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Research Article

Azadirachta indica leaf extract: Phytochemical composition and antioxidant, antibacterial, and antifungal activities

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Abstract: The chemical components and natural traits of *Azadirachta indica* A. Juss. leaf extract from arid environments has been studied. LC-MS analysis identified quercetin, oleanolic acid, salicin, and catechin among the many bioactive compounds in the sample. It was observed that at a concentration of 25.01 ± 0.71 µg/mL, DPPH exhibited antioxidant activity with an IC_{50} value that is higher than that of β-carotene bleaching (22.29 ± 0.66 µg/mL), ABTS assay (45.09 ± 1.23 µg/mL), respectively. *Bacillussubtilis*, *Escherichia coli, Staphylococcus aureus,* and *Pseudomonas aeruginosa* showed susceptibility to the extract, with MICs ranging from VHC to 30 mg/mL while *Alternaria* sp., *Fusarium solani*, and *Thielaviopsis paradox* represent the fungal strains tested for their sensitivity to this extract. Therefore, *Azadirachta indica* leaf extract from dry regions exhibits significant effects, potentially explaining its traditional use in medicine and agriculture.

1. INTRODUCTION

Azadirachta indica A. Juss., sometimes referred to as Neem, is perennial tree that belonges to the Meliaceae family (Bolaji *et al.*, 2024). It is extensively found in subtropical and tropical regions (Bolaji *et al.*, 2024). *A. indica* is renowned for its various applications in traditional medicine, agriculture and cosmetics, due to its pharmacological properties and bioactive compounds (Monyela *et al.*, 2024; Sarkar *et al.*, 2021). Among these compounds are alkaloids, triterpenoids, flavonoids, tannins, and phenolic compounds, which have demonstrated various physiological impacts (Singh *et al.*, 2021; Kumar *et al.*, 2022).

In arid environments, where environmental conditions can be extreme, plants have evolved defense mechanisms to survive (Zahedi *et al.*, 2019; Alsharif *et al.*, 2020; Mohammed *et al.*, 2024). These defense mechanisms may include increased production of phytochemicals, which help protect the plant against oxidative stress and microbial attack. As a result of these

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adaptations, plant extracts from arid environments may exhibit distinct phytochemical profiles and biological activities compared to those from humid environments (Hussain *et al.*, 2021; Avasiloaiei *et al.*, 2023; El-Chaghaby*et al.*, 2024; Uysal *et al.*, 2024). The El Oued region of Algeria, characterized by its arid climate, is home to a diverse population of plants, including *A. indica*. *A. indica* leaves from this region may exhibit unique phytochemical characteristics due to the specific environmental conditions, making them particularly interesting for in-depth studies on their bioactive compounds and biological activities (Ariom *et al.*, 2022; Abid *et al.*, 2021).

The intent of this research is to examine the chemical composition as well as evaluate the antioxidant, antibacterial, and antifungal properties of extract obtained from *A. indica* leaves collected in the El Oued region in Algeria. By evaluating the biological properties of these extracts, we seek to understand their pharmacological potential and explore new sources of natural compounds for therapeutic and agricultural applications.

2. MATERIAL and METHODS

2.1. Sample Collection and Preparation

The leaves of *A. indica* (Figure 1) were obtained from the El Oued Province in Algeria, which is considered an arid region with a desert climate. Morphological and botanical characters were used by the Algerian National Institute of Agricultural Research, Sidi Mehdi, to identify the plant. It has been carefully washed to remove any dust or residue. It is then dried for 15 days at 25 ± 2°C in the shade until its weight is constant; then it is ground into a fine powder.



Figure 1. *Azadirachta indica* leaves.

2.2. Extraction Procedure

50 g of powdered *A. indica* leaves were used in a Soxhlet extraction process, using 200 mL of methanol as the solvent. The extraction was done multiple times until the extract no longer had color, which showed that all the necessary components had been extracted. The final crude extract was obtained by removing the solvent under low pressure with a rotary evaporator (Hasnat *et al.*, 2023; Shikov *et al.*, 2022).

2.3. Phytochemical Analysis

2.3.1. *LC-MS analysis*

An analysis was conducted on the phytochemical content of the *A. indica* extract using a Shimadzu 8040 Ultra-High Sensitivity LC-MS system that was fitted with a Nexera XR LC-20AD binary pump. Utilizing a Restek Ultra C18 column (3µm, 150x2.1mm), separation was accomplished. Methanol (solvent B) and 0.1% formic acid (solvent A) in water made up the mobile phase. A gradient elution technique was used, where solvent A was diluted linearly to 5% after ten minutes at 85% solvent A. For 10–18 minutes, solvent A's composition was kept at 5%, and for 18–23 minutes, it was raised to 85% (Khattabi *et al.*, 2023).

2.3.2. *Determination of total phenolic content (TPC)*

The TPC was carried out by spectrophotometry, using the colorimetric method with the Folin-Ciocalteu reagent (El Aanachi *et al.,* 2020). The protocol used is based on that described by Nacer *et al.* (2023).

2.3.3. *Determination of total flavonoid content (TFC)*

The determination of flavonoids is performed using the aluminum trichloride ($AlCl_3$) technique, as previously outlined in studies by Nacer *et al.* (2023) and Ngoumen *et al.* (2022).

2.4. Antioxidant Assays

2.4.1. *DPPH test*

The extract's ability to counteract DPPH radicals was examined following the procedure outlined by (Olugbami *et al.*, 2015). Extract was mixed with a DPPH radical solution (1 mM in methanol) in a 96-well microplate with different concentrations and then incubated in the dark at 25 ± 2 °C for 30 minutes. The measurement of absorbance was conducted at a wavelength of 517 nm. The standard antioxidants BHA and BHT were also evaluated. The effectiveness of the samples in neutralizing DPPH was assessed by determining the inhibition rates using Equation 1.

$$\text{DPPH inhibition } (\%) = \left[\frac{(A_0 - A_1)}{A_0}\right] \times 100 \quad (1)$$

where, A_0 referred to control and A_1 to sample). The IC_{50} was estimated from the graph of inhibition percentages at various doses.

2.4.2. *ABTS radical scavenging assay*

The ability of ABTS to remove free radicals was evaluated by using the methodology illustrated by Nacer *et al.* (2023).

2.4.3. *β-carotene bleaching assay*

The protocol consists of mixing 100 mg of methanolic extract of *A. indica* with 10 mL of a mixture of acetone and hexane and stirring the mixture for one minute. Then the mixture was filtered through Wattman N 4 paper. Measure the absorbance of the filtrates at different wavelengths: 453 nm, 505 nm, 645 nm and 663nm. Results obtained were expressed in µg of β-carotene/mg of dry extract (Nacer *et al.,* 2023). The pigment content is calculated using equation 2.

$$\beta - carotene(mg/100mL) = 0.216 \times A_{663} - 0.304 \times A_{505} + 0.452 \times A_{453} \quad (2)$$

2.5. Biological Activity

2.5.1. *Broth microdilution assay*

The broth microdilution test was carried out as per the recommendations of the Clinical and Laboratory Standards Institute (Humphries *et al.,* 2021; Pierce & Mathers, 2021). A suspension of bacteria and yeast was created with a turbidity of 0.5 McFarland standard (1.5×10^8 CFU/mL for bacteria and 10^6 CFU/mL for yeast). The plant extract solution (80 mg/mL) was prepared by dissolving the extract in dimethyl sulfoxide (DMSO). A microtiter plate was then prepared by adding 100 µL of plant extract solution to each well, along with 50 µL of bacterial or yeast suspension. The wells underwent monitoring for growth and sterility. The microtiter plate was then placed in an incubator at 37°C for 18 to 24 hours for bacteria and 48 hours for yeast. The minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) were determined by transferring a well sample showing no growth onto appropriate agar plates (Benková *et al.,* 2020).

2.5.2. *Antifungal activity assay*

The researchers tested the antifungal properties of the plant extract by creating a solution with a concentration of 4 mg/mL in DMSO. They mixed this solution into potato dextrose agar (PDA) for the poison food method (Boudjaber *et al.*, 2023). Fungal pathogens were then added to Petri dishes with the extract, and areas of inhibition were noted. The fungal pathogens' colony diameter in centimeters was used to calculate the percentage inhibition of mycelium growth. The equation (3) was utilized to obtain the % inhibition.

$$\%\ inhibition = \frac{D_1 - D_2}{D_1} \times 100 \quad (3)$$

where, D_1 and D_2 represent the diameter of the fungal colony in the control plate (without extract treatment) and the treated plate (with extract treatment), respectively.

2.6. Analytical Statistics

The study was done three times, and the average plus-minus standard deviation (SD) was used to explain the results. Tukey's test was carried out for comparing multiple groups after conducting a one-way analysis of variance (ANOVA), with a significance threshold of $p < 0.05$. Each experiment involved three biological repeats, and distinctions between groups were computed using the student's *t*-test.

3. RESULTS and DISCUSSION

3.1. Quantification of TPC and TFC

The extract of *A. indica* has been shown to contain significant levels of flavonoids and phenolic compounds. The measurements show that there are 49.63±0.159 µg GAE) and 12.481±0.242 µg QE per mg of extract. This is similar to what other studies have found in *A. indica* extracts from other places (Batista *et al.*, 2018; Boukeloua *et al.*, 2024; Islas *et al.*, 2020; Oke *et al.*, 2020; Patil *et al.*, 2023; Vats, 2016), which suggests that the plant has the same bioactive compounds in different places. Studies conducted in various locations have reported a wide range of TFC and TPC values for *A. indica* extracts. For instance, Indian *A. indica* extracts have shown TFC values between 40 and 90 µg GAE/mg extract and TPC values ranging from 50 to 120 µg GAE/mg extract. On the other hand, African *A. indica* extracts have displayed TFC values ranging from 30 to 80 µg GAE/mg extract and TPC values between 40 and 100 µg GAE/mg extract. The differences in TFC and TPC values are due to climate, soil type, and altitude, all of which can change the chemicals in *A. indica* leaves (Islas *et al.*, 2020).

The *A. indica* extract contains flavonoids and phenolic compounds that give it antioxidant properties, shown by its ability to neutralize free radicals in antioxidant tests. The consistent levels of TFC and TPC in *A. indica* leaves across various regions indicate that these beneficial compounds are widely distributed and may have positive effects on health (Oke *et al.*, 2020).

3.2. Identification of Phenolic Compounds by LC-MS

The LC-MS analysis revealed the presence of several bioactive compounds, indicating the diverse phytochemical composition of this plant species. Table 1 illustrates the identified compounds, including ascorbic acid, 8-hydroxyquinoline, β-carotene, curcumin, rutin, oleanolic acid, chrysin, salicin, quercetin, catechin, and thymol, known for their various biological activities and potential health benefits (Gupta *et al.*, 2017).

Among the identified compounds, catechin was found to be the most abundant, representing 64.85% of the total compounds detected. Catechin is a flavonoid compound with strong antioxidant properties. It may be this compound that makes *A. indica* extract so effective (Manach *et al.*, 2004). Another important compound, Oleanolic acid (5.76% of all compounds), has been shown to protect the liver, reduce inflammation, and fight free radicals (Boukeloua *et al.*, 2024; Islas *et al.*, 2020). Salicin, a natural phenolic glycoside, was also abundant in the *A.*

indica extract (17.84% of total compounds). Quercetin, detected at 4.06% of total compounds, further highlights the antioxidant potential of *A. indica* extract (Ahmad, 2023).

Table 1. Data set pertaining to the methanolic extract from *A. indica*'s aerial parts as analyzed by LC-MS.

Compound	Ret. Time	Amount (%)
Curcumin	1.939	0.27
8-hydroxyquinoline	1.986	1.91
Chrysin	2.503	0.23
Thymol	9.033	0.69
Ascorbic acid	9.143	0.97
Quercetin	12.592	4.06
Catechin	13.765	64.85
Oleanolic Acid	14.276	5.76
Salicin	14.818	17.84
Beta caroten	15.862	0.87
Rutin	17.015	2.54

While our study provides insights into the phytochemical composition of *A. indica* from the El Oued region, comparative analyses with extracts from other regions are warranted. Variations in environmental factors and genetic diversity may contribute to differences in the phytochemical makeup of *A. indica* extracts. Future research could explore potential synergistic effects between the identified compounds and investigate the variation in phytochemical composition across different geographical locations. Such studies are crucial for fully understanding the health benefits of *A. indica* extracts and optimizing their utilization in various industries, including medications, cosmetics, and agriculture (Telli *et al.*, 2024).

3.3. Antioxidant Activity

The extract of *A. indica* from the arid environment was found to be moderate in the ABTS assay, with an IC_{50} value of 45.09±1.23µg/mL, indicating its ability to scavenge ABTS radicals (see Table 2). This finding is consistent with Ahmed *et al.*'s study (2023), which also reported the mild antioxidant activity of *A. indica* leaf extract using the ABTS test. The *A. indica* extract also showed strong antioxidant activity in the DPPH assay, with an IC_{50} of 25.01±0.71 µg/mL.

Table 2. Antioxidant activity of *A. indica* leaves.

	IC_{50} µg/mL		
	A. indica extract	BHT	BHA
ABTS assay	45.09±1.23	1.29±0.30	1.81±0.10
β-carotene assay	22.29±0.66	1.05±0.01	22.32±1.19
DPPH assay	25.01±0.71	0.90±0.02	5.73±0.41

This suggests that it might be able to lower inflammation and oxidative stress by scavenging DPPH radicals. Additionally, the *A. indica* extract showed the ability to reduce lipid peroxidation by significantly inhibiting the oxidation of β-carotene (IC_{50} = 22.29±0.66 µg/mL). This aligns with research conducted by Biney *et al.* (2020), where they used HPLC analysis to demonstrate the antioxidant properties of *A. indica* extract. The distinct mechanisms by which each assay operates account for the observed differences between the results of the antioxidant assays (ABTS, DPPH, and β-carotene). The ABTS assay measures the antioxidant's ability to scavenge the ABTS radical cation, which may explain the moderate activity observed in our extract. The DPPH assay, on the other hand, checks how well the extract can donate hydrogen atoms to neutralize the DPPH radical, which results in stronger activity. The β-carotene bleaching assay assesses the inhibition of lipid peroxidation, which indicates the extract's effectiveness in protecting cell membranes from oxidative damage. Based on these differences,

it seems that the antioxidant compounds in *A. indica* may react in different ways depending on the type of free radical or oxidative stress being studied.

Arid environments, characterized by low rainfall and high temperatures, pose challenges to plant growth, leading to oxidative stress (Llauradó *et al.*, 2020; Chaudhry & Sidhu, 2021). Plants may make more antioxidants to protect themselves against reactive oxygen species that are made during photosynthesis and other metabolic processes (Seleiman *et al.*, 2021). Consequently, plants grown in arid environments may exhibit higher levels of antioxidants compared to those grown in more temperate or humid regions (Hassan *et al.*, 2020). Understanding the impact of environmental factors on antioxidant production in *A. indica* plants is vital for harnessing their antioxidant potential and maximizing their health benefits (Sood *et al.*, 2021).

3.4. Broth Microdilution Assay

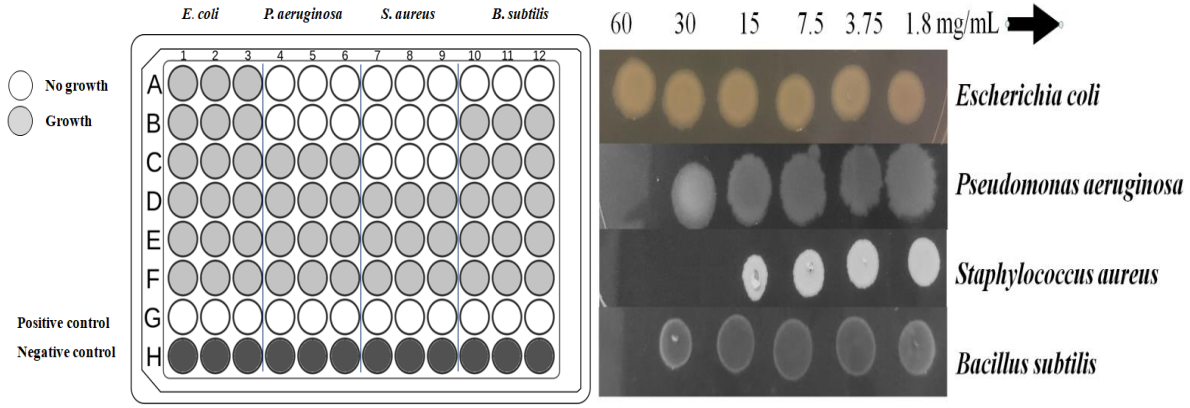
The MIC, MBC, and MBC/MIC ratios of the methanolic extract of *A. indica* were determined against four bacterial strains and results are presented in Table 3 and Figure 2.

Table 3. MBC, MIC, and MBC/MIC ratio of *A. indica* methanolic extract against four bacterial strains.

	MIC (mg/mL)	MBC (mg/mL)	MBC/MIC
Escherichia coli ATCC 25922	VHC	VHC	/
Pseudomonasaeruginosa ATCC 27853	30	60	2
Staphylococcusaureus ATCC 25932	15	30	2
Bacillussubtilis ATCC 25973	30	60	2

*VHC = very high concentration

Figure 2. Antibacterial activity of extract from *A. indica*.



The *A. indica* extract demonstrated limited efficacy against *Escherichia coli* ATCC 25922. Both MIC and MBC required a very high concentration. However, for other studied bacteria, the *A. indica* extract showed promising antibacterial activity, with MIC values of 15 mg/mL, and 30 mg/mL. The MBC values for these strains were double the MIC values, indicating bactericidal activity at higher concentrations. The MBC/MIC ratio of 2 for these strains suggests that the extract is equally effective at inhibiting and killing these bacterial strains at a concentration of twice the MIC.

Comparing these results with studies on *A. indica* extracts from humid environments reveals interesting differences. For example, a study by Prabuseenivasan *et al.* (2006) found that *A. indica* extracts from a humid area have the same antibacterial effect against *E. coli* and *P. aeruginosa* but are less effective against *B. subtilis* and *S. aureus*. This means that *A. indica* extracts from dry places might be just as effective at killing some types of bacteria as *A. indica* extracts from wet places (Gupta *et al.*, 2017).

Variations in the phytochemical makeup of *A. indica* extracts from humid and arid regions may account for the disparity in antibacterial action between them. *A. indica* and other plants produce bioactive chemicals in response to a variety of environmental conditions, including temperature, humidity, and soil composition. Understanding these variations is essential to comprehending the antibacterial qualities of *A. indica* extracts and their effectiveness against bacterial infections in various settings (Alvarez-Caballero & Coy-Barrera, 2019; Kumar *et al.*, 2017).

3.5. Antifungal activity

Table 4 provides a comprehensive view of the antifungal efficacy of *A. indica* leaf extract against *T. paradox*, *Alternaria* sp., and *Fusarium solani*, presenting inhibition rates (%) at concentrations of 1, 2, and 4 mg/mL.

Table 4. Antifungal activity of *A. indica* leaves extract.

Concentraction (mg/mL)	Percentage Mean Mycelial Inhibition (%)		
	Thielaviopsis paradox	*Alternaria* sp.	*Fusariumsolani*
1	66.67±2.94	76.47±4.74	58.43±7.1
2	69.41±1.17	76.47±4.45	59.61±5.8
4	67.45±0.67	88.24±2.96	56.08±8.34

For *T. paradox*, the percentage mean mycelial inhibition ranged from approximately 66.67% to 69.41%, indicating a moderate to high level of inhibition. *A.* sp. showed consistent inhibition ranging from approximately 76.47% to 88.24%, suggesting a strong antifungal effect. *F. solani* exhibited moderate inhibition, with percentages ranging from approximately 56.08% to 59.61%.

Numerous factors, including the sensitivity of the fungal species in the *A. indica* extract and the fact that the extract's impact varies with concentration, might account for the varying percentages of inhibition. The observed variations in susceptibility can potentially result from the distinct fungal cell walls and metabolic activities of the examined fungi.

4. CONCLUSION

In conclusion, this study sheds light on the phytochemical makeup, antioxidant capacity, antibacterial potential, and antifungal characteristics of *A. indica* leaf extracts from dry regions, particularly the El Oued area. Results show that *A. indica* extracts have strong antibacterial and antioxidant properties, with amounts of total flavonoids and phenolics that are similar to what has been found in other *A. indica* extracts.

The biocomponents such as catechin, salicin, quercetin, and oleanolic acid found in the *A. indica* extracts underscore their possible positive health effects. Furthermore, *A. indica* ethanolic extracts are excellent at fighting microbial infections, showing that they can effectively kill both pathogenic fungi and bacterial strains. Comparative analysis shows that *A. indica* extracts from arid regions may have antioxidant and antibacterial effects comparable to or even stronger than those from wet areas, possibly due to differences in phytochemical composition driven by environmental conditions.

To fully harness the medicinal potential of *A. indica* extracts and maximize their application in cosmetics, agriculture, and pharmaceuticals, further research is warranted. Future studies should focus on elucidating the mechanisms of action of *A. indica* bioactive compounds, optimizing formulation strategies, and conducting comparative analyses to identify variations in phytochemical composition and biological activity among *A. indica* extracts from different geographic locations.

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Declaration of Conflicting Interests and Ethics

The authors declare no conflict of interest. This research study complies with research and publishing ethics. The scientific and legal responsibility for manuscripts published in IJSM belongs to the authors.

Authorship Contribution Statement

Abadi Abderrezzak: Investigation, Resources, Formal Analysis, and Writing – original draft. **Wassima Lakhdari:** Methodology, and Data Curation. **Salah Neghmouche Nacer:** Formal Analysis, Investigation, Methodology, Writing – original draft, and Writing – review & editing. **Abderrahmene Dehliz:** Formal Analysis, and Validation. **Hafida Khelafi:** Investigation, and Data curation. **Ridha Ben Salem:** Methodology, and Visualization. **Younes Moussaoui:** Methodology, Writing – original draft, and Writing – review & editing. All authors have read and agreed to the published version of the manuscript.

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