

Original Research

Received 19 June 2026

Revised 16 August 2026

Accepted 25 September 2026

Natural Resources for Human Health 2026, Vol. 6 (5): 1234–1247

KEYWORDS:

Ephedra alata, aqueous extract, antimicrobial activity, phytochemical screening, antibacterial activity, antifungal activity.

Phytochemical Screening and In Vitro Antimicrobial Activity of an Aqueous Extract of *Ephedra Alata* from Southwestern Algeria

Khadidja Fyad^{1*}, Akermi Moulay Mhammed², Nasser Belboukhari¹, Khaled Sekkoum¹

¹ Laboratory of Bioactive Molecules and Chiral Separation (LMBSC), Tahri Mohamed University of Bechar, Algeria.

² Department of Biology, Faculty of Natural and Life Sciences, Tahri Mohamed University of Bechar, Algeria.

*Corresponding email: fyad.khadidja@univ-bechar.dz

ABSTRACT: *Ephedra alata* is a medicinal plant native to arid regions of North Africa and is widely used in traditional medicine. This study characterized its phytochemical profile and evaluated the in vitro antimicrobial activity of an aqueous extract prepared from aerial parts collected in the Béchar region of southwestern Algeria. Dried plant material (30 g) was extracted with 300 mL of distilled water at 90°C, and qualitative phytochemical screening was performed across ether, methanolic, and aqueous preparations. Antibacterial activity was assessed against *Staphylococcus aureus* ATCC 25923, *Bacillus cereus* ATCC 10876, *Escherichia coli* ATCC 8739, and *Pseudomonas aeruginosa* ATCC 27853 using direct-contact, disk-diffusion, and agar-well diffusion assays. Antifungal activity was evaluated against *Fusarium oxysporum* f. sp. *albedinis*, *Rhizopus stolonifer*, and *Candida albicans* ATCC 10231. The aqueous extraction yield was 12.36%. Screening detected several secondary-metabolite classes across the plant preparations, including phenolic compounds, flavonoids, tannins, terpenoids, saponins, free quinones, fatty acids, reducing compounds, and steroidal/triterpenoid glycosides. The direct-contact assay yielded inhibitory concentrations of 0.75 g/mL for *B. cereus* and *P. aeruginosa* and 1.0 g/mL for *S. aureus* and *E. coli*. Diffusion assays produced inhibition zones of 6–19 mm, depending on the organism, concentration, and assay. Radial growth was inhibited by 80.88% for *F. oxysporum* f. sp. *albedinis* and 32.22% for *R. stolonifer*, whereas no detectable inhibition was observed against *C. albicans*. These findings indicate organism- and assay-dependent antimicrobial activity of the crude aqueous extract and support further standardized susceptibility testing and chemical characterization.

1. INTRODUCTION

Medicinal plants remain an important source of chemically diverse natural products with potential antimicrobial activity. Their biological effects, however, are highly context-dependent: plant origin, plant part, extraction procedure, chemical composition, microbial target, and assay design can all influence the observed response. For crude botanical extracts in particular, reproducible interpretation requires attention not only to whether inhibition occurs, but also to the concentration and experimental system in which it is observed.

The genus *Ephedra* (Ephedraceae) comprises species adapted largely to arid and semi-arid environments. Several *Ephedra* species have a long history of medicinal use, and their phytochemistry has attracted interest because the genus contains both alkaloid and non-alkaloid secondary metabolites with diverse biological properties (Caveney et al., 2001; Ibragic & Sofić, 2015; González-Juárez et al., 2020). *Ephedra alata* occurs naturally across parts of North Africa and the Middle East, including the Algerian Sahara, where it retains ethnomedicinal importance among local populations (Hadjadj et al., 2020).

The chemistry of *Ephedra* extends beyond the ephedrine-type alkaloids traditionally associated with the genus. Phenolic compounds, flavonoids, terpenoid-related constituents, saponins, glycosides, and other secondary metabolites have been reported, with profiles varying among species and environmental conditions (Caveney et al., 2001; Hegazi & El-Lamey, 2011; Ibragic & Sofić, 2015). More recent chromatographic studies have further documented phenolic acids and flavonoid-related constituents in *E. alata*, including chlorogenic, gallic, vanillic, caffeic, and rosmarinic acids, with marked compositional variation across plant material and growing conditions (Zaater et al., 2024; Mohammed et al., 2024). This variability is directly relevant to antimicrobial studies because crude extracts are chemically heterogeneous and their effects may reflect both composition and extractability. Findings obtained from one geographical population or solvent system therefore cannot be assumed to apply to another.

Previous investigations have reported antimicrobial activity for *E. alata*, although the magnitude of inhibition has varied substantially across extract types and microbial targets. Chebouat et al. (2014) evaluated antimicrobial activity in a flavonoid-rich extract, whereas Alshalmani et al. (2020) reported activity of a methanolic extract against Gram-positive and Gram-negative bacteria. Algerian studies have also examined essential-oil and aqueous preparations (Chouitah, 2019; Hibi et al., 2022), and a recent leaf-extract study reported concentration-dependent inhibition of *S. aureus* and *P. aeruginosa* in an agar-well assay (Zaater et al., 2024). Collectively, these studies suggest biological activity but also highlight the importance of extraction procedure, concentration, strain identity, and assay format.

Antifungal activity is of particular interest in the Saharan context. Aqueous *E. alata* preparations have inhibited several *Aspergillus* species in previous studies (Al-Qarawi et al., 2011; Hibi et al., 2022), while extracts of *E. alata* aerial parts from southwestern Algeria have also shown activity against filamentous fungi in mycelial-growth, sporulation, and germination assays (Khaldi et al., 2023). Activity against *Fusarium oxysporum* f. sp. *albedinis* is especially relevant because this pathogen causes Bayoud disease of date palm and remains a major phytopathological concern in North African oasis agriculture (Kaddouri et al., 2022; Khayi et al., 2023). At the same time, susceptibility cannot be generalized across fungi, and the responses of filamentous fungi and yeasts should be evaluated separately.

The present study therefore evaluated the in vitro antimicrobial activity of an aqueous extract prepared from aerial parts of *E. alata* collected in southwestern Algeria. Four bacterial species—*Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli*, and *Pseudomonas aeruginosa*—were examined using direct-contact, disk-diffusion, and agar-well diffusion assays. Antifungal activity was assessed against *Fusarium oxysporum* f. sp. *albedinis*, *Rhizopus stolonifer*, and *Candida albicans*. Qualitative phytochemical screening was also performed across plant preparations to provide a chemical context for the biological observations. The study was designed as a descriptive crude-extract investigation and does not infer therapeutic efficacy or identify the compounds responsible for the observed activity.

2. MATERIALS AND METHODS

2.1. Plant material and collection area

Aerial parts of *Ephedra alata* were collected from Taghit, in the southern part of Béchar Province, southwestern Algeria. Plant identification was carried out with the assistance of Abdelkader Guenaia, a specialist in plant ecology and environment. After collection, the material was cleaned to remove impurities and dried for several days at room temperature under ventilated, shaded conditions.

2.2. Preparation of the aqueous extract and extraction yield

Dried plant material (30 g) was mixed with 300 mL of distilled water and maintained at 90°C for 30 min with agitation. The mixture was filtered through Whatman No. 1 filter paper, and the extraction procedure was repeated three times. The resulting filtrates were evaporated to dryness, and the crude extract was stored at 4°C until use.

Extraction yield was calculated as:

$$R (\%) = (\text{mass of dried crude extract} / \text{initial dry plant mass}) \times 100$$

A total of 3.71 g of crude extract was recovered from 30 g of dry plant material, corresponding to a yield of 12.36%.

2.3. Qualitative phytochemical screening

Qualitative phytochemical screening was performed on ether, methanolic, and aqueous plant preparations. Detection was based on characteristic color reactions, precipitation, solubility, or ultraviolet responses, and results were recorded as present (+) or absent (–).

2.3.1. Ether extract

The ether extract was screened for alkaloid bases, emodols, terpenoids, free quinones, and fatty acids. Alkaloid bases were tested with Mayer's reagent after acidification of the dried residue. Emodols were assessed after treatment with 10% ammonium hydroxide. Terpenoids were screened using chloroform and concentrated sulfuric acid, while free quinones were examined after treatment with sodium hydroxide. Fatty acids were assessed after ethanol dissolution, saponification with potassium hydroxide, ether extraction, and evaporation.

2.3.2. Methanolic extract

The methanolic preparation was screened for phenolic compounds, flavonoids, tannins, alkaloid salts, coumarins, sterols/steroids, steroidal and triterpenoid glycosides, reducing compounds, and cardiac glycosides. Phenolic compounds were tested with ferric chloride. Flavonoids were examined using the Wiltstätter reaction with hydrochloric acid and magnesium. Gallotannins and catechin-type tannins were assessed with dilute ferric chloride. Coumarins were evaluated under ultraviolet light after treatment with ammonium hydroxide. Steroidal and triterpenoid constituents were examined using chloroform, acetic anhydride, and concentrated sulfuric acid, including the Liebermann–Burchard reaction. Reducing compounds were tested with Fehling's solution, and cardiac glycosides were assessed after treatment with chloroform and sulfuric acid.

2.3.3. Aqueous extract

The aqueous preparation was screened for saponins and anthocyanins. Saponins were assessed using a foam test after heating the extract with distilled water, followed by vigorous agitation and measurement of the persistent foam layer. Anthocyanins were tested by acidification with 2 N HCl followed by addition of ammonia. Phenolic compounds, flavonoids, and tannins were also examined using the qualitative reactions described above.

2.4. Microbial strains and maintenance

Four bacterial strains and three fungal strains were evaluated. The bacterial panel comprised *Staphylococcus aureus* ATCC 25923, *Bacillus cereus* ATCC 10876, *Escherichia coli* ATCC 8739, and *Pseudomonas aeruginosa* ATCC 27853. These strains were obtained from the teaching laboratory of the Faculty of Natural and Life Sciences, Tahri Mohamed University of Béchar.

The fungal panel comprised *Fusarium oxysporum* f. sp. *albedinis* from the strain collection of Dr. Benlarbi Larbi, *Rhizopus stolonifer* isolated from contaminated bread, and *Candida albicans* ATCC 10231.

Bacterial cultures were checked by colony morphology and microscopic examination after Gram staining. Fungal cultures were examined macroscopically and microscopically, with microculture used for morphological verification of filamentous fungi. Bacterial strains were maintained on nutrient-agar slants at 4°C, while fungal strains were maintained on acidified potato dextrose agar (PDA) slants at 4°C.

Table 1. Microbial strains used in the study

Microorganism	Group	Source / reference
<i>Staphylococcus aureus</i>	Gram-positive bacterium	ATCC 25923
<i>Bacillus cereus</i>	Gram-positive bacterium	ATCC 10876
<i>Escherichia coli</i>	Gram-negative bacterium	ATCC 8739
<i>Pseudomonas aeruginosa</i>	Gram-negative bacterium	ATCC 27853

Microorganism	Group	Source / reference
<i>Fusarium oxysporum</i> f. sp. <i>albedinis</i>	Filamentous fungus	Strain collection of Dr. Benlarbi Larbi
<i>Rhizopus stolonifer</i>	Filamentous fungus	Isolated from contaminated bread
<i>Candida albicans</i>	Yeast	ATCC 10231

Note. ATCC = American Type Culture Collection.

2.5. Preparation of microbial inocula

Bacterial inocula were prepared from young pure cultures. Several colonies were suspended in 9 mL of sterile physiological saline, and turbidity was adjusted to 0.5 McFarland, corresponding to an optical density of approximately 0.08–0.10 and an estimated bacterial density of 10^8 CFU/mL.

For filamentous fungi, inoculum density was adjusted using a Malassez counting chamber to approximately 10^5 propagules/mL.

2.6. Antibacterial activity

Antibacterial activity of the aqueous *E. alata* extract was evaluated using direct contact in Mueller–Hinton agar, disk diffusion, and agar-well diffusion.

2.6.1. Direct-contact assay

The aqueous extract was tested at nominal concentrations of 0.1, 0.2, 0.75, 1.0, and 1.5 g/mL. One milliliter of each extract preparation was mixed with 14 mL of Mueller–Hinton agar, vortexed, poured into Petri dishes, and allowed to solidify. The plates were inoculated with fresh bacterial cultures and incubated at 37°C for 24 h.

For this assay, the inhibitory concentration was defined operationally as the lowest tested concentration at which no visible bacterial growth was observed after incubation. Because the procedure was agar-based rather than a standardized broth-microdilution assay, these values are reported as direct-contact inhibitory concentrations rather than guideline-defined MIC values.

2.6.2. Disk-diffusion assay

Nominal extract concentrations of 0.1, 0.2, 0.3, and 1.0 g/mL were tested. Mueller–Hinton agar plates were surface-inoculated with bacterial suspensions adjusted to approximately 10^8 CFU/mL. Sterile Whatman paper disks (5 mm diameter) were impregnated with the prepared extract concentrations and placed on the inoculated agar.

The plates were held at 4°C for 4 h to allow diffusion and then incubated at 37°C for 24 h. Inhibition-zone diameters were measured across the center of each disk and recorded in millimeters.

2.6.3. Agar-well diffusion assay

Standardized bacterial inocula were spread uniformly over Mueller–Hinton agar. Wells approximately 6–8 mm in diameter were aseptically punched into the agar, and extract preparations at 0.1, 0.2, 0.3, and 1.0 g/mL were introduced into the wells. Plates were incubated at 37°C for 18–24 h, after which inhibition-zone diameters were measured in millimeters.

2.6.4. Antibiotic susceptibility comparator

A conventional antibiotic-disk assay was used as a descriptive susceptibility comparator. The tested antibiotics were rifampicin (RD30), kanamycin (K30), lincomycin (MY2), streptomycin (S10), oxacillin (OX1), and trimethoprim (SXT). Inhibition-zone diameters were recorded for each bacterial strain. Because antibiotic disks contain defined active agents at standardized potencies, these measurements were not interpreted as directly equivalent to inhibition produced by the crude plant extract.

2.7. Antifungal activity

The aqueous extract was tested at nominal concentrations of 0.5 and 1.0 g/mL against *F. oxysporum* f. sp. *albedinis*, *R. stolonifer*, and *C. albicans*.

2.7.1. Radial-growth assay for filamentous fungi

Antifungal activity against *F. oxysporum* f. sp. *albedinis* and *R. stolonifer* was evaluated by radial growth. One milliliter of aqueous extract was added to 14 mL of acidified PDA. A drop of the prepared fungal suspension was placed at the center of each Petri dish, and untreated control plates were prepared without extract. Plates were incubated at 25°C for 7 days.

Mycelial growth inhibition was calculated as:

$$PI_c (\%) = [(DT - D) / DT] \times 100$$

where PI_c is the percentage inhibition of mycelial growth, D_T is the colony diameter in the untreated control, and D is the colony diameter in the presence of the extract.

2.7.2. Assay against *Candida albicans*

For *C. albicans*, 1 mL of each extract preparation was added to 14 mL of molten PDA. After vortex mixing and pouring into Petri dishes, the agar surface was inoculated by uniformly swabbing the fungal suspension over the plate. Growth was then assessed in the presence and absence of the extract.

2.8. Data treatment

The available dataset comprised inhibition-zone diameters, growth/no-growth observations, direct-contact inhibitory concentrations, and percentage inhibition of fungal radial growth. Replicate-level raw measurements, measures of dispersion, and inferential statistical analyses were not available. The results were therefore analyzed descriptively; no unreported standard deviations, confidence intervals, p values, or significance tests were introduced.

3. RESULTS

3.1. Phytochemical profile and aqueous-extract yield

Qualitative screening showed a heterogeneous distribution of secondary-metabolite classes across the ether, methanolic, and aqueous preparations. Terpenoids, free quinones, and fatty acids were detected in the ether extract, whereas alkaloid bases and emodols were not detected. The methanolic preparation was positive for phenolic compounds, flavonoids, gallotannins, steroidal/triterpenoid glycosides, reducing compounds, and cardiac glycosides, while coumarins were not detected. In the aqueous preparation, saponins were detected and anthocyanins were absent.

Table 2. Qualitative phytochemical screening and aqueous-extract yield of *Ephedra alata*

Preparation / measure	Phytochemical class or outcome	Result
Ether extract	Alkaloid bases	—
Ether extract	Emodols	—
Ether extract	Terpenoids	+
Ether extract	Free quinones	+
Ether extract	Fatty acids	+
Methanolic extract	Phenolic compounds	+
Methanolic extract	Flavonoids	+

Preparation / measure	Phytochemical class or outcome	Result
Methanolic extract	Gallotannins	+
Methanolic extract	Coumarins	–
Methanolic extract	Steroidal/triterpenoid glycosides	+
Methanolic extract	Reducing compounds	+
Methanolic extract	Cardiac glycosides	+
Aqueous extract	Anthocyanins	–
Aqueous extract	Saponins	+
Aqueous extraction	Dry plant material	30 g
Aqueous extraction	Crude extract recovered	3.71 g
Aqueous extraction	Yield	12.36%
Aqueous extraction	Appearance	Brown, crystalline

Note. (+) = detected; (–) = not detected. Phytochemical classes were screened across different solvent preparations; detection in one preparation should not be interpreted as presence at the same level in the aqueous antimicrobial extract.

Extraction of 30 g of dried aerial material yielded 3.71 g of crude brown crystalline extract, corresponding to 12.36%.

3.2. Antibacterial activity

3.2.1. Direct-contact assay

Visible bacterial growth decreased as extract concentration increased. The lowest tested concentration associated with absence of visible growth was 0.75 g/mL for *Bacillus cereus* and *Pseudomonas aeruginosa* and 1.0 g/mL for *Staphylococcus aureus* and *Escherichia coli* (Table 3).

Table 3. Direct-contact inhibitory concentrations

Bacterial strain	Inhibitory concentration (g/mL)
<i>Staphylococcus aureus</i> ATCC 25923	1.00
<i>Bacillus cereus</i> ATCC 10876	0.75
<i>Escherichia coli</i> ATCC 8739	1.00
<i>Pseudomonas aeruginosa</i> ATCC 27853	0.75

Note. Values represent the lowest tested concentrations associated with absence of visible growth in the agar-based direct-contact assay. They should not be interpreted as standardized broth-microdilution MIC values.

These values derive from the agar-based direct-contact procedure and are not equivalent to standardized broth-microdilution MIC values.

3.2.2. Disk-diffusion assay

Disk-diffusion responses varied across species and concentrations (Figure 1). At 0.1 g/mL, a 7-mm inhibition zone was recorded only for *S. aureus*. At 0.2 g/mL, a 6-mm zone was observed only for *P. aeruginosa*. At 0.3 g/mL, all four bacteria showed measurable inhibition, with zones ranging from 10 mm for *S. aureus* to 16 mm for *B. cereus*. At 1.0 g/mL, inhibition zones were 15 mm for *S. aureus*, 12 mm for *B. cereus*, 8 mm for *E. coli*, and 15 mm for *P. aeruginosa*.

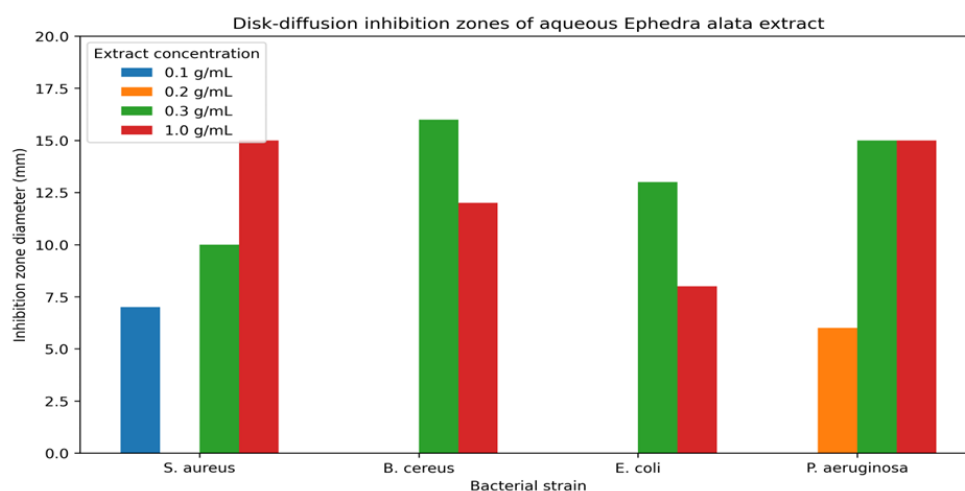


Figure 1. Disk-diffusion inhibition zones of the aqueous *Ephedra alata* extract across bacterial strains and tested concentrations. A value of zero denotes that no inhibition zone was recorded.

The disk-diffusion pattern was not uniformly concentration-dependent. For *B. cereus*, the zone diameter decreased from 16 mm at 0.3 g/mL to 12 mm at 1.0 g/mL; for *E. coli*, it decreased from 13 to 8 mm over the same concentration interval.

3.2.3. Agar-well diffusion assay

Agar-well diffusion also showed species- and concentration-dependent variation (Figure 2). No inhibition zone was recorded at 0.1 g/mL. At 0.2 g/mL, zones of 13 and 7 mm were observed for *S. aureus* and *B. cereus*, respectively. At 0.3 g/mL, inhibition zones were 14 mm for *S. aureus*, 11 mm for *B. cereus*, and 7 mm for *P. aeruginosa*, while no zone was detected for *E. coli*. At 1.0 g/mL, all four bacteria showed measurable inhibition, with the largest zone observed for *S. aureus* (19 mm).

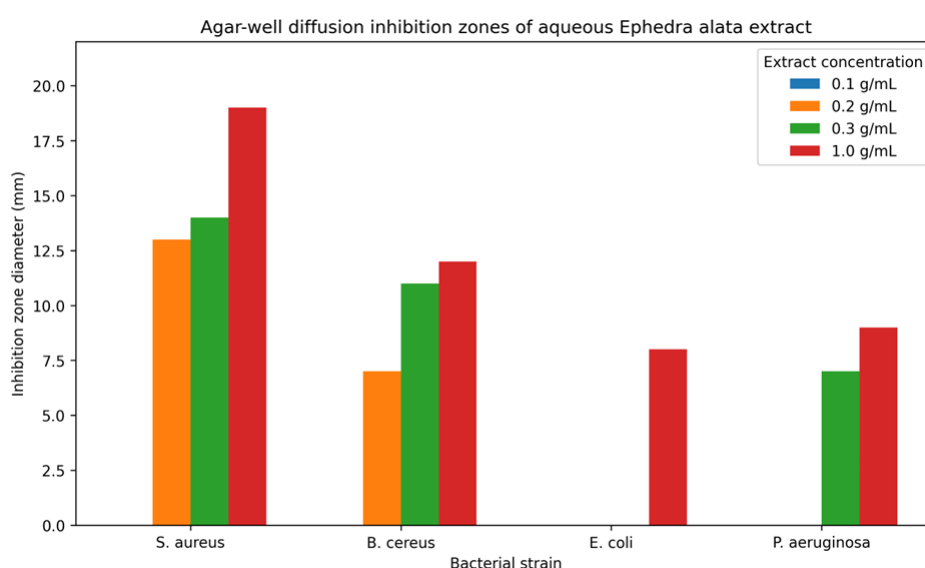


Figure 2. Agar-well diffusion inhibition zones of the aqueous *Ephedra alata* extract across bacterial strains and tested concentrations. A value of zero denotes that no inhibition zone was recorded.

For *S. aureus*, the well-diffusion response increased across the tested concentrations, reaching 19 mm at 1.0 g/mL. At the same concentration, the zones for *B. cereus*, *E. coli*, and *P. aeruginosa* were 12, 8, and 9 mm, respectively.

3.2.4. Antibiotic susceptibility comparator

The antibiotic-disk assay produced heterogeneous susceptibility profiles across the four bacterial strains. Rifampicin generated inhibition zones of 22–50 mm, kanamycin 22–29 mm, and streptomycin 11–26 mm. Lincomycin produced an 11-mm zone only against *S. aureus*. Oxacillin produced zones against *S. aureus*, *B. cereus*, and *P. aeruginosa*, but not *E. coli*. Trimethoprim produced zones against *E. coli* and *P. aeruginosa*, with no zone recorded for *S. aureus* or *B. cereus*. The full matrix is provided in Supplementary Table S1.

3.3. Antifungal activity

The antifungal response differed substantially among the tested organisms. Figure 3 summarizes radial-growth inhibition for the two filamentous fungi, while *C. albicans* is reported separately because it was evaluated using a different assay format.

For *Fusarium oxysporum* f. sp. *albedinis*, the untreated control reached 68 mm by day 7, compared with 13 mm at 1.0 g/mL extract. This corresponded to 80.88% inhibition of radial growth.

For *Rhizopus stolonifer*, the untreated culture reached 90 mm, compared with 61 mm in the presence of the extract, corresponding to 32.22% inhibition. At 0.5 g/mL, extensive growth remained evident, whereas growth retardation was observed at 1.0 g/mL.

By contrast, *Candida albicans* grew in both control and extract-containing plates at the tested concentrations; no detectable inhibitory effect was observed.

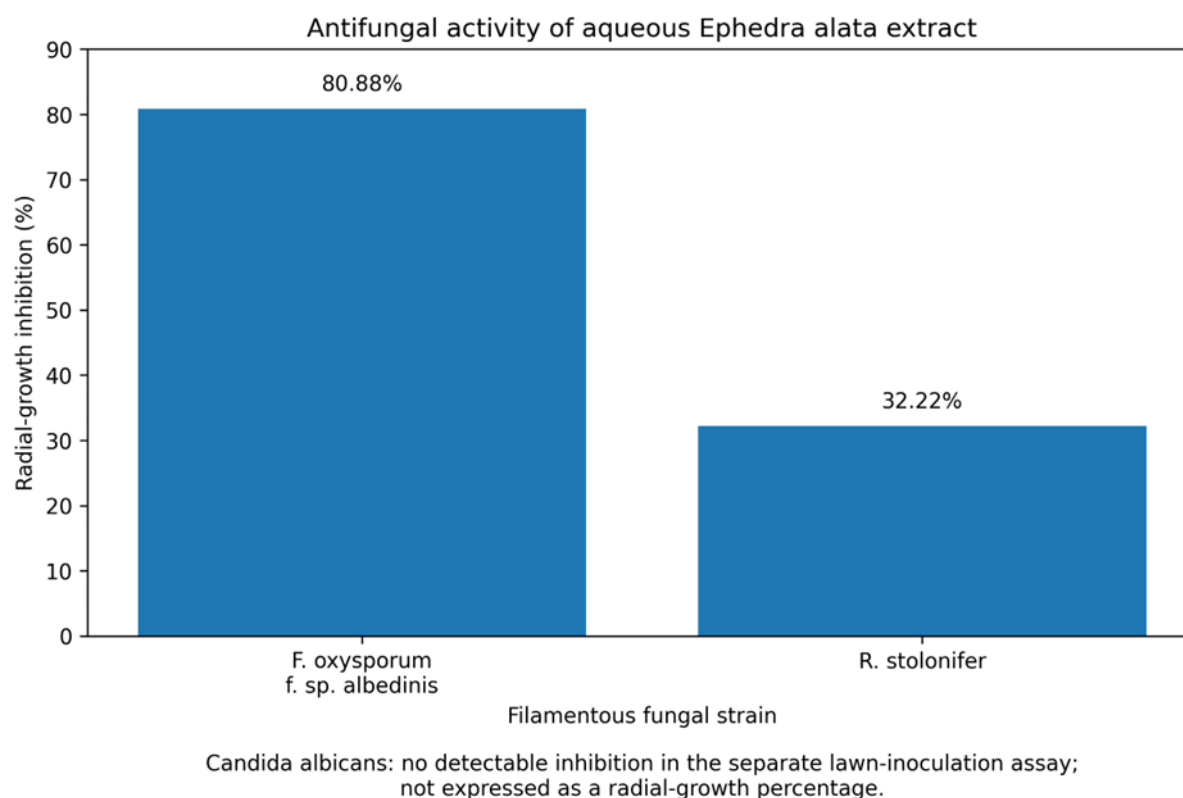


Figure 3. Radial-growth inhibition of the two filamentous fungi tested with the aqueous *Ephedra alata* extract. Inhibition was 80.88% for *F. oxysporum* f. sp. *albedinis* and 32.22% for *R. stolonifer*. *Candida albicans* was evaluated using a separate lawn-inoculation assay and showed no detectable inhibition; it is therefore not assigned a radial-growth percentage.

4. DISCUSSION

This study shows that the antimicrobial response of aqueous *Ephedra alata* extract was neither uniform across organisms nor consistent across assay formats. Measurable antibacterial inhibition was observed for all four bacterial species, but the magnitude and concentration pattern differed between direct-contact, disk-diffusion, and agar-well assays. The strongest antifungal response occurred against *Fusarium oxysporum* f. sp. *albedinis*, whereas *Rhizopus stolonifer* was only partially inhibited and *Candida albicans* showed no detectable response under the assay conditions used. This heterogeneity is central to interpreting the extract: the data support biological activity, but not a generalized claim of broad-spectrum or high-potency antimicrobial efficacy.

4.1. Phytochemical context

Qualitative screening identified several secondary-metabolite classes across the solvent preparations. Terpenoids, free quinones, and fatty acids were detected in the ether fraction; phenolic compounds, flavonoids, gallotannins, steroidal/triterpenoid glycosides, reducing compounds, and cardiac glycosides were detected in the methanolic fraction; and saponins were detected in the aqueous preparation. This broad profile is consistent with the chemical diversity reported for *Ephedra*, including phenolic and other non-alkaloid constituents in addition to the better-known alkaloid fraction (Caveney et al., 2001; Hegazi & El-Lamey, 2011; Ibragic & Sofić, 2015).

The screening nevertheless requires careful interpretation. Because different solvent systems were used, the presence of a compound class in the plant material does not establish that it was present at the same level—or even at a biologically relevant level—in the aqueous extract used for antimicrobial testing. Recent HPLC- and UPLC-based studies of *E. alata* have identified multiple phenolic acids and flavonoid-related constituents, but the detected profiles vary with plant material, extraction strategy, and growing conditions (Zaater et al., 2024; Mohammed et al., 2024). The observed activity therefore cannot be attributed to a particular phytochemical group from the present data alone. Quantitative profiling, fractionation, and compound-specific testing would be required to link the biological response to defined constituents.

The aqueous-extract yield of 12.36% provides an additional practical measure of extraction efficiency but should not be interpreted as a measure of bioactive-compound abundance. Yield in medicinal-plant extraction can vary with geographical origin, developmental stage, environmental conditions, plant part, drying procedure, and extraction protocol. Chemical characterization is therefore necessary before biological activity can be compared confidently across *E. alata* populations or extraction systems.

4.2. Antibacterial activity and assay dependence

The direct-contact assay yielded inhibitory concentrations of 0.75 g/mL for *B. cereus* and *P. aeruginosa* and 1.0 g/mL for *S. aureus* and *E. coli*. These are relatively high concentrations for a crude preparation and therefore do not support characterization of the extract as a highly potent antibacterial agent. Rather, they show that visible growth inhibition occurred when the crude aqueous extract was present at comparatively concentrated levels. This distinction is important because crude extracts contain mixtures of active and inactive constituents and cannot be compared directly, on a mass basis, with purified antimicrobial compounds.

The diffusion assays further demonstrate that apparent antibacterial activity depended on the test format. In disk diffusion, the largest zone was 16 mm for *B. cereus* at 0.3 g/mL, while *S. aureus* and *P. aeruginosa* each reached 15 mm at 1.0 g/mL. In agar-well diffusion, *S. aureus* showed the largest response, reaching 19 mm at 1.0 g/mL. Differences between these two assays are analytically important because inhibition-zone diameter reflects both biological inhibition and movement of extract constituents through the agar matrix. Solubility, diffusibility, viscosity, and local extract loading can all affect the apparent zone size of complex plant preparations (Das et al., 2010). Critical methodological reviews likewise caution that disk- and well-diffusion assays are qualitative, may show poor reproducibility for heterogeneous plant extracts, and need not yield a linear relation between extract concentration and inhibition-zone diameter (Bubonja-Šonje et al., 2020). Zone diameters from different diffusion formats should therefore be treated as assay-specific observations rather than interchangeable measures of intrinsic potency.

The disk-diffusion data also showed non-monotonic responses. For *B. cereus*, the inhibition zone decreased from 16 mm at 0.3 g/mL to 12 mm at 1.0 g/mL; for *E. coli*, it decreased from 13 to 8 mm. These patterns preclude a simple dose-response interpretation. They may reflect biological variability, concentration-dependent diffusion behavior, or both. Because replicate-

level measurements and physicochemical data were unavailable, the present dataset cannot distinguish among these explanations. The appropriate conclusion is therefore that antibacterial activity was detectable but sensitive to organism, concentration, and assay format.

The magnitude of the observed zones is broadly compatible with previous studies of *E. alata*. Hibi et al. (2022), using an aqueous extract prepared under reflux, reported inhibition zones of approximately 15 mm for *B. cereus* and *S. aureus* and 10 mm for *E. coli*. Kebili (2016) reported inhibition-zone diameters ranging from 7 to 19 mm for other *E. alata* preparations, and Alshalmi et al. (2020) observed antibacterial effects against both Gram-positive and Gram-negative bacteria. More recently, Zaater et al. (2024) reported concentration-dependent agar-well inhibition against *S. aureus* and *P. aeruginosa* using a chemically characterized leaf extract. Their reported concentrations and extract system differ substantially from those used here, so direct potency comparisons would be inappropriate; nevertheless, the study reinforces the broader observation that *E. alata* antibacterial responses are highly preparation- and assay-dependent. These comparisons support the plausibility of the present observations but should not be interpreted as direct replication.

The current results also do not support a simple Gram-positive-versus-Gram-negative interpretation. Although *S. aureus* and *B. cereus* produced some of the larger diffusion zones, *P. aeruginosa* also showed measurable inhibition and shared the lowest direct-contact inhibitory concentration with *B. cereus*. The outer membrane of Gram-negative bacteria is an important permeability barrier (Nikaido, 1989; Gupta, 2011), but susceptibility to crude botanical extracts is additionally shaped by strain-specific physiology, extract composition, and test conditions.

4.3. Antifungal activity and organism specificity

The clearest biological response in the study was the 80.88% inhibition of radial growth of *F. oxysporum* f. sp. *albedinis* at 1.0 g/mL. This finding is relevant in the regional context because this pathogen causes Bayoud disease of date palm, a destructive vascular wilt of major importance to North African oasis systems (Kaddouri et al., 2022; Khayi et al., 2023). The result does not establish field efficacy or disease-control potential, but it identifies *F. oxysporum* f. sp. *albedinis* as the most responsive fungal target in the present panel and provides a rationale for confirmatory testing under standardized conditions.

Previous work also supports antifungal activity of *E. alata* preparations against filamentous fungi. Hibi et al. (2022) reported inhibition of *Aspergillus ochraceus*, *A. flavus*, and *A. niger*, while Al-Qarawi et al. (2011) reported concentration-dependent suppression of *A. flavus* radial growth. Khaldi et al. (2023), working with aerial parts of *E. alata* collected in southwestern Algeria, further showed that antifungal responses differed among extract fractions and fungal life-history endpoints, with *F. oxysporum* f. sp. *albedinis* among the tested organisms. The present 80.88% radial-growth inhibition therefore fits within a growing body of evidence that *E. alata* preparations can affect filamentous fungi, while also underscoring substantial dependence on extraction and assay design. Direct numerical comparison should remain cautious because fungal species, extract concentrations, extraction procedures, and endpoint definitions differ across studies.

The response of *R. stolonifer* was substantially weaker. A 32.22% reduction in radial growth indicates partial suppression rather than strong inhibition, and extensive growth remained evident at 0.5 g/mL. The contrast between *R. stolonifer* and *F. oxysporum* f. sp. *albedinis* demonstrates that susceptibility cannot be generalized even among filamentous fungi. Differences in growth rate, cell-wall properties, sporulation, and intrinsic tolerance may contribute, but the present design does not allow these mechanisms to be resolved.

The negative result for *C. albicans* is equally informative. Growth was observed at both tested extract concentrations, indicating that the aqueous preparation did not show detectable anti-*Candida* activity under the conditions used. This finding helps define the biological limits of the extract and argues against describing it as a broad-spectrum antifungal preparation.

4.4. Biological interpretation of the heterogeneous response

The marked variation among microorganisms and assay formats is biologically meaningful because it suggests that the aqueous extract does not act as a nonspecific growth suppressor. Instead, susceptibility appears to depend on the interaction between microbial physiology and the chemical fraction that is recoverable in water. This interpretation is consistent with the broader phytochemical literature on *E. alata*, which shows that the species contains multiple classes of compounds whose abundance and extractability change with solvent system and environmental conditions (Zaater et al., 2024; Mohammed et al., 2024). The present study cannot identify which molecules were active, but the selective pattern of inhibition provides a useful

basis for prioritizing subsequent fractionation rather than treating the crude extract as a chemically homogeneous antimicrobial agent.

The bacterial findings illustrate this point particularly well. *S. aureus* showed the largest agar-well inhibition zone at the highest tested concentration, whereas *B. cereus* produced the largest disk-diffusion zone at 0.3 g/mL. *P. aeruginosa*, despite its Gram-negative envelope and well-recognized permeability barriers, remained responsive in both direct-contact and diffusion assays. *E. coli* was generally less responsive at the highest concentration in the diffusion tests. This pattern cannot be explained by Gram classification alone. Differences in cell-envelope structure, membrane permeability, efflux capacity, growth kinetics, and interactions with poorly diffusible plant constituents may all influence the phenotype observed in agar-based assays. The present data are insufficient to distinguish among these mechanisms, but they clearly show why a single inhibition-zone value should not be used as a universal measure of extract potency.

The contrasting fungal results provide an equally important biological signal. The strong reduction in radial growth of *F. oxysporum* f. sp. *albedinis* compared with the more modest effect on *R. stolonifer* suggests that filamentous fungi may differ substantially in their tolerance to the aqueous phytochemical mixture. Differences in hyphal growth rate, cell-wall architecture, membrane composition, sporulation biology, and oxidative-stress responses could plausibly contribute to this variation. These possibilities remain hypotheses rather than demonstrated mechanisms, but they provide a rational framework for future work. In particular, testing germination, sporulation, and mycelial extension separately could determine whether the extract acts primarily on early developmental stages or on established hyphal growth. Khaldi et al. (2023) similarly showed that different *E. alata* fractions can affect fungal endpoints unequally, supporting the value of moving beyond a single radial-growth metric.

The lack of detectable activity against *C. albicans* is also instructive because it prevents overgeneralization from the filamentous-fungal findings. Yeasts and filamentous fungi differ markedly in morphology, growth mode, cell-wall organization, and assay response. In the present study, *C. albicans* was evaluated by lawn inoculation rather than radial growth, so its negative result should be interpreted within that assay context. Nevertheless, retaining this negative finding strengthens the study by demonstrating biological selectivity and by reducing the risk of overstating the spectrum of action.

From a translational perspective, the relatively high crude-extract concentrations required for inhibition are an important boundary condition. They indicate that the current aqueous preparation is better regarded as a source for bioactivity-guided investigation than as a candidate antimicrobial preparation in its present form. A logical next step would be to fractionate the crude extract, quantify the major constituents, and test each fraction using standardized broth-based methods. If activity becomes concentrated in a particular fraction, subsequent chemical characterization could identify whether the effect is associated with phenolic, flavonoid, saponin, or other constituents. Such a workflow would convert the present descriptive observations into a more mechanistically informative investigation while avoiding premature therapeutic claims.

4.5. Role of the antibiotic comparator

The antibiotic susceptibility assay provides useful context but should not be used to rank the crude extract against conventional antibiotics. Standard antibiotic disks contain defined active agents at specified potencies, whereas the *E. alata* preparation was a chemically heterogeneous crude extract applied at much higher bulk concentrations. The larger inhibition zones observed for several antibiotics therefore cannot be interpreted on a common dose basis with the plant extract. The comparator is most appropriately viewed as a descriptive susceptibility profile of the test strains rather than evidence of antibiotic equivalence, synergy, or replacement potential.

4.6. Strengths, limitations, and implications

A strength of this study is the use of multiple antibacterial assays together with a fungal panel that included *F. oxysporum* f. sp. *albedinis*, a pathogen of regional agricultural relevance. This design reveals organism-by-assay heterogeneity that would be obscured by reliance on a single antimicrobial test. The study also retains the negative *C. albicans* finding, which is important for defining the limits of activity.

Several limitations restrict the strength of inference. Replicate-level raw measurements and measures of dispersion were not available, so the results remain descriptive and no inferential statistical claims can be made. The direct-contact inhibitory concentrations were obtained with an agar-based procedure rather than standardized broth microdilution and should not be treated as guideline-defined MIC values. Phytochemical screening was qualitative and distributed across different solvent

fractions, preventing direct attribution of aqueous-extract activity to specific compound classes. Diffusion assays are also influenced by compound mobility through agar, which limits direct comparison across assay types and concentrations. Finally, the study did not include quantitative chemical profiling, compound isolation, toxicity assessment, or in vivo validation.

These limitations define the appropriate evidentiary level of the findings rather than invalidate them. The data are best interpreted as preliminary in vitro evidence that supports targeted follow-up. Future studies should prioritize independent biological replication, standardized broth-based susceptibility testing, quantitative chemical profiling, and fractionation of the aqueous extract. Given the comparatively strong response of *F. oxysporum* f. sp. *albedinis*, confirmatory dose-response experiments against this pathogen would be especially informative, followed by identification of the fraction or constituent(s) responsible for the observed inhibition.

A staged research pathway would strengthen the evidence efficiently. First, the antibacterial results should be reproduced with biological replicates and broth microdilution so that concentration-response relationships can be estimated independently of agar diffusion. Second, the aqueous extract should be chemically profiled using chromatographic methods and separated into fractions with defined polarity. Third, the most active fractions should be reassessed against the same bacterial and fungal panel, including appropriate solvent and growth controls. Finally, the strong anti-*Fusarium* signal should be tested against additional *F. oxysporum* f. sp. *albedinis* isolates and under plant-relevant conditions before any agronomic implication is considered. This progression would preserve the regional relevance of the study while moving from descriptive screening toward reproducible evidence of mechanism and potential application.

5. CONCLUSION

This study provides a consolidated in vitro assessment of the antimicrobial activity of an aqueous extract of *Ephedra alata* collected from southwestern Algeria. The findings demonstrate that the biological response was selective rather than uniform. In the antibacterial assays, direct-contact inhibitory concentrations were 0.75 g/mL for *Bacillus cereus* and *Pseudomonas aeruginosa* and 1.0 g/mL for *Staphylococcus aureus* and *Escherichia coli*, while disk- and agar-well diffusion produced inhibition zones ranging from 6 to 19 mm. These responses varied according to organism, extract concentration, and assay format, indicating that the antimicrobial phenotype cannot be reduced to a single measure of potency.

The antifungal findings were even more differentiated. *Fusarium oxysporum* f. sp. *albedinis* showed the strongest response, with 80.88% inhibition of radial growth at 1.0 g/mL, whereas *Rhizopus stolonifer* showed only partial inhibition (32.22%). No detectable inhibition of *Candida albicans* was observed under the assay conditions used. The contrast among these organisms is a central result of the study because it defines both the potential and the limits of the aqueous preparation. In particular, the response of *F. oxysporum* f. sp. *albedinis* is noteworthy in view of the importance of Bayoud disease in North African date-palm systems, although the present in vitro result should not be interpreted as evidence of field efficacy.

The phytochemical screening provides a plausible chemical context for the observed activity, but it does not identify the active principle. Because the detected compound classes were distributed across ether, methanolic, and aqueous preparations, attribution of the antimicrobial response to a specific metabolite group would be premature. The relatively high concentrations required for inhibition further indicate that the crude aqueous extract should be viewed as a starting material for bioactivity-guided investigation rather than as a directly applicable antimicrobial product.

Accordingly, the principal contribution of this work lies in identifying an organism- and assay-dependent antimicrobial profile for Saharan *E. alata* and in highlighting *F. oxysporum* f. sp. *albedinis* as the most responsive fungal target in the tested panel. The next research stage should combine independent biological replication, standardized broth-based susceptibility testing, quantitative chromatographic profiling, and fractionation of the aqueous extract. Re-testing active fractions against the same microbial panel would allow the biological signal observed here to be linked more directly to defined chemical constituents. For the antifungal component, confirmation across additional *F. oxysporum* f. sp. *albedinis* isolates and plant-relevant experimental systems would be particularly valuable. Until such evidence is available, the present findings are best interpreted as preliminary but regionally relevant evidence that supports a focused program of chemical and microbiological validation.

REFERENCES

- [1] Al-Qarawi, A. A., Abd Allah, E. F., & Hashem, A. (2011). *Ephedra alata* as biologically-based strategy inhibit aflatoxigenic seedborne mold. *African Journal of Microbiology Research*, 5(16), 2297–2303. <https://doi.org/10.5897/AJMR11.616>
- [2] Alshalmani, S. K., Bengleil, M. S., & Elshibani, F. A. (2020). Antimicrobial, antioxidant and anticancer activity of *Ephedra alata* growing in East of Libya. *Libyan Journal of Science & Technology*, 11(2), 87–90. <https://doi.org/10.37376/ljst.v11i2.2408>
- [3] Bubonja-Šonje, M., Knežević, S., & Abram, M. (2020). Challenges to antimicrobial susceptibility testing of plant-derived polyphenolic compounds. *Archives of Industrial Hygiene and Toxicology*, 71(4), 300–311. <https://doi.org/10.2478/aiht-2020-71-3396>
- [4] Caveney, S., Charlet, D. A., Freitag, H., Maier-Stolte, M., & Starratt, A. N. (2001). New observations on the secondary chemistry of world *Ephedra* (Ephedraceae). *American Journal of Botany*, 88(7), 1199–1208. <https://doi.org/10.2307/3558330>
- [5] Chebouat, E., Dadamoussa, B., Gharabli, S., Gherraf, N., Allaoui, M., Cheriti, A., Lahham, A., & Zellagui, A. (2014). Assessment of antimicrobial activity of flavonoids extract from *Ephedra alata*. *Der Pharmacia Lettre*, 6(3), 27–30.
- [6] Chouitah, O. (2019). The essential oil of Algerian *Ephedra alata* subsp. *alenda* and its antimicrobial properties. *Journal on New Biological Reports*, 8(3), 190–193.
- [7] Das, K., Tiwari, R. K. S., & Shrivastava, D. K. (2010). Techniques for evaluation of medicinal plant products as antimicrobial agent: Current methods and future trends. *Journal of Medicinal Plants Research*, 4(2), 104–111.
- [8] González-Juárez, D. E., Escobedo-Moratilla, A., Flores, J., Hidalgo-Figueroa, S., Martínez-Tagüena, N., Morales-Jiménez, J., Muñiz-Ramírez, A., Pastor-Palacios, G., Pérez-Miranda, S., Ramírez-Hernández, A., Trujillo, J., & Bautista, E. (2020). A review of the *Ephedra* genus: Distribution, ecology, ethnobotany, phytochemistry and pharmacological properties. *Molecules*, 25(14), 3283. <https://doi.org/10.3390/molecules25143283>
- [9] Gupta, R. S. (2011). Origin of diderm (Gram-negative) bacteria: Antibiotic selection pressure rather than endosymbiosis likely led to the evolution of bacterial cells with two membranes. *Antonie van Leeuwenhoek*, 100(2), 171–182. <https://doi.org/10.1007/s10482-011-9616-8>
- [10] Hadjadj, K., Daoudi, B. B., & Guerine, L. (2020). Importance thérapeutique de la plante *Ephedra alata* subsp. *alenda* dans la médecine traditionnelle pour la population de la région de Guettara (Djelfa, Algérie). *Lejeunia, Revue de Botanique*, 201. <https://doi.org/10.25518/0457-4184.1956>
- [11] Hegazi, G. A. E., & El-Lamey, T. M. (2011). In vitro production of some phenolic compounds from *Ephedra alata* Decne. *Journal of Applied Environmental and Biological Sciences*, 1(8), 158–163.
- [12] Hibi, Z., Makhlofi, A., & Azzi, R. (2022). Ethnobotanical, phytochemical characterization and biological activities of *Ephedra alata* Decne extracts, growing wild in Bechar region, south west of Algeria. *South Asian Journal of Experimental Biology*, 12(1), 35–45. [https://doi.org/10.38150/sajeb.12\(1\).p35-45](https://doi.org/10.38150/sajeb.12(1).p35-45)
- [13] Ibragic, S., & Sofić, E. (2015). Chemical composition of various *Ephedra* species. *Bosnian Journal of Basic Medical Sciences*, 15(3), 21–27. <https://doi.org/10.17305/bjbms.2015.539>
- [14] Kaddouri, Y., Benabbes, R., Ouahhoud, S., Abdellattif, M., Hammouti, B., & Touzani, R. (2022). An insight into all tested small molecules against *Fusarium oxysporum* f. sp. *albedinis*: A comparative review. *Molecules*, 27(9), 2698. <https://doi.org/10.3390/molecules27092698>
- [15] Kebili, Z. (2016). *Contribution à l'étude de quelques activités biologiques des extraits de Ephedra alata de la région de Ouargla* [Magister thesis, Université Kasdi Merbah Ouargla].
- [16] Khaldi, A., Mehdi, K., Cheikhi, A., Abachi, M., & Moussaoui, A. (2023). Phytochemical content, antioxidant and antifungal activities of *Ephedra alata* Decne. aerial parts grown wild in South-west Algeria. *Indian Journal of Agricultural Research*, 57(2), 164–169. <https://doi.org/10.18805/IJAR.AF-728>
- [17] Khayi, S., Armitage, A. D., Gaboun, F., Meftah-Kadmiri, I., Lahlali, R., Fokar, M., & Mentag, R. (2023). Chromosome-scale assembly uncovers genomic compartmentation of *Fusarium oxysporum* f. sp. *albedinis*, the causal agent of Bayoud disease in date palm. *Frontiers in Microbiology*, 14, 1268051. <https://doi.org/10.3389/fmicb.2023.1268051>
- [18] Mohammed, H. A., Said, R., Abbas, M. M., Al-Najjar, B. O., Abd-Elmoniem, E., Khan, R. A., Alsohim, A. S., Almahmoud, S. A., Kedra, T. A., Shehata, S. M., & Ismail, A. (2024). Phytochemical, biological, and computational

- investigations of *Ephedra alata* Decne. growing in salinity conditions of Arabian Peninsula. *Scientific Reports*, 14, 21987. <https://doi.org/10.1038/s41598-024-69607-w>
- [19] Nikaido, H. (1989). Outer membrane barrier as a mechanism of antimicrobial resistance. *Antimicrobial Agents and Chemotherapy*, 33(11), 1831–1836. <https://doi.org/10.1128/AAC.33.11.1831>
- [20] Zaater, A., Serhoud, M. O., Ben Amor, I., Zeghoud, S., Hemmami, A., Rebiai, A., Bouras, Y., Laiche, A. T., Alsalmé, A., Cornu, D., Bechelany, M., & Barhoum, A. (2024). Exploring the potential of a *Ephedra alata* leaf extract: Phytochemical analysis, antioxidant activity, antibacterial properties, and green synthesis of ZnO nanoparticles for photocatalytic degradation of methylene blue. *Frontiers in Chemistry*, 12, 1367552. <https://doi.org/10.3389/fchem.2024.1367552>

SUPPLEMENTARY MATERIAL

Supplementary Table S1. Antibiotic susceptibility comparator

Antibiotic	Code	<i>S. aureus</i> (mm)	<i>B. cereus</i> (mm)	<i>E. coli</i> (mm)	<i>P. aeruginosa</i> (mm)
Rifampicin	RD30	50	27	22	39
Kanamycin	K30	26	22	25	29
Lincomycin	MY2	11	—	—	—
Streptomycin	S10	11	21	18	26
Oxacillin	OX1	30	22	—	31
Trimethoprim	SXT	—	—	23	42

Note. — = no inhibition zone recorded. Antibiotic disk results are descriptive comparators and are not directly comparable on a dose basis with the crude plant extract.