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Methanolic extract of *Artemisia campestris*: HPLC analysis, assessing antioxidant, anti hemolysis and antibacterial properties

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Abstract

This study aims to assess the phytochemical composition and evaluate the antioxidant and antibacterial activities of the methanolic extract from the aerial parts of *Artemisia campestris*, which grows in Southeast Algeria. The results indicate that the plant is rich in proteins and lipids while exhibiting low carbohydrate content. Additionally, there were notable variations in the levels of polyphenols and flavonoids. The crude extracts were high in polyphenols content in the ethanol extract 446.7±5.11 mg EAG/g Extract, and the best value of flavonoids in methanol extract 66.8±0.93 mg QE/g Extract and 5.19±2.60 mg CT/g Extract. The HPLC analysis revealed that Rutin (16042.036 µg/g) and Chlorogenic acid (14224.476 µg/g) were the most abundant element with a high amount of Quercetin and Naringin (5884.808 µg /g, 2109.307 µg/g respectively), a moderate quantity of Vanilin, Gallic acid, Vanilic acid and Caffiec acid (864.363 µg/g, 740.516 µg/g, 733.753 µg/g and 669.865 µg/g), a little amount of p- Coumaric acid (216.626 µg/g). To evaluate the antioxidant activity, the results of the DPPH• test showed that methanol extract had a scavenging effect (IC50: 552± 0.7 µg/ml), 50% of erythrocytes could be protected at a concentration of 245.17± 5.8 µg/mg; this value was comparable to ascorbic acid's activity (IC50=85.29± 2.3 µg/mL). The high concentration of extract (25 mg/ml) had the best antibacterial activity using the disc diffusion method on two bacterial strains: *Escherichia coli* (13.66± 1.3 mm) and *Enterococcus faecalis* (18.33± 2.1 mm). The findings underscore the potential applications of *Artemisia campestris* in the agro- food and pharmaceutical industries.

Keywords: *Artemisia campestris*, anti-oxidant activity, erythrocytes, antibacterial activity, pharmaceutical.

Introduction

Medicinal plants have been esteemed across cultures for their nutritional and therapeutic properties, serving as crucial sources for drug development and treating various health conditions (Chaachouay & Zidane, 2024). According to a World Health Organization survey, these plants play a pivotal role in contemporary medicine, highlighting their importance in

discovering new pharmaceuticals and natural remedies (Muscolo, Mariateresa, Giulio, & Mariateresa, 2024). Among the numerous benefits attributed to medicinal plants, their phytochemical constituents are recognized for their antioxidant properties, which can mitigate oxidative stress and its associated health risks (Mow et al., 2024). This has sparked an increasing interest in investigating bioactive compounds and natural antioxidants to validate traditional healing practices, innovate new therapeutic agents, and address various health issues (Limam et al., 2024).

One notable example of such a plant is *Artemisia campestris*, a perennial herb belonging to the Asteraceae family. This species is widely distributed across various continents and is particularly significant in traditional medicine. Its aromatic properties and polymorphic nature, encompassing multiple subspecies and variations, contribute to its diverse applications (Dib & El Alaoui-Faris, 2019). In Algeria, especially in the Hoggar region (Tamanrasset, South Algeria), the local population utilizes the aerial parts and roots of *Artemisia campestris* to manage chronic conditions such as diabetes, skin diseases, and hypertension (Quezel & Santa, 1963; Zahnit et al., 2022). Furthermore, its culinary applications include food preservation, showcasing its versatility (Tardío, Pardo-de-Santayana, & Morales, 2006).

The pharmacological potential of *Artemisia campestris* is extensive and multifaceted. Numerous studies have confirmed its efficacy across various therapeutic domains, including antifungal, antihypertensive, hypotensive, and vasorelaxant activities (Dib, Angenot, Mihamou, Ziyyat, & Tits, 2017; Webster, Taschereau, Belland, Sand, & Rennie, 2008). Additionally, this plant exhibits promising antidiabetic and antioxidant properties and notable antibacterial and anti-inflammatory effects (Ghlissi, Sayari, Kallel, Bougatef, & Sahnoun, 2016; Sefi et al., 2012). Particularly noteworthy are its neuroprotective properties, which have garnered increasing attention due to evidence suggesting its ability to support neuronal health. Recent investigations have further elucidated its anticancer potential, especially in multiple myeloma, where it has been shown to induce apoptotic and necrotic cell death in resistant cancer cells. This phenomenon is believed to be associated with its rich phenolic content (Golubkina et al., 2022; Limam et al., 2024; Younsi et al., 2017).

This research aims to comprehensively analyze the phenolic compounds in *Artemisia campestris* sourced from the Djelfa region of Algeria. This study will focus on quantifying polyphenols, flavonoids, and tannins using High-Performance Liquid Chromatography (HPLC) analysis. Additionally, we will assess the plant's antioxidant, anti-hemolytic, and

antibacterial activities, thereby contributing to a deeper understanding of its therapeutic potential and validating its traditional uses.

Materials and methods

Chemical reagents :

Methanol, DPPH (2,2-Diphenyl-1-picrylhydrazyl), Gallic acid, Sodium carbonate, Folin-Ciocalteu reagent, Ascorbic acid, Vanillin, Quercetin, Aluminum chloride ($AlCl_3$), DMSO (Dimethyl sulfoxide).

Microbial Strains Used

The bacterial strains utilized in this study are reference strains obtained from the American Type Culture Collection (ATCC). These include *Escherichia coli* (ATCC 25922) and *Enterococcus faecalis* (ATCC 51299). The El-Majed El-Oued Medical Analysis Laboratory kindly provided all strains.

Plant Material

Artemisia campestris was manually harvested in July 2021 from M'Liliha, from Djelfa Province, located in the central part of northern Algeria, within the Ouled Nail mountains. It is situated 300 km south of the capital, between 2° and 5° East longitude and 33° and 35° North latitude. This region is characterized by a semi-arid, dry, and cold climate. The leaves were dried in the open air. Subsequently, mechanical means were used to grind the samples into a powdered form.

Methanolic extract preparation

The plant material was subjected to maceration in a hydroalcoholic solution (methanol/water; 70/30; v/v), with the process repeated three times and the solvent refreshed each time. The three extracts were combined after careful decantation through the Whatman filter paper. The filtrate was then evaporated using a rotary evaporator (Büchi) at 45°C until all methanol was removed. The remaining residue was dried in an oven at a maximum temperature of 40°C. Finally, the hydro-methanolic residue was diluted with methanol after vacuum concentration (Alara, Abdurahman, & Ukaegbu, 2021).

Quantification of Total Polyphenol Content (TPC)

The total polyphenol content was determined with the Folin-Ciocalteu reagent (Bobková et al., 2020). A calibration curve was created using gallic acid as the standard. The results are expressed as mg of gallic acid equivalents per gram of plant extract.

Quantification of Total Flavonoids (TFQ)

The quantification of flavonoids was carried out using a modified method involving aluminum chloride (AlCl3) and sodium hydroxide (NaOH). Aluminum chloride forms a yellow complex with flavonoids, while sodium hydroxide produces a pink complex that absorbs in the visible range at 430 nm (Rebiai & Lanez, 2012).

Quantification of Condensed Tannins (CT)

Condensed tannins are quantified through the vanillin assay in an acidic medium. This method leverages vanillin's reactivity with the monomeric units of condensed tannins, forming a colored complex measurable at 500 nm. The interaction is specific to the initial unit of the polymer, enabling accurate estimation of tannin quantities using this approach (Kılıç, Gürgen, Yıldız, Can, & Değirmenci, 2024).

Qualitative analysis by HPLC

We used Shimadzu Prominence LC-20AL High-Performance Liquid Chromatography (HPLC) to measure the phenolic components in *Artemisia campestris* leaves. Non-polar aliphatic residues were used in the reverse-phase chromatography investigations. The mobile phase consisted of a gradient elution using acetonitrile and 0.1% acetic acid. They were outfitted with a Shim-pack VP-ODSC18 analytical column (4,6mm x 250mm, particle size of 5mm) and a Hamilton 25l universal injector. The injection volume of the sample and reference were both 20µL and the flow rate was 1 mL/min.

The number of phenolics in *Artemisia campestris* leaves was measured by utilizing their regression equation to compare the retention periods of the leaves with those of the respective standards, detecting absorbance at 268 nm and obtaining UV spectra using an SPD-20A UV-vis detector (Shimadzu).

Antioxidant Activity

A volume of 1 ml from each extract is combined with 2 ml of a 0.1 mM DPPH• solution in methanol. The mixture is shaken immediately and stored in the dark at room temperature for 30 minutes to facilitate the reaction. Absorbance is measured at 515 nm against a blank.

Samples and controls are prepared under identical conditions. Optical density measurements of each extract at different dilutions enable the calculation of the inhibition parameter and the subsequent determination of IC50 (Wibawa, Pramitha, Sanjiwani, & Adrianta, 2024).

The percentage of inhibition is calculated using the following formula:

$$I\ \%DPPH\ radical\ scavenging = [(A0-As)/A0]*100 \quad (1)$$

A_0: Absorbance of the control (without antioxidant) after 15 minutes
As: Absorbance of the extracts measured after 15 minutes

Inhibition concentration (IC50) represents the percentage of inhibition at different concentrations of the tested fractions and is the concentration of the tested sample required to reduce 50% of the DPPH• radical.

Hemolysis test

This test evaluates the ability of plant extracts to protect erythrocytes from oxidative stress and free radical damage by calculating the proportion of dissolved erythrocytes, indicating cell membrane integrity (Larrán et al., 2024).

Forty microliters of human erythrocytes were mixed with 2 ml of plant extract and incubated at 37 °C for five minutes. Subsequently, 40 µl of H2O2 (30×10^{-3} M), 40 µl of FeCl3 (80×10^{-3} M), and 40 µl of ascorbic acid solution (50×10^{-3} M) were added. After one hour of incubation at 37 °C, the mixture was centrifuged at 700 rpm for 10 minutes. The absorbance of the supernatant was measured at λ=540 nm. The following formula was used to calculate the percentage of hemolysis:

$$Hemolysis\% = (Acontrol / Asample) \times 100 \quad (2)$$

Ascorbic acid served as the standard in this experiment. The results were expressed using the Hly50 value, which indicates the concentration at which 50% of red blood cells were lysed (Carpenter & van Hoek, 2024).

Antibacterial Activity

The diffusion method used the standard disc diffusion test (Bashetti, Vernekar, Devaraju, & Hiremath, 2024). *Escherichia coli* (ATCC 25922) and *Enterococcus faecalis* (ATCC 29212)

strains were first cultured in nutrient agar (composition: agar 15 g, peptone 5 g, sodium chloride (NaCl) 5 g, beef extract 1.5 g, and yeast extract 1.5 g prepared in 1000 ml distilled water, pH 7.0). Using sterile disks (6 mm) impregnated with methanol or the antibiotic gentamicin served as negative or positive controls for an antibacterial study. Agar plates underwent a 37 °C overnight incubation before being evaluated for inhibition zones.

Results and discussion

Determination of Total Polyphenols

The selection of *Artemisia campestris* as the focus of this study is underscored by its traditional applications in the Djelfa region for the treatment of various human ailments and its notable biological activities, which have not been extensively investigated. This medicinal plant presents a valuable opportunity for further research.

In our study, methanol (MeOH) was the most effective solvent for extracting phenolic acids. From the aerial parts (leaves and stems) of *Artemisia campestris* sourced from Foussana, Tunisia, ten out of thirteen phenolic compounds were successfully recovered, yielding a cumulative concentration of 46.07 mg/g of extract. The remarkable efficacy of MeOH can be attributed to its high polarity, which facilitates the extraction process (Limam et al., 2024).

The total polyphenol content was quantified using the Folin-Ciocalteu method, with gallic acid as the standard. The methanolic extract demonstrated a total polyphenol content of 446.7 ± 5.11 mg GAE/g of extract. This is notably higher than values reported for accessions from Laghouat in Algeria, which exhibited a polyphenol content of only 20.38 ± 0.30 mg GAE/g (BAKCHICHE, GÖREN, AYDOĞMUŞ, MATARACIKARA, & GHAREEB, 2022). Another analysis of *Artemisia campestris* from the same region revealed a polyphenol content of 53.84 ± 4.59 mg GAE/g (Djeridane et al., 2006).

These discrepancies highlight the significant variation in polyphenol content, which may be attributed to differences in cultivation sites, extraction methodologies, and environmental factors. Our results, as presented in Table 1, indicate significantly elevated levels of polyphenols, underscoring the potential of *Artemisia campestris* as a rich source of bioactive compounds.

Flavonoid Quantification

Table 1 presents the extract's flavonoid content. Flavonoid concentrations were quantified as mg QE/g extract using the aluminum chloride ($AlCl_3$) method. Our results indicate that the methanolic extract from the Djelfa region demonstrates a notably high flavonoid concentration of 66.8 ± 0.93 mg QE/g extract. In comparison, *Artemisia campestris* samples from Boussaâda and Oum El Bouagh in Algeria exhibited significantly lower flavonoid levels, measuring 12.91 ± 0.01 mg QE/g extract and 13.72 ± 0.00 mg QE/g extract, respectively (Parham et al., 2020).

This substantial difference in flavonoid content may be attributed to various factors, including environmental conditions, soil composition, and cultivation practices in the respective regions. For instance, previous studies have shown that flavonoid levels in medicinal plants can vary widely based on geographic and climatic influences (Pant, Pandey, & Dall'Acqua, 2021). These findings suggest that the Djelfa region may offer unique advantages for cultivating *Artemisia campestris*, potentially enhancing its flavonoid-rich profile and reinforcing its medicinal value.

Quantification of Tannins

In our study, we quantified condensed tannins using the Julkunen-Titto (1985) method (Vergun, Svydenko, Grygorieva, Hauptvogel, & Brindza, 2023), which generates a complex chromophore with a maximum absorbance at 500 nm. We determined a condensed tannin concentration of 5.19 ± 2.60 mg CTE/g extract using catechin as a calibration standard.

When comparing our results with those of a recent study (Sriti, Hammami, Fares, Selmi, & Limam, 2024), we found that our measured concentration of 5.19 mg CTE/g extract is lower than their reported value of 6.35 mg CE/g dry matter for extracts using methanol, with ethanol yielding 2.54 mg CE/g dry matter. This indicates that while *A. campestris* may not be particularly rich in condensed tannins, but the choice of solvent plays a vital role in optimizing extraction efficiency and affecting the final tannin concentration.

Table 1. Total phenolic, flavonoid, and tannin contents in *A. campestris* extract

Estimation	**total phenolics mg GAE/g Ex**	**total flavonoids mg QCE/g Ex**	**condensed tannins mg CTE/g Ex**
Artemisia campestris leaves	446.7 ± 5.11	66.8 ± 0. 93	5.19 ± 2.60

Values expressed as mean ± standard erreur (n=3)

Qualitative analysis by HPLC

The results of HPLC analysis chromatograms showed that we have identified 09 phenolic compounds in the *Artemisia campestris* leaves (Figure. 1) . The analysis revealed that Rutin (16042.036 µg/g) and Chlorogenic acid (14224.476 µg/g) were the most abundant element with a high amount of Quercetin and Naringin (5884.808 µg /g, 2109.307 µg/g respectively), a moderate quantity of Vanilin, Gallic acid, Vanilic acid and Caffiec acid (864.363 µg/g, 740.516 µg/g, 733.753 µg/g and 669.865 µg/g), a little amount of p- Coumaric acid (216.626 µg/g), (Table 2) were detected in *Artemisia campestris* leaves extract.

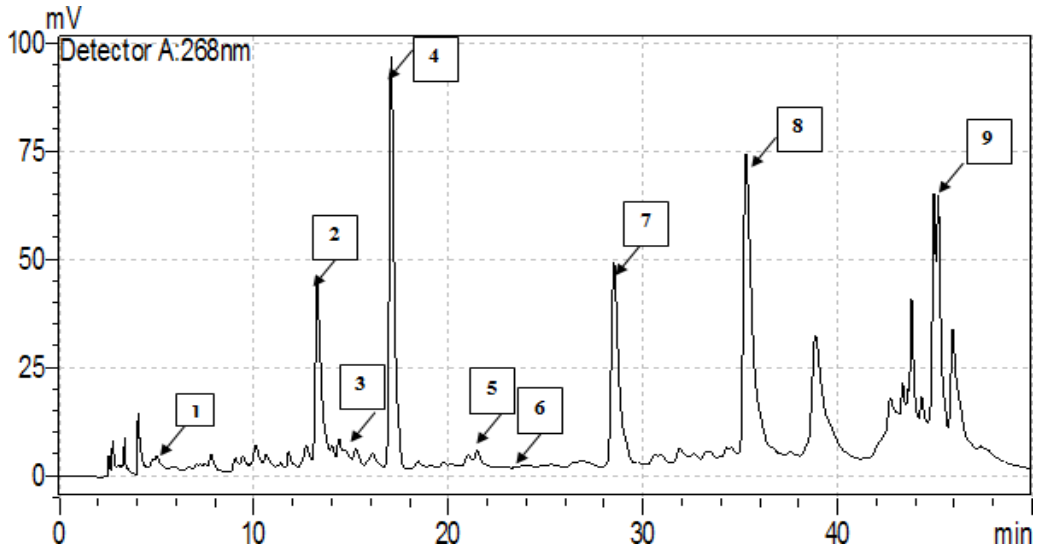


Figure 1. HPLC chromatograms of the extract of *A.campestris* of 1:Gallic acid, 2: Chlorogenic Acid, 3: Vanillic acid, 4: Caffiec Acid, 5: Vanilin, 6:p-Coumaric Acid, 7: Rutin, 8: Naringin, 9: Quercetin, respectively.

Table 2. The concentration of phenolic compounds identified in *A.campestris* extract

.

Number	Compounds	Concentration (µg/g)
1	**Gallic acid**	740.5166
2	**Chlorogenic acid**	14224.4760
3	**Vanilic acid**	733.7530
4	**Caffiec acid**	669.8654
5	**Vanilin**	864.3634
6	**p-Coumarin**	216.6263
7	**Rutin**	16042.0369
8	**Naringin**	2109.3078
9	**Quercetin**	5884.8085

Antioxidant activity of *Artemisia campestris* extracts

Antioxidants are molecules that, at low concentrations relative to the oxidizable substrate, delay or stop oxidation processes, thus regulating cellular redox balance (Gulcin & Alwasel, 2023). The radical scavenging mechanism, characterized by the interaction between antioxidants and DPPH, is contingent upon the antioxidant's structural conformation. Some compounds exhibit rapid reactions with DPPH, reducing the amount of DPPH proportional to their hydroxyl group count (Gulcin, 2020).

A more significant decrease in DPPH absorbance indicates a higher antioxidant power of the extracts. This test provides information on the direct radical-scavenging capacity of various phenolic substances in the extracts (Hendel et al., 2024). This study estimated the DPPH IC50 of *Artemisia campestris* extract at 552 ± 7.0 µg/ml. A previous study on the antioxidant capacity of methanolic extracts of *A. campestris* measured by DPPH showed an IC50 ranging between 48,42 ± 13,19 and 320,60 ± 22,58 µg/ml (Amel et al., 2022).

Table 3. DPPH (IC50 µg/ ml) and % hemolysis of the *Artemisia campestris* leaf extract

Estimation	**DPPH IC50 µg/ml**	**HyIC50 µg/ml**
Artemisia campestris	552 ± 0. 7	245.17± 5.8
Gallic acid	1.01 ± 0. 3	/
Ascorbic acid	/	85.29± 2.3

Values expressed as mean ± standard erreur (n=3)

The anti-hemolysis assay assesses antioxidant activity by determining the effectiveness of antioxidants in protecting red blood cells from hemolysis (rupture). H2O2 can degrade hemoglobin, releasing Fe^{2+} ions from the reaction with hydroxyl radicals (OH). The antihemolytic activity of plant extracts may stem from inhibiting these radicals by bioactive compounds in the extracts, which donate electrons to H2O2, effectively neutralizing it to form water (Salem, Mahfouz, & Fathy, 2021). This test evaluates the ability of antioxidants, such as phenols, alkaloids, terpenes, and tannins, to protect red blood cells from oxidative damage caused by hydrogen peroxide (H2O2) (Hmidani et al., 2021). The extract showed excellent efficacy in shielding erythrocytes from oxidizing agents (heat and H2O2) (table 2). 50% of erythrocytes could be protected at a concentration of 245.17± 5.8 µg/mg; this value was comparable to ascorbic acid's activity (IC50=85.29± 2.3 µg/mL), one of the substances authorized for protection against oxidative damage (Cruz et al., 2021).

As far as we know, *Artemisia campestris* has not been reported to have any anti-hemolytic properties. Based on this finding, we infer that the extract might have solid and lipophilic antioxidants. For certain chemicals, such as flavonoids and catechols, The intramolecular hydrogen bond renders one -OH group unreactive while simultaneously reducing the unbound O-H bond dissociation enthalpy, enhancing its reactivity. The resulting semiquinone radical is stabilized by intermolecular hydrogen bonding, which inhibits its reaction with O_2 (Elizondo-Luevano et al., 2024).

α-tocopherol has the biological potential to impede lipid peroxidation (Ain, Fatima, Naseer, Kanwal, & Mahmood, 2024). This leads to the penetration of membrane permeability and, ultimately, membrane disintegration. In addition to the phenolic acids and flavonoids, the extract's content was verified using quantitative estimations and RP-HPLC-UV analysis (Table. 2). One of the most significant flavonoids that inhibits hemolysis, for instance, is rutin (Al-Maghrabi, 2024).

Antimicrobial Activity

Artemisia campestris extract in this study was found to exhibit significant antimicrobial activity. Two bacteria, namely *Enterococcus faecalis* and *Escherichia coli*, were selected for evaluating the antibacterial effects of the methanolic extract (Figure. 1). The diameter of the inhibition zones was measured. At a higher concentration of *Artemisia campestris* extract (25 mg/mL) compared to the positive control Amoxicillin and Cefazolin (Table 4),

Enterococcus faecalis (18.33± 2.1 mm) and *Escherichia coli* (13.66± 1.3 mm). The antibacterial activity was dose-dependent at concentrations of 10, 15, 20, and 25 µg/mL, with an increase in the inhibitory zone size corresponding to higher concentrations of *Artemisia campestris* extract. Furthermore, our findings supported the previously published findings (Megdiche-Ksouri et al., 2015) that an *A. campestris* extract at 125 mg ml^1 inhibited the growth of *S. aureus* and *E. coli*. Variations in AC extracts' qualitative and quantitative active component contents can cause variations in their antibacterial activity.

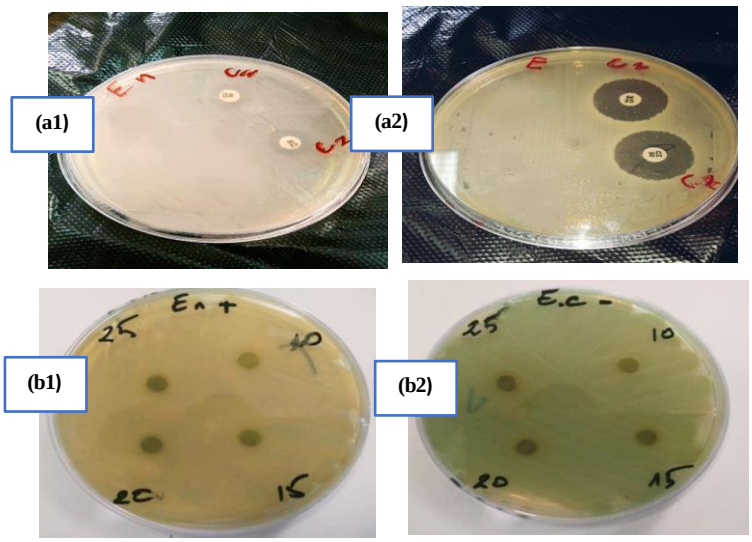
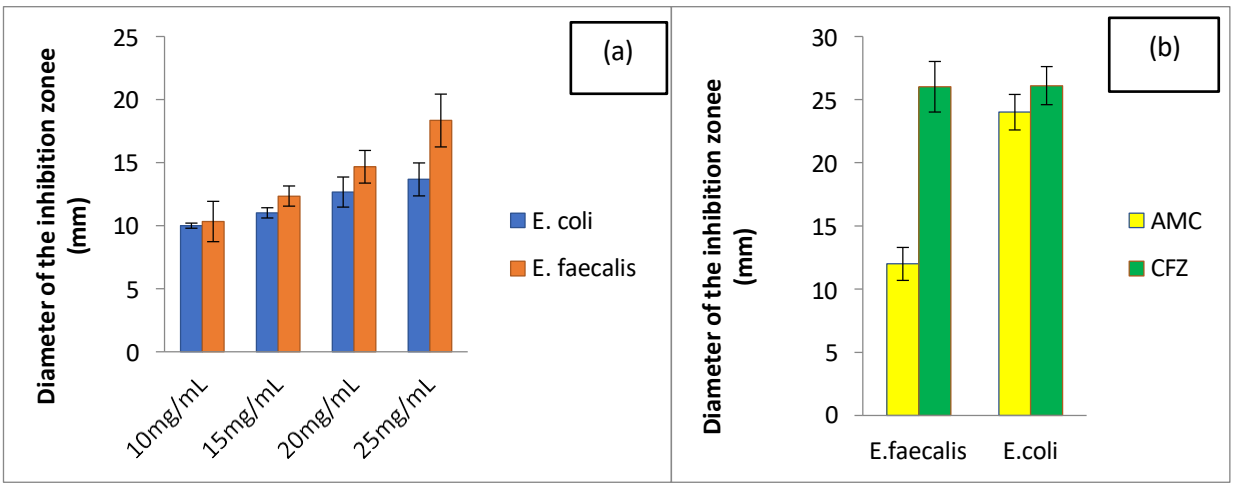


Figure 2. (a1) Inhibitory effect of antibiotics on the bacterial strain *Enterococcus faecalis*, **(a2)** *Escherichia coli* **E**: *Escherichia coli* **En**: *Enterococcus faecalis* **Am**: Amoxicillin **Cz**: Cefazolin**. (b1):** Inhibitory effect of *Artemisia campestris* on the bacterial strain



Enterococcus faecalis. **(b2):** *Escherichia coli* with different concentration (10,15,20, 25mg/ml)

Figure 3. Diameter of the inhibition zone (mm) of (a) *A. campestris* extract with different concentrations (10,15,20, 25mg/ml) - (b) antibiotics (Amoxicillin, Cefazolin**)** against *Enterococcus faecalis* and *Escherichia coli.*

According to a study (Brahmi, Berrached, Kebbouche Gana, Kadik, & Lenchi, 2024; Naili, Alghazeer, Saleh, & Al-Najjar, 2010), the antibacterial activity of the methanolic extract of *A. campestris* leaves and found it to have an inhibitory effect on all tested bacteria, including *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*. Plants contain many antimicrobial compounds, known as phytochemicals, including phenolic compounds, flavonoids, and tannins. Plant extracts' antimicrobial power depends on their chemical compositions (Benamar-Aissa, Gourine, Ouinten, & Yousfi, 2024). The antimicrobial properties of phytochemicals are partly related to their lipophilic nature, leading to their accumulation at bacterial cell walls, disrupting membrane function and permeability, causing membrane degradation, cytoplasmic membrane damage, protein damage, and leakage of cellular contents (Benamar-Aissa, Gourine, Ouinten, & Yousfi,2024).

Table 4. Diameter of the inhibition zone (mm) of *A. campestris* methanolic extract with different concentrations (10,15,20, 25mg/ml) and antibiotics (Amoxicillin, Cefazolin**)** against *Enterococcus faecalis* and *Escherichia coli*.

Sample	Concentration (mg/mL)	Bacterial strain	
		Escherichia coli	*Enterococcus faecalis*
***Artemisia campestris* methanolic extract**	10 (mg/mL)	10 ± 0.2	10.33± 1.3
	15(mg/mL)	11± 0.5	12.33± 0.9
	20 (mg/mL)	12.66± 0.9	14.66± 1.2
	25 (mg/mL)	13.66± 1.3	18.33± 2.1
Amoxicillin	30 µg/disk	26± 1.4	26± 1.6
Cefazolin	30 µg/disk	24± 1.3	12± 1.2

Values expressed as mean ± standard erreur (n=3)

Conclusion

This study highlights the significant pharmacological potential of *Artemisia campestris* as a valuable source of bioactive compounds. The high total polyphenol and flavonoid content

underscores the plant's capability as an antioxidant, supporting its traditional use in treating various ailments in the Djelfa region of Algeria. The choice of methanol as a solvent demonstrates its effectiveness in optimizing extraction and enhancing compound recovery.

The analysis identified critical phenolic compounds, such as Rutin and Chlorogenic acid, known for their potent antioxidant and therapeutic properties. The extract's notable antioxidant activity and protective effect against oxidative stress suggest potential applications in health and medicine. Additionally, the antimicrobial efficacy observed against specific bacterial strains underscores the plant's promise as a natural antimicrobial agent. The observed dose-dependent inhibition highlights the need for further investigation into the mechanisms of action and the clinical relevance of these extracts. Variations in phytochemical concentrations across different regions indicate that environmental factors, including soil composition and climate, significantly influence the bioactive profiles of *Artemisia campestris*. This suggests optimizing cultivation practices to enhance the plant's medicinal properties.

In summary, the multifaceted bioactivity of *Artemisia campestris* positions it as a promising candidate for further research aimed at isolating and characterizing its active components. Comprehensive studies on its pharmacological properties and sustainable cultivation strategies could facilitate its integration into modern herbal medicine and nutraceuticals. The rich phytochemical composition of this plant warrants further exploration to unlock its therapeutic potential fully.

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