Tuijin Jishu/Journal of Propulsion Technology
ISSN: 1001-4055
Vol. 45 No. 4 (2024)

Phytochemical composition, antioxidant, antibacterial, and antifungal activities of the methanoic extract of neem seeds (*Azadirachta indica*) grown in El Oued

Abderrezzak Abadi[1], Salah Neghmouche Nacer[1], Lakhdari Wassima[2,3], Walid Boussebaa[4],Ibtissem Benyahia[5], Djawahir Mouhoubi[6], Amel Ladjal[3], Hamida Hammi[2], Hakim Bachir[7], Abderrahmene Dehliz[2]

[1] *University of El Oued, Faculty of Exact Sciences, Chemistry Department, P.O. Box 789, El Oued 39000, Algeria.*

[2] *National Institute of Agronomic Research of Algeria, Station of Sidi Mehdi, Touggourt 30200, Algeria.*

[3] *Faculty of Life and Nature Sciences, Biology Department, Valcore Laboratory, University of Boumerdes, Algeria.*

[4] *Scientific and Technical Research Center in Physicochemical Analysis, CRAPC Bou-Ismail, Tipaza, Algeria.*

[5] *University of Kasdi Merbah, Faculty of Mathematics and Material Sciences, Department of Chemistry, Laboratory of Biogeochemistry and Desert environments, Ouargla, Algeria.*

[6] *SARL SINAL, Oran, Algeria.*

[7] *Division of Hydraulic and Bioclimatology, National Institute of Agronomic Research (INRA), Algers 16000, Algeria.*

Abstract:

This study investigates the phytochemical composition, antioxidant, and antimicrobial activities of methanolic extracts from the aerial parts of neem (Azadirachta indica) seeds. The extract yielded 4.95% and exhibited total phenolic and flavonoid concentrations of 58.32±0.87 µg QE/mg and 49.03±0.99 µg GAE/mg, respectively. The antioxidant activity was evaluated using the β-carotene bleaching assay, DPPH assay, and ABTS radical scavenging assay, with results presented as IC50 values, demonstrating substantial antioxidant potential despite being lower than that of synthetic antioxidants such as BHT and BHA. Antifungal activity was assessed against pathogenic fungi, revealing significant inhibition rates, while antibacterial activity tests indicated very high concentrations (VHC) required for effective inhibition of various bacterial strains, including *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and Bacillus subtilis. LC-MS analysis identified a range of bioactive compounds, with catechin being the most predominant, suggesting the extract's therapeutic potential. These findings highlight the significance of neem seeds as a source of natural antioxidants and antimicrobial agents, supporting their traditional medicinal applications and potential use in food preservation and health products. Further research is warranted to explore their mechanisms of action and efficacy in clinical settings.

.**Keywords:** Neem seeds, *Azadirachta indica*, phytochemical composition, antioxidant activity,

1.Introduction

The growing interest in medicinal plants stems from the increasing demand for natural products with therapeutic potential, particularly in the context of rising antimicrobial resistance and the search for novel antioxidant agents. Among these plants, *Azadirachta indica* A. Juss., commonly known as neem, has garnered significant attention for its broad spectrum of biological activities. Native to South Asia but now widely cultivated in

tropical and subtropical regions, including Algeria, neem is renowned for its extensive use in traditional medicine systems, such as Ayurveda, due to its wide range of bioactive constituents and pharmacological properties [1,2].

The bioactivity of neem is attributed to its rich phytochemical composition, particularly the presence of limonoids, flavonoids, polyphenols, and terpenoids, which contribute to its diverse biological properties [3]. These compounds, especially those extracted from neem seeds, exhibit potent antioxidant, antibacterial, antifungal, and anti-inflammatory effects. The methanolic extract of neem seeds, in particular, has shown considerable promise as a natural source of antioxidants, capable of mitigating oxidative stress by neutralizing free radicals [4]. Oxidative stress is a key factor implicated in the pathogenesis of numerous chronic conditions, including cancer, cardiovascular diseases, and neurodegenerative disorders [5]. As such, investigating the antioxidant potential of neem seeds holds great promise for developing natural therapeutic agents.

In addition to its antioxidant properties, neem has demonstrated significant antimicrobial activity, which is crucial in the face of increasing bacterial and fungal resistance to conventional drugs. The presence of limonoids such as azadirachtin, nimbolide, and gedunin has been identified as key to neem's antibacterial and antifungal properties, which have been shown to be effective against a broad spectrum of pathogenic microorganisms [6,7]. This positions neem as a promising candidate for the development of natural antimicrobial agents, particularly for use in food preservation and pharmaceutical applications.

The environmental conditions under which medicinal plants are cultivated can significantly influence their phytochemical composition and bioactivity. Given the arid and semi-arid climate of the El Oued region in southeastern Algeria, it is hypothesized that local environmental factors may impact the concentration and efficacy of the bioactive compounds in neem seeds. Studies have suggested that abiotic stresses, such as temperature and water availability, can modulate the synthesis of secondary metabolites in plants, potentially enhancing their therapeutic potential [8].

In this study, we seek to investigate the phytochemical composition, antioxidant, antibacterial, and antifungal activities of the methanolic extract of *Azadirachta indica* seeds grown in El Oued. This research aims to provide a comprehensive analysis of the bioactive compounds present in neem seeds under local environmental conditions, contributing to a deeper understanding of its pharmacological potential and supporting its use in both traditional and modern medicinal applications. The findings of this study are expected to offer valuable insights into the role of environmental factors in shaping the bioactivity of medicinal plants and may pave the way for the development of neem-based natural products with enhanced therapeutic efficacy.

2.Material and methods

2.1.Sample collection:

The seeds of *Azadirachta indica* A. Juss. (neem) were harvested at full maturity from neem plantations located in the El Oued region of southeastern Algeria, an area characterized by its arid climate and unique soil conditions. The selection of this region is of particular significance, as previous studies have suggested that environmental factors such as temperature and water availability can influence the phytochemical profiles and bioactivity of medicinal plants [9]. The collected seeds were carefully inspected to ensure uniformity in maturity and absence of visible contamination.

Following collection, the neem seeds were shade-dried at ambient temperature to preserve the integrity of thermolabile phytochemicals. The drying process lasted until a consistent weight was achieved, indicating the complete removal of moisture content. Once dried, the seeds were finely ground using a mechanical grinder to produce a homogenous powder, facilitating the extraction of bioactive compounds. Grinding the seeds to a fine powder increases the surface area available for solvent contact, thus enhancing the efficiency of the extraction process [10].

2.3. Preparation of methanoic extract:

To prepare the methanolic extract, 100 g of powdered neem seeds were macerated in 500 mL of analytical grade methanol at room temperature (25°C) for a period of 72 hours. Maceration, a widely used technique for

phytochemical extraction, allows for the passive diffusion of bioactive compounds into the solvent over an extended period (Do et al., 2014). During this time, the mixture was intermittently stirred to ensure optimal solvent penetration. After the extraction period, the resulting mixture was filtered through Whatman No. 1 filter paper to remove any residual plant material.

The solvent was subsequently evaporated under reduced pressure using a rotary evaporator at 40°C to yield the methanolic extract, which was stored in an airtight container at 4°C until further analysis. The use of vacuum evaporation at low temperatures is crucial for preserving thermolabile constituents such as polyphenols and flavonoids, which may degrade upon exposure to higher temperatures [11]. The yield of the extract was calculated as a percentage of the initial seed powder weight.

2.4. Phytochemical Composition Analysis:

he analysis of polyphenol standards was conducted using a Shimadzu 8040 UPLC-ESI-MS-MS system equipped with ultra-high sensitivity UFMS technology. For initial optimization, direct injection was performed without a column. The system's electro spray ionization (ESI) settings were as follows: collision-induced dissociation (CID) gas at 230 KPs, conversion dynode at -6.00 kV, desolvation line temperature at 250 °C, nebulizing gas flow rate at 3.00 L/min, heat block temperature at 400 °C, and drying gas flow rate at 15.00 L/min. Chromatographic separation was achieved using a Restek Ultra C18 column (150 × 2.1 mm, 3 µm) with a mobile phase comprising solvent A (water with 0.1% formic acid) and solvent B (methanol) at a flow rate of 0.4 mL/min and injection volume of 5 µL. The gradient program was structured as follows: 0–2 min, 95% A; 2–15 min, 5% A; 15–18 min, 5% A; 18–20 min, 95% A; and 20–30 min, 95% A for re-equilibration. Data acquisition focused on the accurate detection and quantification of polyphenolic compounds[12].

2.5. Total Phenol Content

To ascertain the Total Phenolic Content (TPC), a modified microplate test was employed [13]. A 96-well microplate was loaded with 100 µL of 7.5% sodium carbonate and 20 µL of the sample. Folin-Ciocalteu Reagent was then added to the mixture 100 times its initial concentration in a tenth of distilled water. After two hours of room temperature incubation in the dark, the absorbance at 765 nm was measured. To make a blank, the extract was replaced with methanol. Three runs of each experiment were conducted to ensure accuracy. Using a calibration curve for gallic acid (25–200 µg/mL), TPC was determined under identical circumstances as the extracts. The results are expressed as µg of gallic acid equivalents per milligram of extract (µg GAE/mg extract), with equation (1) representing the linear regression equation corresponding to the calibration curve [14].

$$TPC = \frac{C \times V}{m} \quad (1)$$

where, V: volume of extract (mL); m: mass of the extract (mg); C: concentration of gallic acid (µg/mL).

2.6. Total Flavonoid Content (TFC)

The aluminum nitrate colorimetric test was used to calculate the Total Flavonoid Content (TFC) [15]. 130 µL of ethanol, 10 µL of 10% aluminum nitrate, 50 µL of extract solution (1 mg/mL), and 10 µL of potassium acetate (1M) made up the reaction mixture. Following a 40-minute room temperature incubation period, 415 nm absorbance was recorded. The calibration range of 25–200 µg/mL was established using quercetin as the positive control. Equation (2) and the linear regression equation obtained from the calibration curve built with quercetin standards were used to compute the TFC. The findings are shown as milligrams of quercetin equivalents (µg QE/mg extract) per milligram of extract.

$$TFC = \frac{C \times V}{m} \quad (2)$$

where, V: volume of extract (mL); m: mass of the extract (mg); C: concentration of quercetin (µg/mL).

2.7.Antioxidant activity

2.7.1. DPPH test

A solution of 4 mg of DPPH was dissolved in 100 mL of methanol and stored at -20°C in the dark. For the DPPH assay, each well of a 96-well microplate was filled with 40 µL of the sample containing different concentrations of the test material. Subsequently, 160 µL of the methanolic DPPH solution was added to each well. Absorbance was measured at 517 nm after a half-hour incubation at room temperature in the absence of light. As a negative control, a mixture of 50 µL of methanol and 5 mL of the DPPH solution was prepared, resulting in a total volume of 5 mL. The anti-radical activity of each sample was calculated using Equation (3), expressed as a percentage of DPPH inhibition. The antioxidant activity of the extracts was compared to that of two standard antioxidants, BHT and BHA, at various concentrations[16-19].

$$DPPH\ inhibition\ (\%) = \frac{A_0 - A_1}{A_0} \quad (3)$$

A_0 signifies the absorbance of the control, while A_1 represents the absorbance of the sample subsequent to a 30 min incubation with the DPPH solution. The standards employed in this investigation encompassed butylhydroxyanisole and butylhydroxytoluene.

2.7.2. β-carotene bleaching assay

In the β-carotene bleaching assay, the absorbance of the methanolic extract, which is dissolved in a 6:4 (v/v) acetone/hexane mixture, is measured at three wavelengths: 453 nm (A453), 505 nm (A505), and 663 nm (A663). The total amounts of lycopene and β-carotene are calculated using equations (4) and (5). The carotenoid content is expressed as micrograms per milligram of extract [19].

$$lycopene\ (mg/100mL) = 0.0584 \times A_{663} + 0.372 \times A_{505} - 0.0806 \times A_{453} \quad (4)$$

$$\beta - carotene\ (mg/100mL) = 0.216 \times A_{663} - 0.304 \times A_{505} + 0.452 \times A_{453} \quad (5)$$

2.7.3. ABTS Radical Scavenging

The ABTS•+ radical is generated by removing an electron from one of the nitrogen atoms of ABTS, resulting in absorbance at 734 nm. When an antioxidant donates a hydrogen atom, the ABTS+ solution becomes colored as the nitrogen atom of ABTS+ accepts the hydrogen. To generate the cationic radical ABTS•+, a mixture of 2.45 mM potassium persulfate and 7 mM ABTS is prepared in water and incubated for 16 hours at room temperature in the dark. Following this incubation, ethanol is added to the resulting ABTS+ solution until it reaches an absorbance of 0.70 ± 0.020 at 734 nm.

Subsequently, 160 µL of the ABTS+ solution is mixed with 40 µL of the extract solution at various concentrations. After 10 minutes, the absorbance at 734 nm is measured using a 96-well microplate reader. A negative control is prepared by combining 160 µL of the ABTS+ solution with 40 µL of methanol. The percentage inhibition at each concentration is calculated using the absorbance of ethanol as a blank sample. The scavenging capacity of ABTS is determined using equation (6) [20].

$$ABTS^{\cdot+}scavenging\ activity\ (\%) = \frac{A_{Control} - A_{Sample}}{A_{Control}} \times 100 \quad (6)$$

$A_{Control}$ is the starting concentration of $ABTS^+$ and A_{Sample} is the absorbance of the remaining $ABTS^+$ concentration in the sample.

2.8. Antimicrobial Activity

The Clinical and Laboratory Standards Institute has delineated guidelines for the broth microdilution assay, a standardized method for determining the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of plant extracts against *Bacillus subtilis* ATCC 25973, *Staphylococcus aureus* ATCC 25932, *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 [21,22]. Bacterial strains are cultured on Mueller-Hinton agar and then inoculated into cation-adjusted Mueller-Hinton broth. Cultures are incubated until they reach a distinct turbidity, and subsequently, they are diluted to a turbidity equivalent to 0.5 McFarland (1.5×10^4CFU/mL) using BioMerieux DensiCHEK Plus for VITEK 2 Systems. For yeast cultivation, the strain is diluted to a concentration of 10^6 CFU/mL and grown in Sabouraud dextrose broth.

The plant extract was dissolved in dimethyl sulfoxide to achieve a concentration of 80 mg/mL. The solution was then vortexed for one minute to ensure homogeneity. Subsequently, 100 µL of the plant extract solution was added to each well of the microtiter plate, followed by the addition of 50 µL of the bacterial or yeast suspension. Each isolate was accompanied by a growth control and a sterile control [23]. The microtiter plate is incubated at 37°C for bacteria for 18 to 24 hours, and for yeast, at 48 hours. The minimum concentration of plant extract that halts the growth of bacteria or yeast is determined as the minimum inhibitory concentration (MIC) after incubation. To determine the MBC, 5 µL of each well showing no apparent bacterial growth is plated on Mueller-Hinton agar for bacteria and Sabouraud dextrose agar for yeast. The MBC concentration is defined as the extract concentration with the lowest concentration that, with 99% accuracy, exhibits no bacterial growth [24].

2.9. Antifungal Activity Assessment

The antifungal activity of neem seed extract was evaluated using the poison food technique against two pathogens: *Thielaviopsis paradox* and *Fusarium solani*. Each extract was diluted to a concentration of 4 mg/mL in 20 mL of Potato Dextrose Agar supplemented with dimethyl sulfoxide. A 6 mm pathogen disk was then placed at the center of each Petri dish. The plates were incubated at 27°C until the growth reached the edges of the control plate. Antifungal activity was assessed by measuring the inhibitory zone widths in centimeters. Each experiment was performed in duplicate, and results were compared to controls [25-27].

Three concentrations of neem seed extract (4 mg/mL, 2 mg/mL, and 1 mg/mL) were prepared. The percentage suppression of mycelial growth was calculated by measuring the colony diameter of the fungal pathogens in centimeters. The percentage inhibition was determined using the following formula (7):

$$\%\ inhibition = \frac{D_{Control} - D_{Treated}}{D_{Control}} \times 100 \qquad (7)$$

where, $D_{Control}$ is the diameter of the fungal colony in the control plate (without extract treatment), $D_{Treated}$ is the diameter of the fungal colony in the treated plate (with extract treatment).

2.12. Analytical Statistics

A trilogy of carefully designed experiments was conducted, with results presented as mean ± standard deviation (SD). Statistical significance among treatment groups was assessed using one-way analysis of variance (ANOVA), followed by Tukey's multiple comparison test, with a threshold of $p < 0.05$ for significance. The Student's t-test was also employed to compare means between two specific groups, providing detailed insights into subtle differences. Each experiment was replicated three times with three biological replicates per titration, ensuring robustness. Significance levels for Tukey's test and the Student's t-test were calculated with precision at $p < 0.05$, confirming that observed differences were statistically significant.

3. Results and Discussion

3.1. Yield of extract and phytochemistry

The methanolic extract from the aerial parts of neem seeds yielded 4.95%. Analysis revealed the extract contained 49.03 ± 0.99 µg GAE/mg of total phenolics and 58.32 ± 0.87 µg QE/mg of total flavonoids. Plants produce these phenolic compounds and flavonoids as secondary metabolites, serving as protective agents against environmental stresses such as UV radiation and pathogens.

3.2. LC-MS analysis

The LC-MS analysis of the methanolic extract from the aerial parts of neem seeds revealed a rich and diverse phytochemical profile, comprising 20 identified compounds, each contributing to the extract's potential health benefits. Notably, catechin emerged as the predominant compound, constituting an impressive 66.44% of the extract. This flavonoid is widely recognized for its potent antioxidant properties, which may play a significant role in mitigating oxidative stress and inflammation. The presence of salicin (18.12%) further enhances the extract's therapeutic potential, as salicin is known for its analgesic and anti-inflammatory effects. Additionally, quercetin (4.83%) and rutin (2.45%) were identified, both of which are celebrated for their health-

promoting effects, including anti-cancer and cardioprotective properties. Other minor yet important compounds, such as ascorbic acid (1.70%) and chlorogenic acid (0.48%), contribute to the extract's overall bioactivity, reinforcing its antioxidant capabilities. However, several compounds, including riboflavin and caffeic acid, were absent or undetectable, suggesting that their low concentrations may have limited their recovery during the extraction process. Collectively, the findings underscore the potential of neem seeds as a valuable source of natural antioxidants and other therapeutic agents, warranting further research into their mechanisms of action, potential health applications, and the exploration of isolation techniques to maximize the extraction of these beneficial compounds for future pharmaceutical and nutraceutical developments.

Table 4. Data set related to mass spectrometry analysis of the methanolic extract from the aerial parts of *neem seeds*.

ID#	Name	Molecular Formula	Ret. Time	Height	Area	Relative amounts (%)
1	**Ascorbic Acid**	C6H8O6	9.14	9183	94212	1.70
2	**8-hydroxyquinoline**	C9H7NO	2.053	12224	167252	3.02
3	**Beta caroten**	C40H56	15.859	3901	36372	0.66
4	**Curcumin**	C21H20O6	10.963	980	5314	0.10
5	**Rutin**	C27H30O16	16.995	11123	135866	2.45
6	**Oleanolic Acid**	C30H48O3	14.222	3807	29790	0.54
7	**kojik Acid**	C6H6O4	13.187	5682	12487	0.23
8	**Chrysin**	C15H10O4	2.615	860	4747	0.09
9	**Salicin**	C13H18O7	14.831	93861	1003911	18.12
10	**Riboflavin**	C17H20N4O6	0	0	0	0.00
11	**Quercetine**	C15H10O7	12.605	27128	267285	4.83
12	**Vitexin**	C21H20O10	0	0	0	0.00
13	**Vanillin**	C8H8O3	0	0	0	0.00
14	**Catechin**	C15H14O6	13.764	413312	3679995	66.44
15	**thymol**	C10H14O	9.047	4078	41024	0.74
16	**Caffeic Acid**	C9H8O4	0	0	0	0.00
17	**Chlorogenic Acid**	C16H18O9	6.025	3240	26463	0.48
18	**Vanillic Acid**	C8H8O4	0	0	0	0.00
19	**Gallic acid**	C4H4O4	0	0	0	0.00
20	**Salycilic acid**	C7H6O3	5.977	7434	34304	0.62

3.3. Antioxidant Activity

The antioxidant activity of the methanolic extract from neem seeds was assessed using three assays: the β-carotene bleaching assay, the DPPH assay, and the ABTS radical scavenging assay. The dry extract was diluted in methanol as the solvent, ensuring a final methanol concentration below 1% v/v in all assays. The results, expressed as IC50 (the concentration required to achieve 50% inhibition), are presented in Table 1.

Table 1. Antioxidant activities of the methanolic extract from *neem seeds* using DPPH, ABTS, and β-carotene assays. (BHT and BHA serve as standards).

Extracts	% Inhibition							
	3.125 µg	**6.25 µg**	**12.5 µg**	**25 µg**	**50 µg**	**100 µg**	**200 µg**	**IC_{50} µg/mL**
β-carotene	ND	22,26±0,99	31,71±0,28	36,28±0,81	47,36±0,12	53,46±0,91	65,07±0,31	28,35±0.81
BHT	81.14±0.84	86.0.9±1.04	87.52±4.24	91.67±0.52	94.11±0.42	94.41±0.32	95.28±3.25	1.05±0.01
BHA	84.23±1.14	90.11±0.68	94.59±0.77	96.09±0.02	97.35±1.08	99.59±0.14	99.76±	0.90±0.02
ABTS	ND	ND	ND	ND	ND	51,88±0,90	61,19±1,08	28,09±0,92
BHT	59.22±0.59	78.55±3.43	90.36±0.00	92.18±1.27	93.37±0.86	94.87±0.87	96.68±0.39	1.29±0.30
BHA	83.42±4.09	93.52±0.09	93.58±0.09	93.63±0.16	93.63±0.95	94.20±0.90	95.39±2.62	1.81±0.10
DPPH	ND	6,52±1,04	9,91±0,67	19,91±0,78	36,47±1,05	47,03±0,91	53,88±1,01	28,88±0,49
BHT	11,69±1,88	22,21±1,30	37,12±1,80	52,63±2,70	56,02±0,53	83,60±0,23	87,28±0,26	22.32±1.19
BHA	28,95±1,16	54,33±1,59	76,76±1,65	84,09±0,35	87,53±0,82	87,73±0,15	88,43±0,23	5.73±0.41

*The values are shown as follows: (mean ± SD, $n = 3$, $p<0.05$)..

* ND: not determinate

The methanolic extract from neem seeds showed lower antioxidant activity than BHT and BHA across all assays. Nonetheless, the high levels of flavonoids and phenolic compounds, as previously quantified, suggest that the extract holds considerable antioxidant potential [28,29]. Phenolic compounds are of particular scientific interest due to their ability to scavenge free radicals, displaying significant antioxidant effects beneficial to human health [30]. Antioxidant therapy is emerging as a promising option for treating chronic and neurodegenerative diseases, as oxidative stress is thought to play a key role in their development [31]. Antioxidants protect cells from free radical damage primarily through three mechanisms: radical scavenging, promoting radical breakdown, and inhibiting radical formation [32,33].

3.4. Antimicrobial activity

In this study, the antimicrobial efficacy of neem seed methanolic extract was evaluated against four bacterial strains: *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 25932, and *Bacillus subtilis* ATCC 25973. The minimum inhibitory concentration (MIC) and minimum

bactericidal concentration (MBC) values are presented in Table 4, along with the MBC/MIC ratio for each strain.

Table 4. MIC, MBC, and MBC/MIC ratio of neem seeds extract against four bacteria**.**

Bacteria strains (n = 3)	MIC (mg/ml)	MBC (mg/ml)	MBC/MIC
***Escherichia coli* ATCC 25922**	VHC	VHC	/
***Pseudomonas aeruginosa* ATCC 27853**	VHC	VHC	/
***Staphylococcus aureus* ATCC 25932**	VHC	VHC	/
Bacillus subtilis ATCC 25973	VHC	VHC	/

*VHC = very high concentration

The results indicate that the neem seed extract required a very high concentration (VHC) to inhibit or kill these bacterial strains. An MBC/MIC ratio could not be established, as the concentrations necessary for MIC and MBC were beyond standard limits. This finding suggests that, while neem seed extract contains bioactive compounds with notable antioxidant activity, its antimicrobial potential against the tested strains may be limited or require optimization to achieve effectiveness.

The requirement for high concentrations may be attributed to several factors, such as the resistance profile of the tested bacteria or the specific bioactive components present in the extract. Although neem extracts have been reported to exhibit antimicrobial properties, this study's results underscore the variability in efficacy depending on the bacterial strain and extraction method used. Additionally, factors such as compound stability, solubility, and interaction with microbial cell structures may impact antimicrobial activity.

3.5.Antifungal Activity:

The antifungal activity of neem seed extract was evaluated against two pathogenic fungi, *Thielaviopsis paradox* and *Fusarium solani*, with results presented in Table 3. The extract exhibited significant antifungal effects at varying concentrations.

Table 3. Antifungal activity of *neem seeds* against pathoginic fungi.

Concentration (mg/mL)	Inhibition (%)	
	Thielaviopsis paradox	*Fusarium solani*
4	97.37±0.68	84.85±2.86
2	99.12±2.07	92.94±0.53
1	97.34±1.25	87.82±4.52

At a concentration of 4 mg/mL, the neem seed extract demonstrated inhibition rates of 97.37±0.68% against *Thielaviopsis paradox* and 84.85±2.86% against *Fusarium solani*. Notably, the inhibition percentages increased with lower concentrations, with the extract achieving 99.12±2.07% inhibition against *Thielaviopsis paradox* at 2 mg/mL and 97.34±1.25% at 1 mg/mL. For *Fusarium solani*, the extract showed a notable reduction in fungal growth, with inhibition rates of 92.94±0.53% at 2 mg/mL and 87.82±4.52% at 1 mg/mL.

These findings indicate that neem seed extract possesses strong antifungal properties, particularly against *Thielaviopsis paradox*, which was inhibited nearly completely at the lower concentration. The effectiveness of the neem extract against both fungi suggests that it contains bioactive compounds capable of disrupting fungal growth, potentially through mechanisms such as cell wall disruption or interference with metabolic pathways.

The results support the traditional use of neem in herbal medicine for treating fungal infections and highlight its potential as a natural antifungal agent. Future research should aim to isolate and characterize the specific compounds responsible for these antifungal effects and investigate their mechanisms of action. Additionally, exploring the synergistic effects of neem extract with other antifungal agents could enhance its efficacy and broaden its application in agricultural and pharmaceutical settings.

Conclusion

In conclusion, the methanolic extract from the aerial parts of neem seeds exhibits significant phytochemical properties, characterized by the presence of various bioactive compounds such as flavonoids and phenolics. The extract demonstrated substantial antioxidant activity, although it was lower than that of synthetic antioxidants like BHT and BHA. The results of the antimicrobial assays indicated that while the extract showed considerable antifungal activity against pathogenic fungi, it exhibited very high concentrations (VHC) in antibacterial tests against several bacterial strains, suggesting limited antibacterial efficacy. Mass spectrometry analysis revealed a diverse array of compounds, with catechin being the most abundant, highlighting the extract's potential for therapeutic applications. These findings underscore the importance of neem seeds as a valuable source of natural antioxidants and antimicrobial agents, warranting further investigation into their potential health benefits and applications in food preservation and medicine. Overall, the study contributes to the growing body of evidence supporting the utilization of neem seeds in traditional medicine and their potential role in developing natural therapeutic alternatives.

Acknowledgments

The authors extend their thanks to Al-Majd Laboratory of El Oued and to the Biotechnology Research Center of Constantine for their scientific support.

References

1. Gupta, S., Kataria, M., Gupta, P.K., Murganandan, S. and Yashroy, R.C., 2004. Protective role of extracts of neem seeds in diabetes caused by streptozotocin in rats. *Journal of Ethnopharmacology*, *90*(2-3), pp.185-189.
2. El-Mahmood, A.M., Ogbonna, O.B. and Raji, M., 2010. The antibacterial activity of Azadarichta indica (neem) seeds extracts against bacterial pathogens associated with eye and ear infections. *J. Med. Plants Res*, *4*(14), pp.1414-1421.
3. Tonthubthimthong, P., Chuaprasert, S., Douglas, P. and Luewisutthichat, W., 2001. Supercritical CO2 extraction of nimbin from neem seeds–an experimental study. *Journal of food Engineering*, *47*(4), pp.289-293.
4. Schmahl, G., Al-Rasheid, K.A., Abdel-Ghaffar, F., Klimpel, S. and Mehlhorn, H., 2010. The efficacy of neem seed extracts (Tre-san®, MiteStop®) on a broad spectrum of pests and parasites. *Parasitology research*, *107*, pp.261-269.
5. Brahmachari, G., 2004. Neem—an omnipotent plant: a retrospection. *Chembiochem*, *5*(4), pp.408-421.
6. Nayan, N.K., Kumar, S. and Singh, R.K., 2013. Production of the liquid fuel by thermal pyrolysis of neem seed. *Fuel*, *103*, pp.437-443.
7. Musabyimana, T., Saxena, R.C., Kairu, E.W., Ogol, C.P.K.O. and Khan, Z.R., 2001. Effects of neem seed derivatives on behavioral and physiological responses of the Cosmopolites sordidus (Coleoptera: Curculionidae). *Journal of Economic Entomology*, *94*(2), pp.449-454.
8. Bawa, G.S., Orunmuyi, M., Agbaji, A.S., Ladan, Z. and Okekeifi, U.O., 2007. Effect of different methods of processing neem (Azadirachta indica) seeds on performance of young rabbits. *Pakistan Journal of Nutrition*, *6*(3), pp.212-216.

9. Nacer, S.N., Zobeidi, A., Bensouici, C., Amor, M.L.B., Haouat, A., Louafi, F., Moussaoui, Y., Salem, R.B., Khan, M.I., Ghernaout, D. and Elboughdiri, N., 2023. In vitro antioxidant and antibacterial activities of ethanolic extracts from the leaves and stems of Oudneya Africana R. growing in the El Oued (Algeria). *Biomass Conversion and Biorefinery*, pp.1-12.
10. Nacer, S.N., Wassima, L., Boussebaa, W., Abadi, A., Benyahia, I., Mouhoubi, D., Ladjal, A., Hammi, H., Bachir, H., Dehliz, A. and Moussaoui, Y., 2024. Phytochemical screening, antioxidant, antibacterial, and antifungal properties of the Cymbopogon citratus methanolic extract. *Pharmacological Research-Natural Products*, *5*, p.100094.
11. Lakhdari, W., Bouhenna, M.M., Neghmouche, N.S., Dehliz, A., Benyahia, I., Bendif, H. and Garzoli, S., 2024. Chemical composition and insecticidal activity of Artemisia absinthium L. essential oil against adults of Tenebrio molitor L. *Biochemical Systematics and Ecology*, *116*, p.104881.
12. Wassima, L., Neghmouche Nacer, S., Abderrezzak, A., Bachir, H., Dehliz, A., Hammi, H. and Elsharkawy, E.R., 2024. Unveiling the therapeutic potential of Haloxylon articulatum extract: a comprehensive study on its phytochemical composition, antioxidant, antifungal, and antibacterial activities. *International Journal of Food Properties*, *27*(1), pp.1290-1301.
13. El Aanachi, S., Gali, L., Nacer, S.N., Bensouici, C., Dari, K. and Aassila, H., 2020. Phenolic contents and in vitro investigation of the antioxidant, enzyme inhibitory, photoprotective, and antimicrobial effects of the organic extracts of Pelargonium graveolens growing in Morocco. *Biocatalysis and Agricultural Biotechnology*, *29*, p.101819.
14. Larbi, B.A.M., Naima, B., Elsharkawy, E.R. and Neghmouche, N.S., 2018. Phytochemical characterization, in-vitro cytotoxic and antibacterial activity of Cotula cinerea (Delile) Vis essential oil. *Journal of Natural Remedies*, pp.107-112.
15. Naima, B., Abdelkrim, R., Ouarda, B., Salah, N.N. and Larbi, B.A.M., 2019. Chemical composition, antimicrobial, antioxidant and anticancer activities of essential oil from Ammodaucus leucotrichus Cosson & Durieu (Apiaceae) growing in South Algeria. *Bulletin of the Chemical Society of Ethiopia*, *33*(3), pp.541-549.
16. von Gadow, A., Joubert, E. and Hansmann, C.F., 1997. Comparison of the antioxidant activity of aspalathin with that of other plant phenols of rooibos tea (Aspalathus linearis), α-tocopherol, BHT, and BHA. *Journal of agricultural and food chemistry*, *45*(3), pp.632-638.
17. Durmaz, L., Karagecili, H. and Gulcin, İ., 2023. Evaluation of Carbonic anhydrase, acetylcholinesterase, butyrylcholinesterase, and α-Glycosidase inhibition effects and antioxidant activity of Baicalin Hydrate. *Life*, *13*(11), p.2136.
18. Bektasevic, M., Jurin, M., Roje, M. and Politeo, O., 2023. Phytochemical profile, antioxidant activity and cholinesterase inhibition potential of essential oil and extracts of Teucrium montanum from Bosnia and Herzegovina. *Separations*, *10*(8), p.421.
19. Güzel, A., 2023. Relationship Between Phenolic Content Determined by LC/MS/MS and Antioxidant Capacity and Enzyme Inhibition of Cyclotrichium niveum L. *Chemistry & Biodiversity*, *20*(4), p.e202300027.
20. Inci, H., Izol, E., Yilmaz, M.A., Ilkaya, M., Bingöl, Z. and Gülçin, I., 2023. Comprehensive phytochemical content by LC/MS/MS and anticholinergic, antiglaucoma, antiepilepsy, and antioxidant activity of apilarnil (drone larvae). *Chemistry & Biodiversity*, *20*(10), p.e202300654.
21. Zobeidi, A., Neghmouche Nacer, S., Atia, S., Kribaa, L., Kerassa, A., Kamarchou, A., AlNoaimi, M., Ghernaout, D., Ali, M.A., Lagum, A.A. and Elboughdiri, N., 2023. Corrosion inhibition of azo compounds derived from Schiff bases on mild steel (XC70) in (HCl, 1 M DMSO) medium: An experimental and theoretical study. *ACS omega*, *8*(24), pp.21571-21584.
22. Mecheri, R., Zobeidi, A., Atia, S., Neghmouche Nacer, S., Salih, A.A., Benaissa, M., Ghernaout, D., Arni, S.A., Ghareba, S. and Elboughdiri, N., 2023. Modeling and optimizing the crystal violet dye adsorption on kaolinite mixed with cellulose waste red bean peels: insights into the kinetic, isothermal, thermodynamic, and mechanistic study. *Materials*, *16*(11), p.4082.

23. Atia, D., Zobeidi, A., Neghmouche Nacer, S., Ghernaout, D. and Elboughdiri, N., 2024. Biocomposite of nontronite/Enteromorpha sp. for cationic methylene blue dye removal: optimization, kinetics, and isothermal thermodynamics study. *Biomass Conversion and Biorefinery*, pp.1-18.
24. Nacer, S.N., 2023. The Influence of Temperature on the Kinetics of Antioxidant Activity and Phenolic Compounds in Extracts of Origanum Majorana. *Tobacco Regulatory Science (TRS)*, pp.1674-1687.
25. Cheikh, F.Z.B., Zobeidi, A., Belkhalfa, H., Douadi, A., Nacer, S.N., Ghernaout, D. and Elboughdiri, N., 2023. Transforming waste cooking oil into environmentally friendly biodiesel: a comparative analysis of three transesterification methods. *International Journal of Oil, Gas and Coal Technology*, *34*(4), pp.413-427.
26. Noureddine, T., Nedjimi, M.S., Medjdoub, A., Belalem, M.A., Tlili, S. and Nacersalah, N., 2023. A comparison of the antioxidant and antimicrobial effects of phenolic extracts from plants growing in Oued Souf," Haloxylon articulatum Bioss and Arnebia decumbens Vent Coss and Kral". *NeuroQuantology*, *21*(7), p.842.
27. Bahemmi, S., Zobeidi, A., Atia, S., Nacer, S.N., Ghernaout, D. and Elboughdiri, N., Activating Illite kaolinite clay with CTAB for adsorbing Methylene blue: Isotherms, Kinetics, and thermodynamics studies. *Tuijin Jishu/Journal of Propulsion Technology*, *44*(5), p.2023.
28. Lockhart, S.R., Chowdhary, A. and Gold, J.A., 2023. The rapid emergence of antifungal-resistant human-pathogenic fungi. *Nature Reviews Microbiology*, *21*(12), pp.818-832.
29. Elshafie, H.S., De Martino, L., Formisano, C., Caputo, L., De Feo, V. and Camele, I., 2023. Chemical identification of secondary metabolites from rhizospheric actinomycetes using LC-MS analysis: In silico antifungal evaluation and growth-promoting effects. *Plants*, *12*(9), p.1869.
30. Prusty, J.S. and Kumar, A., 2024. LC-MS/MS profiling and analysis of Bacillus licheniformis extracellular proteins for antifungal potential against Candida albicans. *Journal of Proteomics*, p.105228.
31. Liang, Y., Wu, W., Li, R., Lu, Y., Wang, G., Tan, S., Chen, H., Xi, J., Huang, X., He, C. and Yi, K., 2023. Evaluation of Bacillus subtilis Czk1 Metabolites by LC–MS/MS and Their Antifungal Potential against Pyrrhoderma noxium Causing Brow Rot Disease. *Agriculture*, *13*(7), p.1396.
32. Christopher, F., Ndakidemi, P., Nyalala, S. and Mbega, E., 2023. Antifungal activity of Leonotis nepetifolia extracts against Colletotrichum species causing bean anthracnose and their phytochemical analysis using LC-MS. *Journal of Natural Pesticide Research*, *6*, p.100057.
33. Choi, H., Yim, J., Cha, J.H., Kim, J., Kim, K.J., Nam, M., Nam, M.H., Lee, C.K., Cho, Y. and Yun, S.G., 2023. HPLC-MS/MS Method Validation for Antifungal Agents in Human Serum in Clinical Application. *Clinical Laboratory*, *69*(9).