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Valorization of five medicinal plant species from Khenchela region, Algeria; comparative phytochemical profiling, antioxidant and antimicrobial properties

Oumaima Naili[1]*, Hanane Mellal[1], Khawla Aroua[2], Nouha Aggoun[3], Yasmine Arab[3,4], Sabrina Bakli[5], Miyada Ouanes[1], Naila Chaabna[5], Roufaida Berriche[3], Chahinez Mederegnarou[3], Nour Elhouda Naili[3], Hadjer Heneche[3] and Houria Bouteraa[1]

[1]Laboratory of Biotechnology, Water, Environment and Health, Faculty of Nature and Life Sciences, Abbes Laghrour University, Khenchela 40000, Algeria.
[2]Laboratory of Applied Molecular Biology, Abbes Laghrour University, Khenchela 40000, Algeria
[3]Faculty of Nature and Life Sciences, Abbes Laghrour University Khenchela 40000, Algeria.
[4]Laboratory of Biomolecules and Plant Breeding, Larbi Ben Mhidi University Oum El Bouaghi 04000, Algeria.
[5]Laboratory of Applied Microbiology, Faculty of Nature and life Sciences, Ferhat Abbas University Setif 19000, Algeria.

*Correspondence e-mail: naili.oumaima@univ-khenchela.dz

Abstract

Algeria, a country renowned for its natural resources, has a singularly rich and diverse flora. The main objective of this study was to assess the phytochemical composition, antioxidant, and antimicrobial properties of five selected medicinal plants from the Khenchela region, east of Algeria. Phytochemical tests revealed that M. vulgare, A. campestris, and Z. lotus hydroethanolic extracts possess the most promising phytochemical profile, due to their significantly high levels of phytoconstituents. Colorimetric assays showed polyphenol and flavonoid contents ranging from 57.4 ± 0.02 µg GAE/mg E to 145.89 ± 0.39 µg GAE/mg E, and from 10.05 ± 0.02 µg QE/mg E to 33.42 ± 0.2 µg QE/mg E, respectively. Antioxidant activity was evaluated using DPPH scavenging assay. The results revealed that M. sylvestris exhibited a potent antioxidant capacity with an IC50 of 2.29 ± 0.05 mg/mL. Antimicrobial activity was assessed in vitro using the agar diffusion method. The results showed that S. aureus was highly sensitive, with inhibition zone diameters of 22 ± 0.0 mm, 12.3 ± 1.0 mm, and 11 ± 0.0 mm for Z. lotus, A. campestris and C. spinosa, respectively. A moderate sensitivity was observed for other Gram positive and Gram-negative strains with all extracts. However, they were found to be inactive against Aspergillus niger. The majority of selected plants exhibited notable bioactivity, which can be attributed to the presence of different classes of secondary metabolites. These results offer scientific support for the traditional medicinal use of these species.

Keywords: Phytochemicals, antimicrobial activity, antioxidant activity, medicinal plants, Khenchela region.

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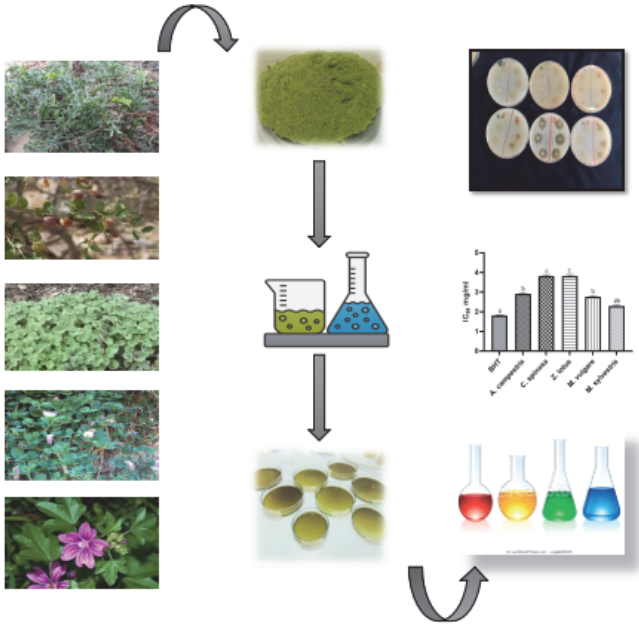
Graphical abstract



1. Introduction

For thousands of years, humans have utilized various plants found in their environment to treat and manage a wide range of ailments (Chaachouay and Zidane, 2024). Approximately 80% of the global population relies on traditional medicine to meet their primary healthcare needs (Oyebode *et al.*, 2016), largely due to poverty and limited access to modern medical systems. Today, despite advancements in synthetic chemistry, medicinal plants continue to hold a prominent role in therapeutic practices due to their proven efficacy in various treatments. They represent a vast group of species rich in bioactive compounds used in the treatment of numerous diseases. Beyond their direct therapeutic applications, these plants are also widely used in the pharmaceutical and cosmetic industries (Ooi and Pak, 2025).

Plants constitute a vast reservoir of potential bioactive molecules attributed to secondary metabolites, which are highly diverse in chemical structure and exhibit a wide range of biological activities (Elbouzidi *et al.*, 2025). It is estimated that plants produce more than 200,000 secondary metabolites, representing immense economic value, particularly for the pharmaceutical and cosmetic sectors. The major groups of these compounds include alkaloids, terpenoids, steroids, and phenolic compounds (Crozier *et al.*, 2006).

Algeria is characterized by a rich flora of medicinal plants, due to its climatic and topographical diversity (Azzi *et al.*, 2012). For centuries, medicinal plants have been used by algerian population to treat various ailments (Reguieg, 2011). Hence the current study aimed to valorize algerian plant species, particularly those from Khenchela region by the evaluation of antimicrobial activity, antioxidant capacity, and phytochemical constituents of hydroethanolic extracts of five medicinal plants (*Artemisia campestris, Capparis spinosa, Ziziphus lotus, Marrubium vulgare L, and Malva sylvestris*). This work provides a comparative analysis of these species, offering new insights into their relative bioactive potential and supporting their ethnopharmacological uses in Khenchela region, which have not been systematically reported before.

2. Materials and methods

2.1. *Collection and identification of plants*

Five plant materials belonging to different plant families were collected from the region of Khenchela (east of Algeria) on the basis of traditional medicinal use, particularly for their use in treating infectious and inflammatory ailments. Plants were authenticated by Dr. Zeraib Azzeddine, University of Khenchela. **Table 1** shows general information about these plants.

2.2. *Plants extraction*

The collected samples were first dried in a non-humid environment at room temperature and away from direct sunlight for 15 to 21 days to avoid degradation of thermolabile and photosensitive compounds. For hydroethanolic extraction, 25g of plant powders were macerated individually to 200 mL of hydroethanolic solvent mixture (water: ethanol, 50:50 v/v) for 48h (Biyiti *et al.*, 2004). After filtration, the solutions were evaporated using a rotary evaporator (SCILOGEX RE 100-PRO) to eliminate as much as possible extra solvent. Then, the obtained extracts were dried at 45°C and the residues were stored in refrigerator until use.

Table 1. Scientific name and traditional use of selected plants.

Scientific name	Family	Parts used	Traditional use
Artemisia campestris	**Asteraceae**	Leaves	obesity, antivenin, anti-inflammatory, antimicrobial, antilithiasic, hypoglycemic, choleretic (Al-Snafi, 2015).
Capparis spinosa	**Capparaceae**	Leaves	Hemorrhoids, headaches, toothache, allergic diseases, rheumatism, gout, digestive, kidney and spleen disorders (Annaz *et al.*, 2022)
Ziziphus lotus	**Rhamnaceae**	Leaves	Bronchitis, diarrhea, intestinal diseases, abscess, diabetes and eye leucomas (Abdoul-Azize, 2016)
Marrubium vulgare L	**Lamiaceae**	Leaves	Pulmonary infections, cough, rheumatoid arthritis, loss of appetite, as diuretic, bitter tonic, cholagogue (Al-Snafi *et al.*, 2020)
Malva sylvestris	**Malvaceae**	Leaves	Cough, burn, tonsillitis, cold, bronchitis, eczema, digestive problems (Pirbalouti *et al.*, 2010)

Table 2. Phytochemical screening results of hydroethanolic extract of selected plants.

Phytochemicals	A. campestris	C. spinosa	Z. lotus	M. vulgare	M. sylvestris
Polyuronoides	—	—	++	+	—
Alkaloids	—	—	—	—	—
Steroids	—	+	—	+	+
Flavonoids	—	—	—	++	+
phenolic Compounds	+++	—	+++	+++	+
Reducing compounds	+++	+	++	+	—
Saponins	+	—	—	+	—
Mucilage	—	—	—	+	—
Terpenoids	++	—	+++	+	—
Tannins	+++	—	+++	+	—
Coumarins	—	—	—	+	+

"—": Not detected, "+": Weak presence, "++": Moderate presence, "+++": Strong presence

2.3. *Determination of extracts yield*

The extracts yield was calculated using the following equation:

Extract yield% = R/S × 100 (where R; Weight of extracted plants residues (g) and S; Weight of dried plat powder (g) (Bagale, 2022).

2.4. *Phytochemical screening*

Phytochemical screening was conducted on the five selected plants using qualitative tests based on visual changes in color or precipitate formation, enabling the identification of major classes of secondary metabolites (Ailli, 2023). These phytochemical tests were conducted according to the protocols described in previous works: phenolic compounds, saponins and steroids (Bruneton, 1999), flavonoids (Lock *et al.*, 2006), Alkaloids (Tiwari and Kakkar, 1990), polyuronides and terpenoids (Ayoola *et al.*, 2008), mucilage (Banu and Catherine, 2015), tannins (Dohou *et al.*, 2003), coumarins and reducing compounds (Zellagui *et al.*, 2012).

2.5. *Determination of total phenolic content*

Total phenolic content was determined using the method described by Li *et al.* (2007). 100 µL of each extract was added to 500 µL of Folin-Ciocalteu reagent (diluted 10 times in distilled water). After incubation for 4 min, 400 µL of 7.5% sodium carbonate solution was added. The mixtures were kept in dark for 2 hours at room temperature. The absorbance of each solution was measured at 765 nm using a UV-VIS spectrophotometer (UV 1900, SHIMADZU). The concentration of total polyphenols was calculated from the regression equation of the calibration curve for gallic acid at different concentrations and expressed as micrograms of gallic acid equivalent per milligram of extract (µg GAE/mg E).

2.6. *Determination of Total flavonoid content*

The quantification of flavonoids was carried out using the method of Quettier-Deleu *et al.* (2000). A volume of 500 µL of each extract was added to an equal volume of $AlCl_3$ solution (2% in methanol). Then, the mixture was stirred using a vortex and the absorbance at 430 nm was measured using a spectrophotometer after 10 minutes of incubation. flavonoid content was quantified based on the quercetin calibration curve at different concentrations. Results are expressed in micrograms of quercetin equivalent per milligram of extract (µg QE/mg E).

2.7. *Antioxidant activity determination by DPPH free radical scavenging method*

The potential of extracts to scavenge DPPH radicals was assessed using the method described by Mansouri *et al.* (2005). A volume of 25 µL of DPPH solution (2.4 mg DPPH in 100 mL methanol) was mixed with 975 µL of extract solutions or standard antioxidant (BHT) at different concentrations. The absorbance was measured at 517 nm after 30 minutes incubation in darkness at room

temperature. The percentage of the DPPH radical scavenging activity is calculated using the following equation: (%) inhibition = [(Ac - As/ Ac] x 100. Where Ac and as denote absorbance of control and the sample, respectively. From the inhibitory activity versus concentration graph, the IC_{50} (mg/mL) values were calculated.

2.8. *Antimicrobial activity*

2.8.1. Microbial strains

To explore the *in vitro* antimicrobial activity of selected plants, two Gram-positive bacteria (*Bacillus cereus* ATCC 11778 and *Staphylococcus aureus* ATCC 25923), three Gram-negative bacteria (*Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 4352 and *Pseudomonas aeruginosa* ATCC 27853) and one strain of filamentous fungi (*Aspergillus niger* 2CA936) were used.

2.8.2. Antibiotic sensitivity testing of bacterial strains

Antibiotic sensitivity of bacterial strains was tested by disc diffusion method on Mueller-Hinton agar. The method consists of preparing an inoculum for each bacterial strain by adjusting the turbidity to a 0.5 Mc Farland standard and spreading it onto Mueller-Hinton agar plates. Then, antibiotic discs were placed on inoculated agar plates and incubated at 37°C for 24 h. The diameter of growth inhibition was measured and bacteria were classified as sensitive or resistant (Hanoun *et al.*, 2023).

2.8.3. Antibacterial potential of plant extracts

The antibacterial test was assessed using the disc diffusion method against the five selected bacteria. Solutions of extracts were prepared in dimethyl sulfoxide (DMSO) at 200 mg/mL, and then sterilized using 0.45 µm millipore filters. Paper discs (6mm) were placed on the inoculated Mueller-Hinton agar plates and impregnated with 10 µL of the extracts. The plates were incubated at 37°C for 24 h, and antimicrobial activity was determined by measuring the diameter of the inhibition zones around the discs. The assay was performed three times.

2.8.4. Antifungal activity

The conidia suspension of *Aspergillus niger* was filtered and adjusted to final concentration of 2-3 × 10^6 spores/mL in sterile distilled water. This suspension was aseptically spread onto Sabouraud dextrose agar plates. Sterile Whatman paper discs were impregnated with 10 µL of each extract (200 mg/mL) and placed on the inoculated agar plates. The plates were incubated at 28°C, and the diameters of the inhibition were measured after 72 hours (Yazdani *et al.* 2012). The test was repeated three times.

2.8.5. Minimum inhibitory concentrations of extracts

The minimum inhibitory concentrations (MICs) of extracts were assessed using the same method (disc diffusion method). Serial dilutions of each extract (200 mg/mL to 6.12 mg/mL) were prepared in DMSO. A volume of 10 µL from each dilution was applied onto sterile discs, which were then placed on Mueller Hinton agar plates previously inoculated with bacteria. The MIC was defined as the lowest concentration of each extract that visibly inhibited microbial growth after 24 hours of incubation (Boughougal *et al.*, 2025).

2.8.6. Minimum bactericidal concentrations of extracts

Streaks were taken from plant extract plates exhibiting invisible growth on MIC plates and subcultured onto Mueller Hinton agar plates and subcultures. MBC was considered as the concentration of extract that did not show any bacterial growth after incubation at 37 °C for 24h.

2.9. *Statistical analysis*

All analyses were conducted in triplicate (n=3), and the results were presented as mean ± standard deviation (SD). The data were evaluated using one-way analysis of variance (ANOVA) and differences between means was calculated by Tukey's multiple comparison test using GraphPad Prism 5 (GraphPad Software, San Diego, CA, USA). Difference among means at 5% level ($p<0.05$) was considered significantly different.

3. Results

3.1. *Extraction yield*

The extraction yield is an indicator of solvent efficiency in extracting specific compounds from the original plant material (Adam *et al.*, 2019). By using the hydroethanolic extraction method, the highest yield was obtained from *Z. lotus* extract (16.52%), while the least yield was that of *M. sylvestris* extract (11.34%). As for the other plant extracts, the extraction yields were 14.86%, 13.83% and 12.64% for *M. vulgare*, *C. spinosa* and *A. campestris*, respectively (**Figure 1**).

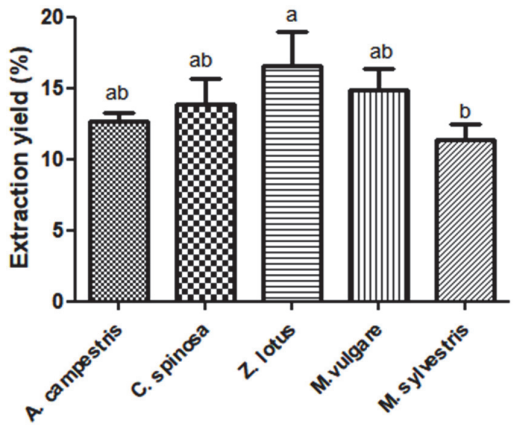


Figure 1. Comparison of extraction yield values of selected plant extracts. Means ± SD of determinations were made in triplicate experiments. Column means followed by different letters (a, b) differ significantly ($p< 0.05$).

3.2. *Phytochemical screening*

The phytochemical profiles of five plant species were qualitatively assessed for the presence of essential secondary metabolites. The results are summarized in **Table 2**.

Among the tested species, *M. vulgare* exhibited the highest phytochemical diversity, with presence of ten out of the eleven tested compound classes. In contrast, *C.*

spinosa showed the least diversity, presenting only two detected compound groups (reducing compounds and steroids). Phenolic compounds and reducing compounds were the most frequently detected phytochemicals. Notably *A. campestris* and *Z. lotus* showed strong reactions for both classes. Tannins followed a similar pattern, with strong presence in *A. campestris* and *Z. lotus*. Saponins and mucilage were less common, detected only in one or two species. Finally, alkaloids were not detected in any of the tested species, which may reflect a low alkaloid content in the aerial parts used.

3.3. *Total phenolic content*

The total phenolic content of five medicinal plant extracts was determined and expressed as micrograms of gallic acid equivalents per milligram of dry weight (µg GAE/mg E). The results are illustrated in **Figure 2** and demonstrate significant variation among studied species ($p< 0.05$), reflecting species-dependent differences in polyphenol accumulation. *Z. lotus* had the highest phenolic content (145.89 ± 0.39 µg GAE/mg E), showing a statistically significant difference compared to the other species. *A. campestris* exhibited a phenolic concentration of 118.1 ± 0.6 µg GAE/mg E, while *M. sylvestris* and *M. vulgare* showed intermediate values of 104.49 ± 0.05 µg GAE/mg E and 102.2 ± 0.11 µg GAE/mg E, respectively. In contrast, *C. spinosa* had the lowest phenolic concentration of 57.4 ± 0.02 µg GAE/mg E.

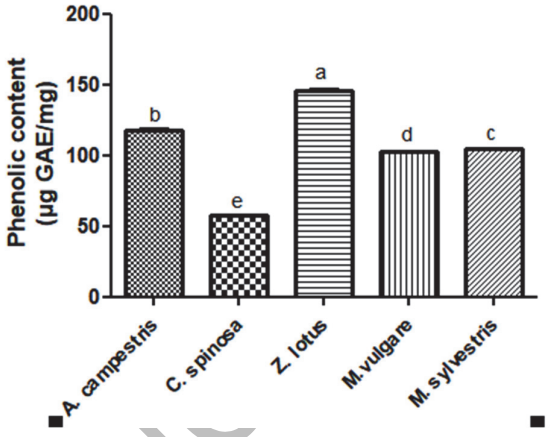


Figure 2. Total phenolic content of tested hydroethanolic plant extracts. Values were expressed as means ± SD of triplicate. Column means followed by different letters (a, b, c, d) differ significantly ($p< 0.05$).

3.4. *Total flavonoid content*

The total flavonoid content of extracts was evaluated and expressed as micrograms of quercetin equivalents per milligram of dry weight (µg QE/mg E). As shown in **Figure 3**, the flavonoid content varied significantly among the five species ($p< 0.05$). *Z. lotus* exhibited the highest flavonoid concentration (33.42 µg QE/mg E), significantly outperforming all other species. *C. spinosa* and *A. campestris* followed with moderate flavonoid levels of around 27.51 and 26.77 µg QE/mg E, respectively. *M. sylvestris* showed a lower content (18.05 µg QE/mg E) while *M. vulgare* recorded the lowest flavonoid level (10.05 µg QE/mg E).

3.5. *Free radical scavenging activity*

The antioxidant activity of the selected extracts was assessed using the DPPH assay, and the results are expressed as the IC_{50} values (**Figure 4**). All extracts exhibited radical scavenging activity ranging from 2.29 ± 0.05 to 3.81 ± 00.9 mg/mL. *M. sylvestris* showed the most potent antioxidant activity which did not differ significantly from the standard antioxidant BHT ($p >0.05$), followed by *M. vulgare* and *A. campestris*. Whereas *C. spinosa* and *Z. lotus* showed comparatively lower activity.

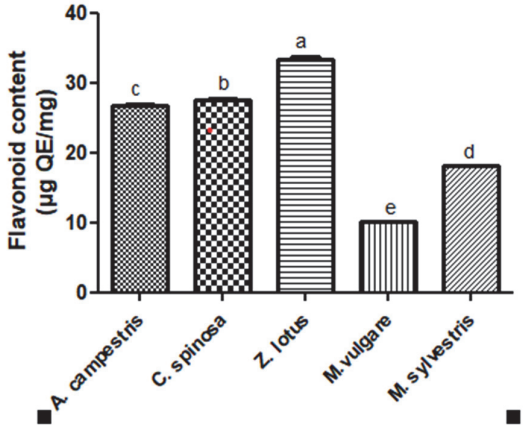


Figure 3. Total flavonoid content of tested hydroethanolic plant extracts. Values were expressed as means ± SD of triplicate. Column means followed by different letters (a, b, c, d, e) differ significantly ($p < 0.05$).

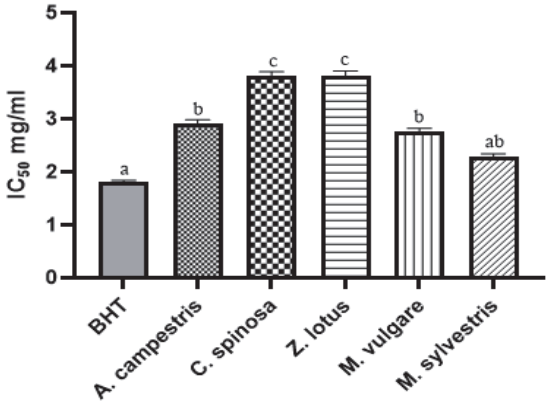


Figure 4. IC50 values of plant extracts and BHT (standard) against DPPH radicals. Values were expressed as means ± SD of triplicate. Column means followed by different letters (a, b, c) differ significantly ($p< 0.05$).

3.6. *Antibiotic susceptibility*

In order to evaluate the sensitivity of the bacterial strains, nine standard antibiotics belonging to different families were used as positive controls, and the results are presented in the **Table 3**.

Staphylococcus aureus showed a resistance to both antibiotics Piperacillin and Erythromycin, and sensitivity to the other antibiotics. The strain *E. coli* was sensitive only

to Ofloxacin, Chloramphenicol, and Tetracycline. As for *P. aeruginosa* and *B. cereus*, they exhibited sensitivity only to Ofloxacin, whereas *K. pneumoniae* was resistant to all the antibiotics tested.

Table 3. Antibiotic sensitivity of bacterial strains.

Strains / ATB	*S. aureus*	*B. cereus*	*E. coli*	*P. aeruginosa*	*K. pneumoniae*
Piperacillin	R	R	R	R	R
Erythromycin	R	R	R	R	R
Pristinamycin	S	R	R	R	R
Fusidic acid	S	R	R	R	R
Vancomycin	S	R	R	R	R
Ofloxacin	S	S	S	S	R
Oxacillin	S	R	R	R	R
Chloramphenicol	S	R	S	R	R
Tetracycline	S	R	S	R	R

R: resistant ; S: sensitive

Table 4. Diameters of inhibition zones (mm) of the tested extracts.

Strains	Plant extracts (200 mg/mL)					DMSO
	A. campestris	*C. spinosa*	*Z. lotus*	*M. vulgare*	*M. sylvestris*	
S. aureus	12.3 ± 1^{a}	11 ± 0.0 ^{ab}	22 ± 0.0^{c}	7 ± 0.0^{b}	—	—
B. cereus	9 ± 0.0^{a}	8 ± 0.0^{a}	—	11 ± 0.12^{b}	—	—
E. coli	—	8 ± 0.0^{a}	—	7 ± 0.0^{a}	7 ± 0.0^{a}	—
P. aeruginosa	11 ± 0.0^{a}	—	—	7 ± 0.0^{b}	12 ± 0.3^{a}	—
K. pneumoniae	—	—	—	7 ± 0.0^{a}	10 ± 0.1^{b}	—
A. niger	—	—	—	—	—	—

Values were expressed as means ± SD (n=3). Means followed by different letters (a,b,c) within the same row differ significantly (p< 0.05), (-): No activity.

Table 5. Minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) of hydroethanolic extracts against sensible strains.

Strains	Plant extracts (mg/mL)									
	A. campestris		*C. spinosa*		*Z. lotus*		*M. vulgare*		*M. sylvestris*	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
S. aureus	25	25	100	100	25	100	ND	ND	ND	ND
B. cereus	100	200	100	100	ND	ND	12.5	50	ND	ND
P. aeruginosa	25	50	ND	ND	ND	ND	ND	ND	12.5	100
K. pneumoniae	ND	ND	ND	ND	ND	ND	ND	ND	25	100

ND: Not determined

3.7. *Antimicrobial activity of extracts*

The five extracts exhibited antibacterial activity with varying effectiveness depending on the tested strain, while no antifungal activity was detected (**Table 4**).

In disc diffusion method, the extracts of *Z. lotus*, *A. campestris* and *C. spinosa* were shown to have antibacterial potency against *S. aureus* with mean inhibition zones of 22 ± 0.0 mm, 12.3 ± 1 mm and 11 ± 0.0 mm, respectively. The *M. vulgare* and *A. campestris* extracts exhibited moderate activity against *B. cereus* with inhibition zones of 11 ± 0.12 mm and 9 ± 0.0 mm, respectively. Extracts from *M. sylvestris* and *A. campestris* displayed potential activity with diameters of 12 ± 0.3 mm and 11 ± 0.0 mm for *P. aeruginosa*. *K. pneumoniae* was resistant to all extracts exception for *M. sylvestris* extract with inhibition zone of 10 ± 0.1 mm. On the other hand, no antibacterial activity was observed for all extracts against *E. coli* strain.

The minimum inhibitory concentrations and minimum bactericidal concentrations of the extracts ranged from 12.5 mg/mL to 200 mg/mL. *S. aureus* revealed MIC values of 25, 100, 25 mg/mL for *A. campestris*, *C. spinosa* and *Z. lotus* while MBC values of the extracts for the same strain were 25, 100, 25 mg/mL, respectively. The MIC and MBC values for *B. cereus* at examination of *A. campestris*, *C. spinosa* and *M. sylvestris* were 100, 200; 100, 100; 12.5, 50 mg/mL, respectively. The MIC values of *A. campestris* and *M. vulgare* were 25, 12.5 mg/mL for *P. aeruginosa*, while the MBC values for the same strain and the same extracts were 50, 100 mg/mL. Finaly, the MIC and MBC of *M. vulgare* against *K. pneumoniae* were 25 and 100 mg/mL (**Table 5**).

4. Discussion

In the present research, results suggest that *Z. lotus* and *A. campestris* have the most promising phytochemical

profile, due to their significantly high levels of phenolics and flavonoids compounds. These constituents are known to play critical roles in plant defence, offering protection against biotic and abiotic stresses (Zaynab *et al.*, 2018) and are associated with different biological activities (Cavasos *et al.*, 2021). These results were compared with those reported by Letaief *et al.* (2021) and Bakchiche *et al.* (2019). In their researches, the ethanolic extracts of *Z. lotus* and *A. campestris* leaves exhibited a relatively low phenolic and flavonoid contents (41.70 ± 0.70 µg GAE/ mg and 28.54 ± 1.89 µg QE/mg) and (102.09 ± 1.65 µg GAE/mg and 17.94 ± 1.26 µg RE/mg), respectively. In contrast, *M. vulgare*, despite having moderate phenolic concentration, exhibited the lowest flavonoid content, indicating a more limited potential from flavonoid-based mechanisms. For *M. sylvestris*, results were significantly higher than those of El- Sayed *et al.* (2018) who found that the total phenolic and flavonoid contents of 70% hydroethanolic extracts were 57.30 ± 0.04 µmg GAE/ mg E and 11 ± 0.01 µg QE/ mg E, respectively. Regarding *C. spinosa*, the hydroethanolic extract demonstrated an important flavonoid content compared to other plants. These finding are consistent with previous reports emphasizing the richness of this plant extract in flavonoid compounds (Tlili *et al.*, 2011). The relatively high variability of total phenolic and flavonoid compounds of plants in this study may be attributed to different factors, plant material, extraction procedures, with environmental and climatic conditions being the most influential determinants of their levels (Pourhosseini *et al.*, 2020).

Given the richness of the studied extracts in various phytochemicals known for their redox potential, the antioxidant activity was further assessed using DPPH radical scavenging assay. Results revealed that *M. sylvestris* and *M. vulgare* hydroethanolic extracts exhibited the strongest radical scavenging capacity compared to other plants. This activity could be related to their richness in phenolic compound especially the presence of coumarins which are considered as one of the most potent antioxidant compounds (Lončarić *et al.*, 2020) and anticancer agent (Paul *et al.*, 2024). It has been reported in several studies the antioxydant potential of *M. sylvestris* (Batiha *et al.*, 2022; Rhimi *et al.*, 2025) howevere in this study, no significant difference was observed between the antioxidant activity of the hydroethanolic extract (2.29 ± 0.05) and BHT (1.8 ± 0.04 mg/mL), indicating that *M. sylvestris* extract can achieve antiradical effects equivalent to those of a synthetic standard. Also, previous researches highlighted its nutritional value and therapeutic potential, particularly the leaves due to their anticancer, anti-ulcerogenic, skin-whitening, and anti-aging properties (Paul *et al.*, 2024).

The emergence of antibiotic-resistant bacteria is one of the greatest problems facing the medical community in both developed and developing countries (Islam, 2021). In Algeria, natural therapies based on plant-derived compounds have gradually increased. Therefore, such plants should be studied to better understand their efficiency. Among the Gram-positive strains, *S. aureus* showed important sensitivity to several extracts, particularly *Z. lotus*, followed by *A. campestris* and *C. spinosa*. Similarly, *B. cereus* was moderately inhibited by *M. vulgare* and *A. campestris*. The notable sensitivity against Gram-positive bacteria may be attributed to flavonoids, which are potent inhibitors of sortase enzymes located in the cytoplasmic membrane of these bacteria (Ghedadba *et al.*, 2015). In contrast, Gram-negative bacteria exhibited a more variable and generally lower sensitivity to the five extracts. *P. aeruginosa* was found to be sensitive to *M. sylvestris* and *A. campestris*. Whereas, *K. pneumoniae* was sensitive only to *M. sylvestris* extract. These bacteria are well known for their high level of resistance than Gram-positive bacteria, primarily due to the presence of the outer membrane and plethora of active efflux pumps (Leus *et al.*, 2023).

The antibacterial activity of the plant tested is highly significant in the context of antibiotic resistance. Many of the tested strains showed reduced susceptibility or resistance to conventional antibiotics. Whereas they were highly sensitive to certain plant extracts. This finding suggests that plant-derived compounds may represent important alternatives to antibiotics. Many researches have demonstrated that certain plants exhibit antibacterial activity due to the presence of phytochemicals that can disrupt bacterial cell membrane, inhibit enzyme involved in DNA replication and transcription, and interfere with bacterial virulence factors (Ibn Awadh and Ahmed, 2025). Furthermore, the antibacterial efficacy is not solely gouverned by the total content of phytochemicals, but rather by their qualitative composition and the structural diversity.

Antibacterial potential of *Z. lotus* leaves hydroalcoholic extracts was studied in several previous works. In the present study, the extract exhibited a notably strong inhibitory effect against *S. aureus*, with an inhibition zone of 22± 0.0 mm, which is considerably higher than the values reported by Yahia *et al.* (2020), who observed inhibition diameters ranging from 12.2 to 13 mm. In addition, the hydroethanolic extract of *M. sylvestris* demonstrated enhanced antibacterial activity against *P. aeruginosa* (12 ± 0.3) compared to the findings of Memdueva *et al.* (2025), who reported an inhibition diameter of 7 mm, whereas comparable antibacterial effects were observed against *E. coli, S. aureus,* and *B. cereus.*

The variability in the bioactivity of medicinal plants may be attributed to extraction methods as well as environmental factors, including soil, climate and topography, which significantly influence the synthesis and accumulation of secondary metabolites (Wang *et al.*, 2024).

5. Conclusion

The five hydroethanolic extracts emerge as promising sources for therapeutic or nutraceutical development due to their dual richness in phytochemicals especially phenolic compounds and flavonoids that are closely related to the observed antioxydant and antibacterial

effect. The clear interspecies differences in biological activities highlight the importance of phytochemical analysis when selecting materials for antioxidant or medicinal purposes. Furthermore, These finding provide scientific support for traditional use of these medicinal plants and underscore their potential as alternatives to synthetic agents. However, future investigations should focus on isolatation and characterisation of the active constituents.

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