3 and 4 respectively.

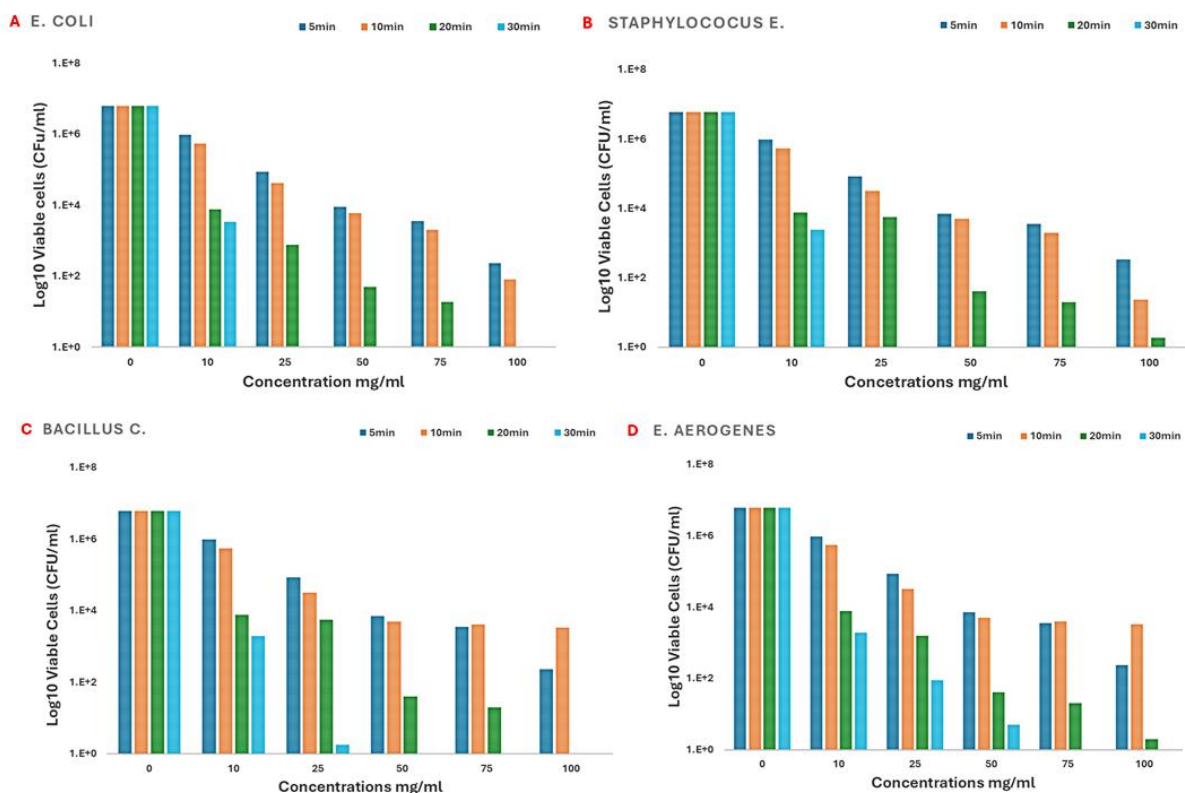


Figure 3: Minimum inhibitory concentration (MIC) values of the methanolic extract of *Azadirachta indica* L. against the examined bacterial species are presented. The remaining viable cell populations following exposure to the extract for 5, 10, 20, and 30 min are also shown.

Statistical analysis was performed using two-way analysis of variance (ANOVA). A moderate correlation is indicated by ** ($p \leq 0.01$), whereas a high correlation is indicated by *** ($p \leq 0.001$).

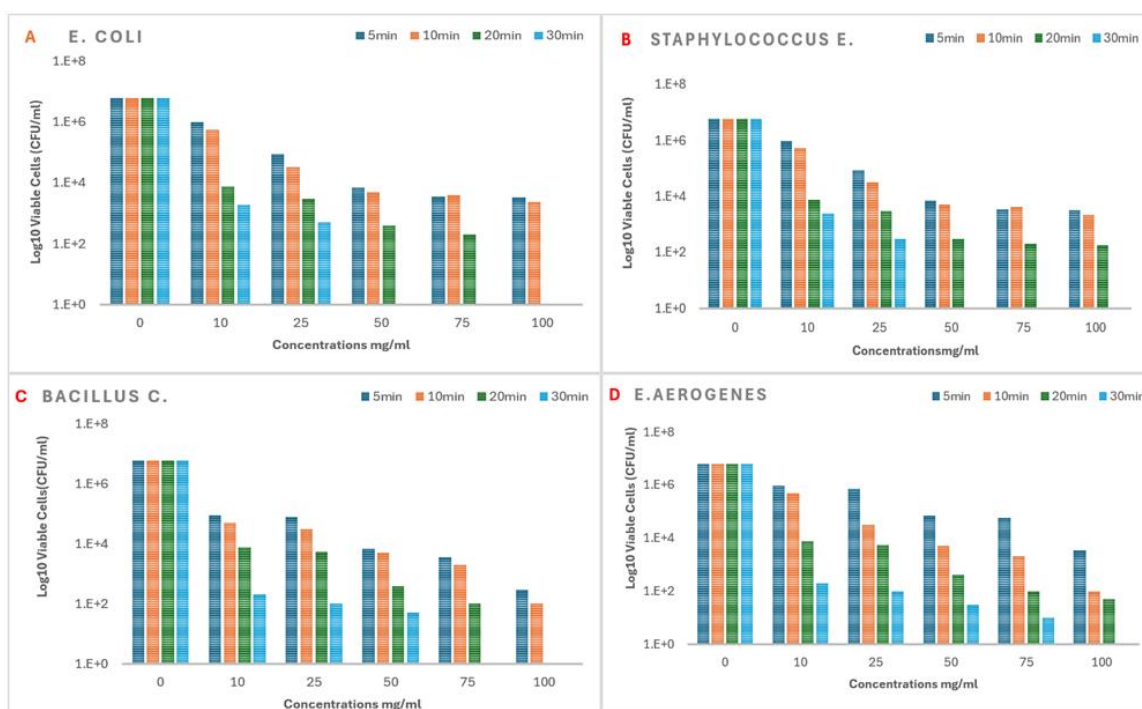


Figure 4: Minimum inhibitory concentration (MIC) values of the methanolic leaf extract of *M. azedarach* L. against the examined bacterial species. The remaining viable cell populations at exposure intervals of 5, 10, 20, and 30 min are also shown.

Two-way analysis of variance (ANOVA) indicated a moderate correlation ($p \leq 0.01$) and a high correlation ($p \leq 0.001$).

The antibacterial activity observed for both *A. indica* leaves and *M. azedarach* leaves extracts demonstrate their potential to inhibit the growth of the tested bacterial strains. The dependence of MIC values on extract concentration and exposure time suggests that the antibacterial effects were concentration- and time-dependent. The greater inhibitory effects observed at longer exposure periods may indicate that prolonged contact between the bioactive constituents and bacterial cells enhanced their antibacterial action.

The differential susceptibility of Gram-negative and Gram-positive bacteria may be associated with differences in their cellular structures and with the chemical composition of the plant extracts. The antibacterial constituents present in these extracts may interact with bacterial cell envelopes, alter membrane integrity, interfere with cellular metabolism, or affect other essential cellular processes [36]. Differences in the composition and accessibility of bacterial cell-wall and membrane structures may therefore contribute to the variation in susceptibility observed among the tested strains.

The pronounced activity of the extracts against Gram-negative bacteria is of particular interest because infections caused by multidrug-resistant Gram-negative bacteria can present substantial challenges. The present findings therefore support the potential of *A. indica* and *M. azedarach* extracts as sources of antibacterial phytochemicals. However, further studies are required to identify the specific bioactive constituents responsible for the observed activity and to clarify their mechanisms of antibacterial action.

Taken together, the findings indicate that both *A. indica* and *M. azedarach* extracts possess concentration- and time-dependent antibacterial activity against the tested bacterial strains, with comparatively greater activity observed against Gram-negative bacteria. The results obtained from the MIC assay further support the antibacterial potential of these medicinal plants and provide a basis for subsequent investigations of their active constituents and mechanisms of action.

Conclusion

Overall, the findings indicate that both *Azadirachta indica* and *Melia azedarach* are valuable sources of biologically active secondary metabolites. Methanol proved to be the most effective extraction solvent, yielding extracts rich in phenolics and flavonoids that exhibited strong antioxidant and antibacterial

activities. Among the two species, *A. indica* demonstrated superior antioxidant capacity and greater antibacterial potency in MIC assays, whereas *M. azedarach* generally produced larger inhibition zones in well diffusion assays. The strong association between total phenolic content and antioxidant activity highlights the important role of phenolic compounds in the pharmacological properties of these plants. These findings support the traditional medicinal use of both species and suggest that they may serve as promising natural sources of antioxidant and antimicrobial agents for future pharmaceutical applications.

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Author Contributions

RY, JY, DY, NY, NY: Investigation and Data Collection; **JPG, GSR, KSD:** Methodology and Supervision; **SJ, AP, MY:** Conceptualization, Data Analysis, Manuscript Drafting, Reviewing and Editing; All authors reviewed the results and approved the final version of the manuscript.

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No conflict of interest is declared by the authors.

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AI language tools (ChatGPT and Grammarly Premium) were used to enhance the linguistic clarity and readability of the manuscript. All generated text was carefully reviewed and edited to ensure accuracy and alignment with the intended meaning of the research.

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