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

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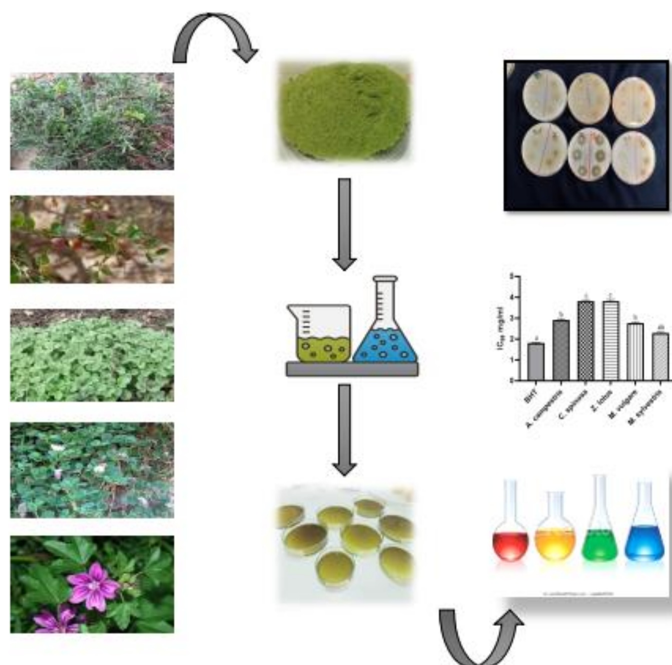

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16 **Abstract**

17 Algeria, a country renowned for its natural resources, has a singularly rich and diverse flora. The main
18 objective of this study was to assess the phytochemical composition, antioxidant, and antimicrobial
19 properties of five selected medicinal plants from the Khenchela region, east of Algeria.
20 Phytochemical tests revealed that *M. vulgare, A. campestris,* and *Z. lotus* hydroethanolic extracts
21 possess the most promising phytochemical profile, due to their significantly high levels of
22 phytoconstituents. Colorimetric assays showed polyphenol and flavonoid contents ranging from 57.4
23 ± 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
24 ± 0.2 µg QE/mg E, respectively. Antioxidant activity was evaluated using DPPH scavenging assay.
25 The results revealed that *M. sylvestris* exhibited a potent antioxidant capacity with an IC50 of 2.29 ±
26 0.05 mg/mL. Antimicrobial activity was assessed *in vitro* using the agar diffusion method. The results
27 showed that *S. aureus* was highly sensitive, with inhibition zone diameters of 22 ± 0.0 mm, 12.3 ±
28 1.0 mm, and 11 ± 0.0 mm for *Z. lotus*, *A. campestris* and *C. spinosa*, respectively. A moderate
29 sensitivity was observed for other Gram positive and Gram-negative strains with all extracts.
30 However, they were found to be inactive against *Aspergillus niger*. The majority of selected plants
31 exhibited notable bioactivity, which can be attributed to the presence of different classes of secondary
32 metabolites. These results offer scientific support for the traditional medicinal use of these species.

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

35 **1. Introduction**

36 For thousands of years, humans have utilized various plants found in their environment to treat and
37 manage a wide range of ailments (Chaachouay and Zidane, 2024). Approximately 80% of the global
38 population relies on traditional medicine to meet their primary healthcare needs (Oyebode et al.,
39 2016), largely due to poverty and limited access to modern medical systems. Today, despite
40 advancements in synthetic chemistry, medicinal plants continue to hold a prominent role in

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41 therapeutic practices due to their proven efficacy in various treatments. They represent a vast group
42 of species rich in bioactive compounds used in the treatment of numerous diseases. Beyond their
43 direct therapeutic applications, these plants are also widely used in the pharmaceutical and cosmetic
44 industries (Ooi and Pak, 2025).

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

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

60 **2. Materials and Methods**

61 **2.1. Collection and identification of plants**

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

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66 **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)

67

68 **2.2. Plants extraction**

69 The collected samples were first dried in a non-humid environment at room temperature and away
70 from direct sunlight for 15 to 21 days to avoid degradation of thermolabile and photosensitive
71 compounds. For hydroethanolic extraction, 25g of plant powders were macerated individually to 200
72 mL of hydroethanolic solvent mixture (water: ethanol, 50:50 v/v) for 48h (Biyiti et al., 2004). After
73 filtration, the solutions were evaporated using a rotary evaporator (SCILOGEX RE 100-PRO) to

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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.

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).

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98 **2.6. Determination of Total flavonoid content**

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

105 **2.7. Antioxidant activity determination by DPPH free radical scavenging method**

106 The potential of extracts to scavenge DPPH radicals was assessed using the method described by
107 Mansouri et al. (2005). A volume of 25 µL of DPPH solution (2.4 mg DPPH in 100 mL methanol)
108 was mixed with 975 µL of extract solutions or standard antioxidant (BHT) at different concentrations.
109 The absorbance was measured at 517 nm after 30 minutes incubation in darkness at room temperature.
110 The percentage of the DPPH radical scavenging activity is calculated using the following equation:

111 (%) inhibition = [(Ac - As/ Ac] x100. Where Ac and As denote absorbance of control and the sample,
112 respectively. From the inhibitory activity versus concentration graph, the IC_{50} (mg/mL) values were
113 calculated.

114 **2.8. Antimicrobial activity**

115 **2.8.1. Microbial strains**

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

120 **2.8.2. Antibiotic sensitivity testing of bacterial strains**

121 Antibiotic sensitivity of bacterial strains was tested by disc diffusion method on Mueller-Hinton agar.
122 The method consists of preparing an inoculum for each bacterial strain by adjusting the turbidity to a

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123 0.5 Mc Farland standard and spreading it onto Mueller-Hinton agar plates. Then, antibiotic discs were
124 placed on inoculated agar plates and incubated at 37°C for 24 h. The diameter of growth inhibition
125 was measured and bacteria were classified as sensitive or resistant (Hanoun et al., 2023).

126 **2.8.3. Antibacterial potential of plant extracts**

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

133 **2.8.4. Antifungal activity**

134 The conidia suspension of *Aspergillus niger* was filtered and adjusted to final concentration of 2-3 ×
135 10^6 spores/mL in sterile distilled water. This suspension was aseptically spread onto Sabouraud
136 dextrose agar plates. Sterile Whatman paper discs were impregnated with 10 µL of each extract (200
137 mg/mL) and placed on the inoculated agar plates. The plates were incubated at 28°C, and the
138 diameters of the inhibition were measured after 72 hours (Yazdani et al. 2012). The test was repeated
139 three times.

140 **2.8.5. Minimum inhibitory concentrations of extracts**

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

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147 **2.8.6. Minimum bactericidal concentrations of extracts**

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

151 **2.9. Statistical analysis**

152 All analyses were conducted in triplicate (n=3), and the results were presented as mean ± standard
153 deviation (SD). The data were evaluated using one-way analysis of variance (ANOVA) and
154 differences between means was calculated by Tukey's multiple comparison test using GraphPad Prism
155 5 (GraphPad Software, San Diego, CA, USA). Difference among means at 5% level ($p<0.05$) was
156 considered significantly different.

157 **3. Results**

158 **3.1. Extraction yield**

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

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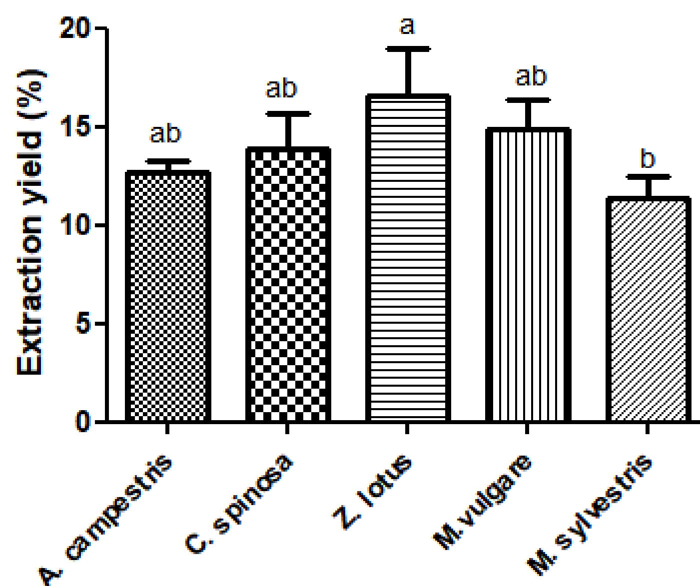

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165 **Figure 1**: Comparison of extraction yield values of selected plant extracts. Means ± SD of
166 determinations were made in triplicate experiments. Column means followed by different letters
167 (a,b) differ significantly ($p< 0.05$)

168 **3.2. Phytochemical screening**

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

171 Among the tested species, *M. vulgare* exhibited the highest phytochemical diversity, with presence
172 of ten out of the eleven tested compound classes. In contrast, *C. spinosa* showed the least diversity,
173 presenting only two detected compound groups (reducing compounds and steroids). Phenolic
174 compounds and reducing compounds were the most frequently detected phytochemicals. Notably *A.*
175 *campestris* and *Z. lotus* showed strong reactions for both classes. Tannins followed a similar pattern,
176 with strong presence in *A. campestris* and *Z. lotus*. Saponins and mucilage were less common,
177 detected only in one or two species. Finally, alkaloids were not detected in any of the tested species,
178 which may reflect a low alkaloid content in the aerial parts used.

179 **Table 2:** Phytochemical screening results of hydroethanolic extract of selected plants

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

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

181 **3.3. Total phenolic content**

182 The total phenolic content of five medicinal plant extracts was determined and expressed as
183 micrograms of gallic acid equivalents per milligram of dry weight (µg GAE/mg E). The results are
184 illustrated in **figure 2** and demonstrate significant variation among studied species ($p < 0.05$),
185 reflecting species-dependent differences in polyphenol accumulation. *Z. lotus* had the highest
186 phenolic content (145.89 ± 0.39 µg GAE/mg E), showing a statistically significant difference

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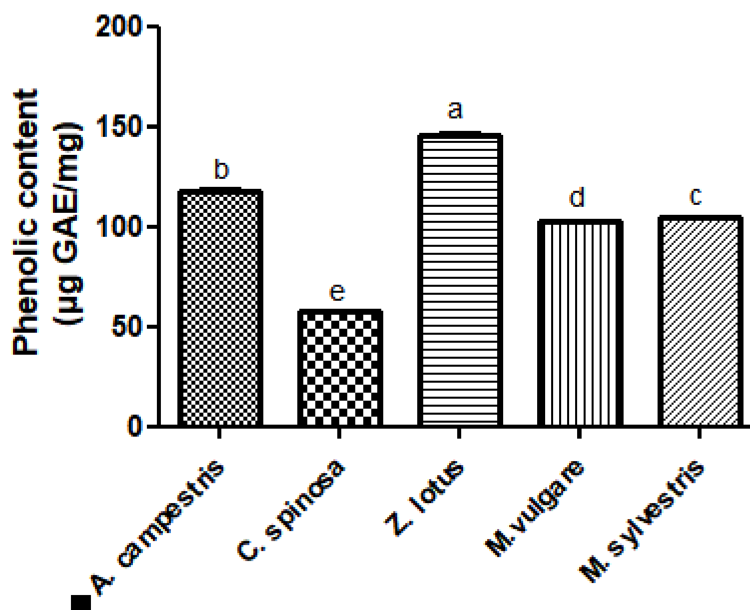
187 compared to the other species. *A. campestris* exhibited a phenolic concentration of 118.1 ± 0.6 µg
188 GAE/mg E, while *M. sylvestris* and *M. vulgare* showed intermediate values of 104.49 ± 0.05 µg
189 GAE/mg E and 102.2 ± 0.11 µg GAE/mg E, respectively. In contrast, *C. spinosa* had the lowest
190 phenolic concentration of 57.4 ± 0.02 µg GAE/mg E.



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

195 **3.4. Total flavonoid content**

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

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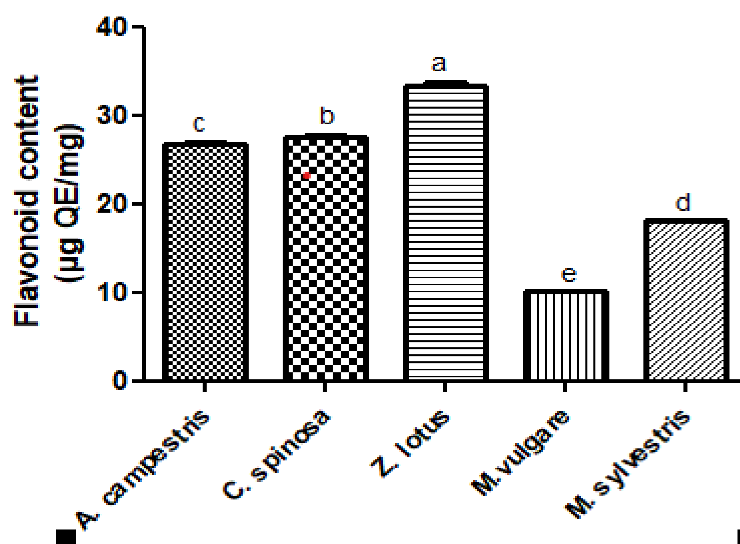

204 **Figure 3**: Total flavonoid content of tested hydroethanolic plant extracts. Values were expressed as

205 means ± SD of triplicate. Column means followed by different letters (a,b,c,d,e) differ significantly

206 ($p < 0.05$)

207 **3.5. Free radical scavenging activity**

208 The antioxidant activity of the selected extracts was assessed using the DPPH assay, and the results

209 are expressed as the IC_{50} values (**Figure 4**). All extracts exhibited radical scavenging activity ranging

210 from 2.29 ± 0.05 to 3.81 ± 00.9 mg/mL. *M. sylvestris* showed the most potent antioxidant activity

211 which did not differ significantly from the standard antioxidant BHT ($p > 0.05$), followed by *M.*

212 *vulgare* and *A. campestris*. Whereas *C. spinosa* and *Z. lotus* showed comparatively lower activity.

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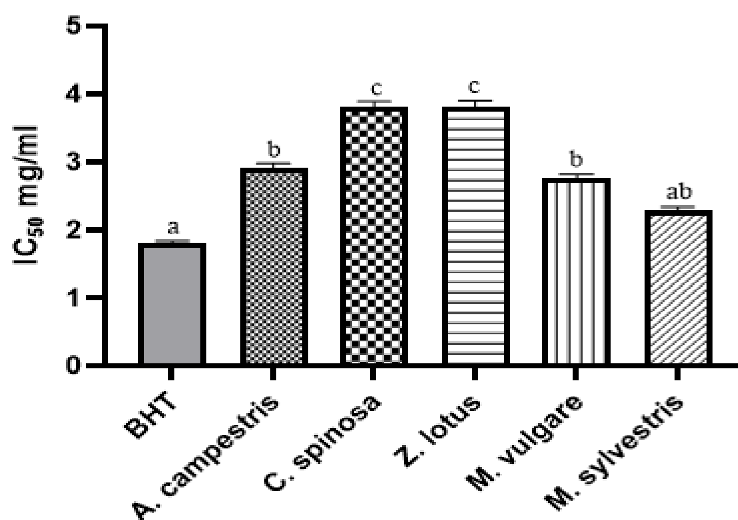

214 **Figure 4:** IC50 values of plant extracts and BHT (standard) against DPPH radicals. Values were

215 expressed as means ± SD of triplicate. Column means followed by different letters (a,b,c) differ

216 significantly ($p< 0.05$)

217 **3.6. Antibiotic susceptibility**

218 In order to evaluate the sensitivity of the bacterial strains, nine standard antibiotics belonging to

219 different families were used as positive controls, and the results are presented in the **table 3**.

220 *Staphylococcus aureus* showed a resistance to both antibiotics Piperacillin and Erythromycin, and

221 sensitivity to the other antibiotics. The strain *E. coli* was sensitive only to Ofloxacin,

222 Chloramphenicol, and Tetracycline. As for *P. aeruginosa* and *B. cereus*, they exhibited sensitivity

223 only to Ofloxacin, whereas *K. pneumoniae* was resistant to all the antibiotics tested.

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229 **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

230 R: resistant ; S: sensitive

231 **3.7. Antimicrobial activity of extracts**

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

234 In disc diffusion method, the extracts of *Z. lotus*, *A. campestris* and *C. spinosa* were shown to have
235 antibacterial potency against *S. aureus* with mean inhibition zones of 22 ± 0.0 mm, 12.3 ± 1 mm and
236 11 ± 0.0 mm, respectively. The *M. vulgare* and *A. campestris* extracts exhibited moderate activity
237 against *B. cereus* with inhibition zones of 11 ± 0.12 mm and 9 ± 0.0 mm, respectively. Extracts from
238 *M. sylvestris* and *A. campestris* displayed potential activity with diameters of 12 ± 0.3 mm and 11 ±
239 0.0 mm for *P. aeruginosa*. *K. pneumoniae* was resistant to all extracts exception for *M. sylvestris*
240 extract with inhibition zone of 10 ± 0.1 mm. On the other hand, no antibacterial activity was observed
241 for all extracts against *E. coli* strain.

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242 **Table 4:** Diameters of inhibition zones (mm) of the tested extracts

243

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	—	—	—	—	—	—

244 Values were expressed as means ± SD (n=3). Means followed by different letters (a,b,c) within the

245 same row differ significantly ($p< 0.05$), (-): No activity

246 The minimum inhibitory concentrations and minimum bactericidal concentrations of the extracts

247 ranged from 12.5 mg/mL to 200 mg/mL. *S. aureus* revealed MIC values of 25, 100, 25 mg/mL for *A.*

248 *campestris*, *C. spinosa* and *Z. lotus* while MBC values of the extracts for the same strain were 25,

249 100, 25 mg/mL, respectively. The MIC and MBC values for *B. cereus* at examination of *A. campestris*,

250 *C. spinosa* and *M. sylvestris* were 100, 200; 100, 100; 12.5, 50 mg/mL, respectively. The MIC values

251 of *A. campestris* and *M. vulgare* were 25, 12.5 mg/mL for *P. aeruginosa*, while the MBC values for

252 the same strain and the same extracts were 50, 100 mg/mL. Finaly, the MIC and MBC of *M. vulgare*

253 against *K. pneumoniae* were 25 and 100 mg/mL (**Table 5**).

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254 **Table 5:** Minimum inhibitory concentrations (MICs) and minimum bactericidal
255 concentrations (MBCs) of hydroethanolic extracts against sensible strains.
256 ND: Not determined

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

257

258 **4. Discussion**

259 In the present research, results suggest that *Z. lotus* and *A. campestris* have the most promising
260 phytochemical profile, due to their significantly high levels of phenolics and flavonoids compounds.
261 These constituents are known to play critical roles in plant defence, offering protection against biotic
262 and abiotic stresses (Zaynab et al., 2018) and are associated with different biological activities
263 (Cavasos et al., 2021). These results were compared with those reported by Letaief et al. (2021) and
264 Bakchiche et al. (2019). In their researches, the ethanolic extracts of *Z. lotus* and *A. campestris* leaves
265 exhibited a relatively low phenolic and flavonoid contents (41.70 ± 0.70 µg GAE/ mg and 28.54 ±
266 1.89 µg QE/mg) and (102.09 ± 1.65 µg GAE/mg and 17.94 ± 1.26 µg RE/mg), respectively. In
267 contrast, *M. vulgare*, despite having moderate phenolic concentration, exhibited the lowest flavonoid

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268 content, indicating a more limited potential from flavonoid-based mechanisms. For *M. sylvestris*,
269 results were significantly higher than those of El- Sayed et al. (2018) who found that the total phenolic
270 and flavonoid contents of 70% hydroethanolic extracts were 57.30 ± 0.04 µmg GAE/ mg E and 11 ±
271 0.01 µg QE/ mg E, respectively. Regarding *C. spinosa*, the hydroethanolic extract demonstrated an
272 important flavonoid content compared to other plants. These finding are consistent with previous
273 reports emphasizing the richness of this plant extract in flavonoid compounds (Tlili et al., 2011). The
274 relatively high variability of total phenolic and flavonoid compounds of plants in this study may be
275 attributed to different factors, plant material, extraction procedures, with environmental and climatic
276 conditions being the most influential determinants of their levels (Pourhosseini et al., 2020).

277 Given the richness of the studied extracts in various phytochemicals known for their redox potential,
278 the antioxidant activity was further assessed using DPPH radical scavenging assay. Results revealed
279 that *M. sylvestris* and *M. vulgare* hydroethanolic extracts exhibited the strongest radical scavenging
280 capacity compared to other plants. This activity could be related to their richness in phenolic
281 compound especially the presence of coumarins which are considered as one of the most potent
282 antioxidant compounds (Lončarić et al., 2020) and anticancer agent (Paul et al., 2024). It has been
283 reported in several studies the antioxydant potential of *M. sylvestris* (Batiha et al., 2022; Rhimi et al.,
284 2025) howevere in this study, no significant difference was observed between the antioxidant activity
285 of the hydroethanolic extract (2.29 ± 0.05) and BHT (1.8 ± 0.04 mg/mL), indicating that *M. sylvestris*
286 extract can achieve antiradical effects equivalent to those of a synthetic standard. Also, previous
287 researches highlighted its nutritional value and therapeutic potential, particularly the leaves due to
288 their anticancer, anti-ulcerogenic, skin-whitening, and anti-aging properties (Paul et al., 2024).

289 The emergence of antibiotic-resistant bacteria is one of the greatest problems facing the medical
290 community in both developed and developing countries (Islam, 2021). In Algeria, natural therapies
291 based on plant-derived compounds have gradually increased. Therefore, such plants should be studied
292 to better understand their efficiency. Among the Gram-positive strains, *S. aureus* showed important
293 sensitivity to several extracts, particularly *Z. lotus*, followed by *A. campestris* and *C. spinosa*.

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294 Similarly, *B. cereus* was moderately inhibited by *M. vulgare* and *A. campestris*. The notable sensitivity
295 against Gram-positive bacteria may be attributed to flavonoids, which are potent inhibitors of sortase
296 enzymes located in the cytoplasmic membrane of these bacteria (Ghedadba et al., 2015). In contrast,
297 Gram-negative bacteria exhibited a more variable and generally lower sensitivity to the five extracts.
298 *P. aeruginosa* was found to be sensitive to *M. sylvestris* and *A. campestris*. Whereas, *K. pneumoniae*
299 was sensitive only to *M. sylvestris* extract. These bacteria are well known for their high level of
300 resistance than Gram-positive bacteria, primarily due to the presence of the outer membrane and
301 plethora of active efflux pumps (Leus et al., 2023).

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

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

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

322 **Conclusion**

323 The five hydroethanolic extracts emerge as promising sources for therapeutic or nutraceutical
324 development due to their dual richness in phytochemicals especially phenolic compounds and
325 flavonoids that are closely related to the observed antioxydant and antibacterial effect.. The clear
326 interspecies differences in biological activities highlight the importance of phytochemical analysis
327 when selecting materials for antioxidant or medicinal purposes. Furthermore,These finding provide
328 scientific support for traditional use of these medicinal plants and underscore their potential as
329 alternatives to synthetic agents. However, future investigations should focus on isolatation and
330 characterisation of the active constituents.

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