

Research Article

Variation of Phenolic Content and Bioactivity between the Different Organs of the Algerian *Capparis Spinosa* L.HAKIMA BELDI^{1*}, LYNDIA GALI², AMEL BENBOTT³, AMEL BOUDECHICHA⁴, CHAWKI BENSOUICI², ABDELOUAHAB YAHIA¹, NADIA BENZIDANE⁴¹ Laboratory of Natural Sciences and Materials (LSNM), Abdelhafid Boussouf University Center Mila, Algeria.² Biotechnology Research Center, Ali Mendjli New Town UV 03, BP E73, Constantine, Algeria.³ Laboratory of Natural Materials, Biomolecules and Biotechnology Applications, Department of Natural and Life Sciences, Larbi Ben M'hidi University, Oum El Bouaghi, Algeria.⁴ Laboratory of Applied Microbiology, Department of Microbiology, Faculty of Natural and Life Sciences, Ferhat Abbas University Setif1, Algeria.**ARTICLE DETAILS***Article history:*

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ABSTRACT

El-Kabbar or Caper (*Capparis spinosa* L.) is a xerophilic shrub that has a great capacity to resist extreme environmental conditions and can therefore be of great interest for the discovery of new molecules. Thus, the present study evaluated the antioxidant, anticholinesterase, and antibacterial activities of the methanolic extract of different parts (leaves, stems, and roots) of *Capparis spinosa* L. DPPH, ABTS, CUPRAC, reducing power, and β -carotene bleaching test were adopted to study the antioxidant activity. The extracts were also examined for their inhibitory capacity on acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) and for their antibacterial activity assessed by the disk diffusion method against four bacterial strains. The extracts have substantial levels of phenolic compounds mainly concentrated in the leaves. Significant antioxidant activity was displayed by the different plant organs, mainly the leaves which showed the strongest effect. This activity showed a strong correlation with their phenolic content. The different parts of the plant showed no AChE inhibition, while only the roots showed a slight BChE inhibition effect ($IC_{50} = 150.77 \pm 80.56 \mu\text{g/mL}$). Moreover, all extracts were inactive against *E. faecalis* and *K. pneumoniae*, while only leaves and roots at 100 mg/mL exerted weak inhibition against *E. coli* and *S. aureus*. Extracts from this plant have shown. The results indicated that the methanolic extract of *Capparis spinosa* L. leaves possessed strong antibacterial and antioxidant properties and could be an important source of natural compounds for the development of new drugs.

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INTRODUCTION

In recent years, there has been considerable interest in herbal medicine for the treatment of various diseases [1]. The pharmacological and therapeutic properties of medicinal plants have been attributed to chemical compounds isolated from their crude extracts, which play different physiological roles at low concentrations, ranging from cell signal transduction to defense against pathogens [2].

Reactive oxygen species (ROS) have been implicated in the pathology of many diseases, including cancer, hypertension, diabetes, and neurodegenerative disorders, as well as in aging.

Antioxidants are used in food and cosmetic products for preservation purposes. However, many synthetic antioxidant compounds have been shown to have toxic and/or mutagenic effects, which have encouraged research into the properties of natural antioxidants [3]. On the other hand, the consumption of foods rich in antioxidants can significantly delay or prevent damage caused by free radicals on tissues and biological molecules and interrupt the mechanisms leading to various diseases [4]. Alzheimer's disease is the most dominant form of dementia in the world, mainly affecting the elderly, in whom oxidative stress is one of the essential factors leading to the degeneration of cholinergic neurons leading to cognitive disorders. Inhibition of acetylcholinesterase and butyrylcholinesterase remains the main

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approach to improve the symptoms of the disease. However, there are enormous efforts to discover new molecules that are more effective and with less toxicity. In fact, substances targeting more than one factor are appreciated, such as both anticholinesterase and antioxidant effects [5]. Among plant-derived antioxidants, polyphenols have been widely appreciated for their pharmacological affects mainly their great antioxidant capacity [6].

For a long time, bacterial resistance has been a major problem in effectively treating infections. This resistance was born from the massive use of antibiotics in the food industries and in therapy. Recently, a great deal of attention to herbal medicines as alternative antimicrobials has seen great momentum in raising resistance by using them in combination with antibiotics. This is why it is very important to discover new sources of antioxidants and antimicrobial compounds from natural sources [7].

Capparis spinosa L. commonly known as capers is a spontaneous shrub that belongs to the Capparaceae family, which is a large family and includes about 470 species belonging to 17 genera, distributed in tropical and subtropical areas, the species are well diversified in vegetative and floral characteristics [8]. Its flower buds (Capers) and immature fruits are consumed as food or condiments in cooking [9]. This plant grows in different parts of the world, especially in Asian and African countries. *Capparis spinosa* has countless of pharmaceutical and ethnobotanical importance, which contains important bioactive agents and has the potential to produce valuable useful biochemical compounds for various pharmaceutical and food industries [10]. Caper with its different parts, flower buds, fruits, seeds, shoots and skinned roots, has several medicinal qualities, which explains its intense use in traditional medicine. This species contains many biologically active chemical families including alkaloids, glycosides, tannins, phenolic compounds, flavonoids, triterpenoids, steroids, carbohydrates, saponins, and a wide range of elemental minerals and electrolytes [11]. It has numerous pharmacological effects, including antimicrobial [12], cytotoxic, antidiabetic [13], anti-inflammatory [14], cardiovascular, broncho-relaxant [15], hepatoprotective, antioxidant, cardiovascular, anti-cancer and hypoglycemic properties [16 - 18].

Despite the significant medical benefits and therapeutic effects of *Capparis spinosa* L., there are few reports on the pharmacological effects of the plant grown in Algeria. Moreover, to our knowledge, to date there are no systematic studies on the *in vitro* antioxidant activity of the stem extract of this species. Therefore, the main objective of the present study was to determine the total content of phenols and flavonoids and to evaluate the antibacterial, enzymatic and antioxidant activities of methanolic extracts from stems, leaves, and roots of *C. spinosa* L.

MATERIALS AND METHODS

1. Chemicals

Folin-Ciocalteu reagent, sodium carbonate, gallic acid, quercetin, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azinobis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS), linoleic acid, β -carotene, 2,6-di-tert-butyl-4-hydroxytoluene (BHA), Tween 40, neocuproine, acetylcholinesterase (AChE) type VI-S, from electric eel <1000 U/mg solid, Butyrylcholinesterase (BChE) from equine serum 100 U/mg solid, 5,50-dithiobis [2-nitrobenzoic acid] (DTNB), butyrylthiocholine chloride, galanthamine were purchased from Sigma Aldrich, Germany. The antioxidant and anticholinesterase activities were performed on 96-well microplate and the absorbance was recorded using a microplate multimode reader (PerkinElmer Enspire, Singapore).

2. Plant Material

The plant *Capparis spinosa* L was collected at the flowering stage, in July 2018, in the commune of Aftis (Latitude: 36°23' 17"; Longitude: 4°27' 41") wilaya of Jijel (eastern Algeria). The taxonomic identification of the plant sample was carried out by the botany laboratory of the Institute of Biology, Abdelhafid Boussouf University Center - Mila (Algeria). The leaves, stems, and roots were separated, air-dried, and ground into a fine powder using an electric grinder.

3. Preparation of Plant Extracts

A quantity of 20g of powder was soaked in 100 mL of pure methanol for 48 hours in the dark and with stirring (700 rpm). The mixture is then filtered on Whatman paper. The methanol was evaporated using a rotary evaporator at 35°C (BUCHI, R215, Switzerland). The residue obtained was stored at -20°C in the dark until their analysis [14].

4. Phytochemical Analysis

4.1. Qualitative Analysis

To detect the main groups of secondary metabolites, present in the methanolic extracts of *Capparis spinosa* L., preliminary tests were carried out according to classic techniques based on the observation of color changes or formation of precipitations either with the vegetable powder or its 5% infused [19].

4.2. Determination of the Content of Phenolic Compound (TPC)

The quantification of total phenolics in the different extracts of *Capparis spinosa* L. was carried out spectrophotometrically using the Follin-Ciocalteu method according to the protocol described by Tlili [14]. Briefly, in glass hemolysis tubes, a volume of 200 μ L of the extracts of the species under study were mixed with 1 mL of 10-fold diluted Folin-Ciocalteu reagent. After 5 min in the dark, 800 μ L of sodium carbonate solution (7.5%) was added. The absorbance was read at 765 nm. After 2h of incubation in the dark. Total phenols were calculated using a gallic acid etalon curve (1-10 μ g/mL).

4.3. Determination of Total Flavonoids Content (TFC)

In hemolysis tubes, one milliliter of extract was added to one milliliter of methanolic solution of $AlCl_3$ (2%). Spectrophotometric reading of the absorbance was taken at 430nm after ten minutes of incubation in the dark. Quercetin (2.5-25 μ g/mL) was used as a standard to perform the standard curve [20]. Flavonoids concentrations were expressed as μ g quercetin equivalents per mg of extract (μ g QE/mg).

5. Antioxidant Activity

5.1. 2,2-diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging Activity

To study the anti-free radical activity of the extracts against the DPPH radical, the colorimetric method reported by El Aanachi [6] was applied. The DPPH solution (1 mM) was mixed with the samples at different concentrations. The transformation from violet color of the solution to yellow was followed at 517 nm. BHA, BHT, and α tocopherol were used as positive standards for comparison. Results were given as inhibition percentages and as 50% inhibition concentration (IC_{50}) (μ g/mL).

5.2. ABTS⁺ Radical Cation Decolorization Assay

The ABTS⁺ scavenging effect of tested samples was determined according to the method described by Gali and Bedjou [5]. The solution of the cation ABTS⁺ previously prepared was added to the different concentrations of plant samples (12.5-800 μ g/mL) in 96-well microplate. The discoloration of the solution was monitored at 734 nm. The results were compared to BHA and BHT standards.

5.3. Ferric-Reducing Antioxidant Potential (FRAP) Assay

The reducing power activity of the different samples was detected by applying the protocol described by Yakoubi et al. [21]. The protocol consists of reducing ferric Fe^{3+} ions from potassium ferricyanide (1%) to ferrous iron Fe^{2+} with the development of a blue-green color whose absorbance was recorded at 700nm. Results were reported as absorbance and $A_{0.5}$ values which correspond to the concentration giving an absorbance of 0.5.

5.4. Cupric Reducing Antioxidant capacity (CUPRAC)

To assess the ability to reduce copper ions of different extracts of *Capparis spinosa* L., the CUPRAC test was performed with slight modifications [22]. Neocupronine (7.5 mM) forms a complex with copper ions to which samples have been mixed at different concentrations (12.5-800 μ g/mL) inducing the development of a yellow-orange color. The intensity of the color thus developed was recorded at 450 nm. BHA and BHT at various concentrations were used as standards.

5.5. β -Carotene Bleaching Assay

The β -carotene bleaching test was performed according to the protocol previously reported by [23]. This test was used to predict the potential of different extracts of *Capparis spinosa* L. to counteract peroxide radicals. A linoleic acid/ β -carotene emulsion was prepared using tween 40 as an emulsifier. The oxidation reaction was started by adding a 30% solution of hydrogen peroxide. The solution thus obtained was mixed with samples and incubated at 50°C for 2 hours. The absorbance of the reaction was at 470 nm. The results were expressed as percentage inhibition and IC_{50} values and compared to BHA and BHT used as standards.

6. Anticholinesterase Activity

The ability of *Capparis spinosa* L. extracts to inhibit acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) was examined according to Ellman's method as described by [24]. AChE (5.32×10^{-3} U) or BChE (6.85×10^{-3} U) in phosphate buffer (0.1 mM, pH 8) were mixed with the galantamine extracts as standard. Thiocholine production from acetylthiocholine iodide (0.71 mM) or butyrylthiocholine chloride (0.2 mM) was monitored at 415 nm by adding DTNB. Inhibitory capacity was expressed as percentage inhibition.

7. Antibacterial Activity

7.1. Bacterial Strains

The antibacterial effect of *Capparis spinosa* L. was evaluated on two Gram-positive strains (*Staphylococcus aureus* ATCC 25923 and *Enterococcus faecalis* ATCC 51299) and two Gram-negative bacteria (*Klebsiella pneumoniae* ATCC 700603 and *Escherichia coli* ATCC 25922) obtained from the Microbiology Laboratory, Department of Microbiology, Faculty of Natural and Life Sciences, Ferhat Abbas Sétif 1 University (Algeria). Bacterial strains were subcultured in Brain Heart Infusion Broth (BHIB) nutrient broth at 37°C for 18-24 hours to obtain young cultures which were then used to prepare the inoculum by transferring some colonies to tubes containing physiological water (0.9%) to have an initial cell density of 108 colony forming units (CFU) corresponding to an optical density of 0.08-0.1 at 625 nm [7].

7.2. Agar Disk Diffusion Assay

The agar disk diffusion method was used to assess the antibacterial activity of *Capparis spinosa* L. extracts based on the measurement of the diameter of the apparent inhibition halos around the disks loaded with plant extract [25]. Methanolic extracts were prepared at concentrations of 10 mg/mL, 50 mg/mL, and 100 mg/mL in dimethyl sulfoxide (DMSO). Bacterial suspensions were spread out using sterile swabs on the surface of Petri dishes containing Mueller Hinton (MHA) agar. Then discs of Whatman No. 3 filter paper, 6 mm in diameter, were prepared and impregnated with 20 µL of the different concentrations of extracts of *Capparis spinosa* L., which were therefore delicately deposited with sterile forceps on the surface of the seeded agar. A disc impregnated with DMSO and a ready-to-use disc with Gentamicin (10 mg), were used respectively as negative control and positive control. The Petri dishes are incubated in an

oven at 37°C between 18 and 24 hours. The antibacterial activity was determined by measuring, with a ruler, the diameter of the zone of inhibition. All measurements were performed in triplicate [26].

8. Statistical Analysis

Data were reported as the mean \pm SD of three measurements. Statistical analysis was performed on GraphPad Prism software (version 6) using one-way ANOVA followed by Turkey's multiple comparisons post hoc test. Values were considered statistically significant at $p < 0.05$. The values of IC_{50} and $A_{0.5}$ were determined from the regression curves obtained from the percentages of inhibition or the absorbance at different concentrations using the Excel software. The Pearson correlation coefficient (r) between the biological activities and the polyphenol/flavonoid contents was calculated using the "PYTHON" programming language.

RESULTS AND DISCUSSION

1. Phytochemical Analysis

1.1. Qualitative Screening

As shown in Table 1, the qualitative analysis of the main phytochemical groups in the different parts of the plant revealed the presence of polyphenols and tannins in the roots and leaves.

Table 1: Preliminary analysis of the phytochemical composition of different extracts of *Capparis spinosa* L..

Phytochemicals	Parts of plant		
	Roots	Leaves	Stems
Polyphenols	+	++	-
Flavonoids	-	++	-
Tannins	+++	+++	-
Proteins	+	-	-
Terpenes	-	-	+
Steroids	-	-	-
Alkaloids	-	++	-
Saponosides	-	+	-
Quinones	-	-	-
Carbohydrates	-	++	-
Anthraquinones	-	-	-
Anthocyanins	-	-	-
Mucilage	-	++	-

Symbols (-, +, ++, +++) represent the following: (-) = negative, (+) = few concentration, (++) = moderate concentration, (+++) = high concentration.

Flavonoids, alkaloids, carbohydrates, and mucilage were only observed in the leaves, while

terpenes were only detected in the stems. Whereas, steroids, quinones, anthraquinones and anthocyanins were absent (negative reaction) in all three organs.

Previous studies reported the presence of several components in *Capparis spinosa* L. including flavonoids and alkaloids [27,28], tannins and terpenoids [29,30]. Additionally, Muraih et al. [31] revealed the richness of the stems in alkaloids, flavonoids, terpenoids, tannins and proteins.

3.2. Phenolic Compounds Contents

Fig. 1 presents the results for the quantification of total phenolics and flavonoids in the three organs. The total phenolic content of *C. spinosa* L. showed important levels in different parts, the highest content being recorded with the leaf extract (160.09 ± 3.13 $\mu\text{g GAE/mg}$), and the stem extract (120.44 ± 6.32 $\mu\text{g GAE/mg}$), while the lowest value was recorded with roots ($101.76 \pm$ $\mu\text{g GAE/mg}$). Similarly, the determination of total flavonoids in the extracts showed that the leaves had the highest content with a value of 74.11 ± 1.19 $\mu\text{g EQ/mg}$, followed by the stems which also had a remarkable content (37.70 ± 1.14 $\mu\text{g EQ/mg}$). In contrast, the roots contained a low content of flavonoids (9.05 ± 0.55 $\mu\text{g EQ/mg}$). These observations indicate that phenolic compounds were more concentrated in the leaves of *Capparis spinosa* L. than in the roots and stems.

Earlier reports also described the variation of phenolic contents according to the organ of the plant. Rajhi et al. [32] found that polyphenols and flavonoids are more abundant in leaves, flowers, fruits and finally roots confirming the results of the present study. Furthermore, the polyphenol content of flower buds has also been reported and found to be 65.13 ± 5.53 mg RE/g extract [14]. Recently, Khojasteh Rad et al. [33] showed that the hydro-methanolic extract of the leaves of *C. spinosa* from Iran was rich in flavonoids (128.88 $\text{mg RE/g of extract}$).

The variation in the content of phenolic compounds among plant organs is mainly explained by the role of these compounds in the plant. Flavonoids play several functions in plants such as coloring and play an essential role in the absorption of UV radiation and therefore intervene in the defense mechanisms of plants, which explains the high levels of these compounds in the aerial parts compared to the

roots. Previously, the same results had been reported by Bakhouch et al. [34] indicating the great influence of plant parts on total phenolics and flavonoids whose levels were higher in the leaves than in the roots. However, the latter have the highest contents of condensed tannins suggesting the role of these compounds in the defense against pathogens.

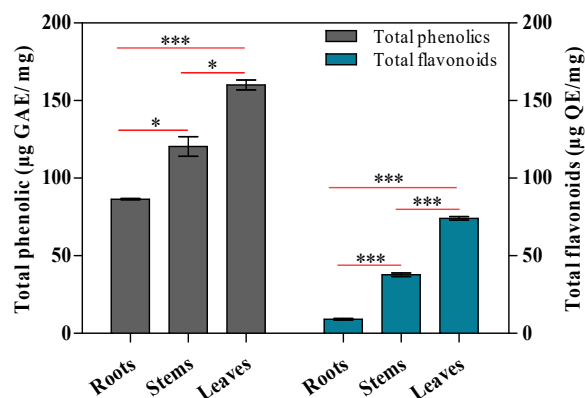


Figure 1: Total phenolics and flavonoids contents in leaf, stem, and root extracts of *Capparis spinosa* L. each column present the mean of three determinations. *, **, *** indicate a significant difference at $p < 0.05$ (One-way ANOVA/Tukey test).

3. In Vitro Antioxidant Capacity

The antioxidant activity analysis of the different extracts of the three parts of *Capparis spinosa* L. was determined and evaluated using five different methods. The results reported as IC_{50} and $\text{A}_{0.5}$ values were depicted in Table 2. The use of the different methods is mandatory for more reliable results because herbal extracts present a mixture of compounds that can exert various antioxidant mechanisms and to consider the conditions of each assay.

As shown in the Table 2, antioxidant activity assessed by DPPH assay revealed that leaves have the greatest effect compared to roots and stems with an IC_{50} value of 74.04 ± 3.17 $\mu\text{g/mL}$, followed by stems and finally by roots giving the highest IC_{50} value (474.57 ± 35.45 $\mu\text{g/mL}$). Similarly, leaf extract was the most potent in scavenging ABTS radicals ($\text{IC}_{50} = 17.67 \pm 0.74$ $\mu\text{g/mL}$), followed by stem and root extracts, respectively.

The leaves were also found to be the strongest in reducing iron and copper ions compared to stems and roots.

Table 2: Antioxidant activity of the different parts of *Capparis spinosa* L..

	DPPH IC ₅₀ (µg/mL)	ABTS IC ₅₀ (µg/mL)	Reducing Power A _{0.5} (µg/mL)	CUPRAC A _{0.5} (µg/mL)	β-Carotene IC ₅₀ (µg/mL)
Roots	474.57±35.45 ^e	254.62±5.03 ^d	>200	639.15±9.49 ^d	501.94±401.44 ^d
Stems	150.30±7.06 ^d	85.29±7.11 ^c	>200	108.34±2.54 ^c	333.02±113.16 ^c
Leaves	74.04±3.17 ^c	17.67±0.74 ^b	36.86±2.45 ^b	38.29±3.84 ^b	169.21±4.75 ^b
BHA	6.14±0.41 ^a	1.81±0.10 ^a	nt	5.35±0.71 ^a	1.05±0.03 ^a
BHT	12.99±0.41 ^b	1.29±0.30 ^a	nt	8.97±3.94 ^a	0.91±0.01 ^a
α-Tocopherol	nt	nt	34.93±2.38 ^b	nt	nt
Ascorbic acid	nt	nt	6.77±1.15 ^a	nt	nt

Values are reported as mean ± SD of three measurements. nt; not tested, na: not active, the results were analyzed by One-way ANOVA followed by the multi-comparison Tukey test (p<0.05).

In this context, leaf extract produced an absorbance of 0.5 upon reduction of iron ions with a concentration of 36.86±2.45 µg/mL, while the absorbance recorded at the highest concentration (200 µg/mL) in the case of the stems and roots was < 0.5 (A_{0.5}> 200 µg/mL). Uniformly, a low value of A_{0.5} was given by the leaf extract (38.29 ± 3.84 µg/mL) in the cupric reducing capacity, indicating strong reducing ability of copper ions, followed by stems and finally roots showing the weakest effect (A_{0.5}= 108.34±2.54 and 639.15±9.49 µg/mL, respectively).

To better understand the antioxidant effect of the extracts, the β-carotene bleaching test was also used. Similarly, for the other tests, the leaves significantly prevented the oxidation of β-carotene compared to the stems and roots which showed a weak effect. Thus, the extracts exerted their effect in the following decreasing order:

leaves (IC₅₀= 169.21±4.75 µg/mL) > stems (IC₅₀=333.02±113.16 µg/mL) > roots (IC₅₀ = 501.94±401.44 µg/mL).

The antioxidant effect of the extracts is mainly due to the presence of phenolic compounds capable of exerting various antioxidant mechanisms, including free radical scavenging activity, chelation of transition metals and breaking of the hydrogen chain of subtraction during lipid oxidation, etc. the structure of polyphenols characterized by the presence of hydroxyl groups make them powerful donors of electrons and hydrogen which constitute the main mechanism of antioxidant activity [35]. The study of the correlation between the antioxidant activity and total phenolics and total flavonoids contents was carried out using the IC₅₀ or A_{0.5} values and therefore more the value of these parameters is low more the antioxidant capacity is important (negative relationships) (Fig. 2).

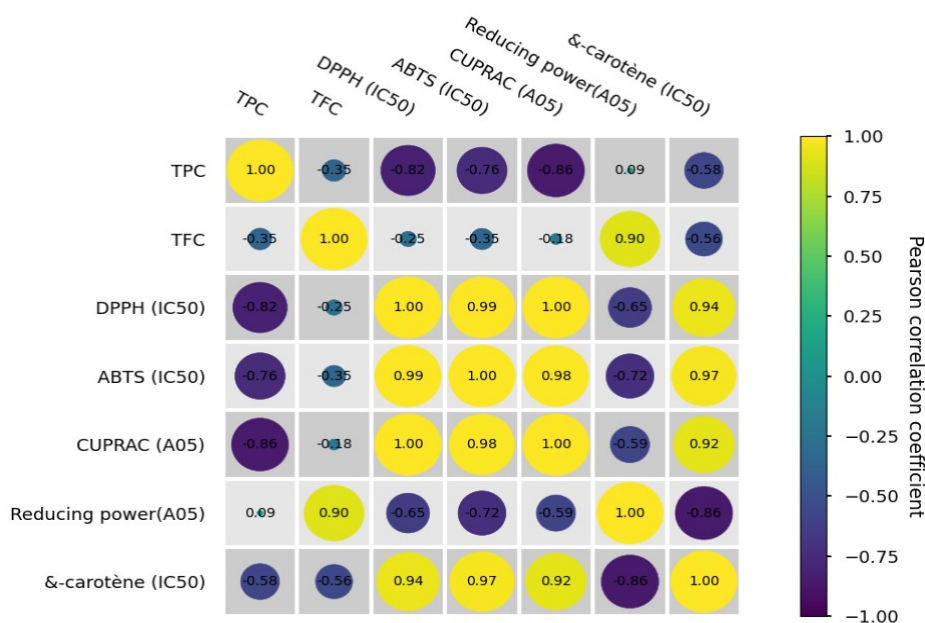


Figure 2: Pearson's correlations between total phenolic content (TPC), total flavonoids content (TFC) and antioxidant (DPPH, ABTS, Reducing power, CUPRAC, β-carotene bleaching) activities.

Strong negative correlation was observed between TPC/TFC and the antioxidant activity evaluated using DPPH ($r = -0.82$ (TPC) and $r = -0.25$ (TFC)), which suggests that this activity could be attributed to the presence of phenolic compounds and mainly total polyphenols. In addition, important negative correlation between ABTS scavenging action and phenolic compounds contents with $r = -0.76$ (TPC) and $r = -0.35$ (TFC) was observed. A positive correlation was observed between iron reducing power and polyphenol content ($r = 0.09$ (TPC) and $r = 0.90$ (TFC)), therefore the antioxidant activity of the extracts can be explained by the presence of phenolic compounds (mainly flavonoids). This correlation justifies the activity of the extracts and confirms the great contribution of phenolic compounds in the antioxidant activity mainly by electron/hydrogen atom transfer. These results agree with previous studies reporting a close relationship between the content of phenolic compounds in extracts and their antioxidant activity [36, 37].

4. Anticholinesterase Activity

The anticholinesterase activity of various extracts examined against acetylcholinesterase and butyrylcholinesterase has been shown in Table 3. The extracts of the three parts were found to be inactive against AChE, while weak activity was observed only in the root extract against BChE with an $IC_{50} = 150.77 \pm 80.56 \mu\text{g/mL}$.

The capacity of *Capparis spinosa* L., both AChE and BChE has been already reported by few papers. Stefanucci [38] and Mollica [18] reported remarkable anticholinesterase effect in capparid buds. More recently, significant inhibitory effect was exhibited by the aerial parts and the roots of

the plant. This activity was essentially assigned to the presence of different phytochemical compounds including phenolic compounds and alkaloids, which were identified in the roots [39].

Table 3: Anticholinesterase effect of *Capparis spinosa* L methanolic extract.

	AChE IC_{50} ($\mu\text{g/mL}$)	BChE IC_{50} ($\mu\text{g/mL}$)
Roots	Na	150.77 \pm 80.56
Stems	Na	na
Leaves	Na	na
Galantamine	6.27 \pm 1.2	34.75 \pm 1.99

Values are means \pm SD of three determinations.

5. Antibacterial Activity

The antibacterial effect of *Capparis spinosa* L. extracts was showed very weak effect using the disk diffusion method (Table 4). All the extracts didn't show an effect on the bacterial growth against *E. faecalis* and *K. pneumonia*. The leaf extract exerted only a slight inhibition at 100mg/mL against *S. aureus* with a halo of 8.66 \pm 0.00 mm and against *E. coli* (zone of inhibition of 10.32 \pm 0.5mm). Moreover, the root extract showed weak inhibition against *S. aureus* and *E. coli* (ZI = 9.01 \pm 0.22 and 10.21 \pm 0.44 mm respectively). On the other hand, the stem extract was found to be inactive against all the strains tested.

The antibacterial effect of *Capparis spinosa* has been widely studied in several countries conducted on different parts of the plant. Most of these studies described the non-activity of the plant against the strains tested including *E. coli*, *S. aureus*, and *P. aeruginosa* [12, 11, 40].

Table 4: Antibacterial activity of methanolic extract of different parts of *Capparis spinosa* L..

		Diameter of Inhibition Zone (mm)			
		Gram ⁺ bacteria		Gram ⁻ bacteria	
		<i>S. aureus</i>	<i>E. faecalis</i>	<i>E. coli</i>	<i>K. pneumonia</i>
Leaves	25 mg/mL	-	-	-	-
	50 mg/mL	-	-	-	-
	100mg/mL	8.66 \pm 0.00	-	10.32 \pm 0.5	-
Stems	25mg/mL	-	-	-	-
	50mg/mL	-	-	-	-
	100mg/mL	-	-	-	-
Roots	25mg/mL	-	-	-	-
	50mg/mL	-	-	-	-
	100mg/mL	9.01 \pm 0.22	-	10.21 \pm 0.44	-

Data were reported as means \pm SD of three measurements.

However, many other works have reported the significant antibacterial activity of *C. spinosa* extracts against a wide range of bacteria [41,42,31]. This variation in antibacterial activity can be attributed to several possible factors such as the extraction procedure, the method used to assess the antibacterial activity and the strain tested [43,44].

CONCLUSION

Throughout the article, some biological effects of different parts of *Capparis spinosa* L have been discussed. The results demonstrate substantial levels of phenolic compounds and total flavonoids which are believed to be responsible for the remarkable antioxidant activity of the extracts, as confirmed by a correlation study. Additionally, plant extracts were inactive or weak against AChE, BChE, and as antibacterial agents. It can be concluded that this plant may be a strong candidate as a source of natural antioxidants.

LIST OF ABBREVIATIONS

ABTS:	(2,2'-azino-bis (3-ethylbenzthiazoline - 6-sulphonic acid)
A _{0.5} :	Concentration corresponding to 0.5 of absorbance
AChE :	Acetylcholinesterase Enzyme
AD:	Alzheimer's Disease
BHA:	Butylated Hydroxyanisole
BHIB:	Brain Heart Infusion Broth
BHT:	Butylated Hydroxytoluene
BChE:	Butyrylcholinesterase Enzyme
DMSO:	Dimethyl Sulfoxide
DNTB:	5,5-dinitro-bis-nitrobenzoic acid
DPPH:	1, 1-diphenyl-2-picrylhydrazyl
IC ₅₀ :	Inhibition Concentration of 50%
ROS:	Reactive Oxygen Species
TPC:	Total Phenolic Content
TFC:	Total Flavonoid Content
GAE:	Gallic Acid Equivalent
QE:	Quercetin Equivalent

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

REFERENCES

- [1] Bolkent S, Yanardag R, Karabulut-Bulan O, Yesilyaprak B. Protective role of *Melissa officinalis* L. extract on liver of hyperlipidemic rats: A morphological and biochemical study. *J. Ethnopharmacol.* 2005; 99: 391–398.
- [2] Aicha B, Ben Rouabhi R, Gasmi S, Bensouici C, Mohammedi H, Mennai I. Lc-MS Analysis and Antioxidant Activity of the Hydro-Alcoholic Extract of *Melissa Officinalis* L. From Algeria. *Chem. J. Mold.* 2020; 15:78–87.
- [3] Safari M, Ahmady Asbchin S. Evaluation of antioxidant and antibacterial activities of methanolic extract of medlar (*Mespilus germanica* L.) leaves. *Biotechnol. Biotechnol. Equip.* 2019; 33:372–378.
- [4] Lugasi A, Hóvári J, Sági K V, Bíró L. The role of antioxidant phytonutrients in the prevention of diseases. *Acta Biol.* 2003;47: 119–125.
- [5] Gali L, Bedjou F. Antioxidant and anticholinesterase effects of the ethanol extract, ethanol extract fractions and total alkaloids from the cultivated *Ruta chalepensis*. *South African J. Bot.* 2019a; 120:163–169.
- [6] El Aanachi S, Gali L, Neghmouche Nacer S, Bensouici C, Dari K, Aassila H, Phenolic contents and *in vitro* investigation of the antioxidant, enzyme inhibitory, photoprotective, and antimicrobial effects of the organic extracts of *Pelargonium graveolens* growing in Morocco, Biocatalysis and Agricultural Biotechnology. 2020;29: 1-13.
- [7] Gali L, Bedjou F. Antibacterial and Cytotoxic Effects of the Cultivated *Ruta chalepensis*. *Curr. Bioact. Compd.* 2019b; 16: 654–660.
- [8] Alzahrani D, Albokhari E, Yaradua S, Abba A. The complete plastome sequence for the medicinal species *Capparis spinosa* L. (Capparaceae). *Gene Reports.* 2021; 23:101059.
- [9] Tesoriere L, Butera D, Gentile C, Livrea MA. Bioactive components of caper (*Capparis spinosa* L.) from sicily and antioxidant

- effects in a red meat simulated gastric digestion. *J. Agric. Food Chem.* 2007; 55: 8465–8471.
- [10] Ibrahim OMS. Evaluation of anti-bacterial activity of Capparis spinosa (Al-Kabara) and Aloe vera extracts against Isolates Bacterial Skin Wound Infections *in-vitro* and *in-vivo*. *Kufa J. Vet. Med. Sci.* 2012.
- [11] Benzidane N, Aichour R, Guettaf S, Laadel N, Khennouf S, Baghiani A, and AL. Chemical investigation, the antibacterial and antifungal activity of different parts of Capparis spinosa extracts. *J. Drug Deliv. Ther.* 2020; 10: 118–125.
- [12] Boga C, Forlani L, Calienni R, Hindley T, Hochkoeppler A, Tozzi S, Zanna N. On the antibacterial activity of roots of Capparis spinosa L. *Nat. Prod. Res.* 2011; 25: 417–421.
- [13] Eddouks M, Ajebli M, Hebi M. Ethnopharmacological survey of medicinal plants used in Daraa-Tafilalet region (Province of Errachidia), Morocco. *J Ethnopharmacol.* 2017; 23: 516–530.
- [14] Tlili N, Feriani A, Saadoui E, Nasri N, Khaldi A. Capparis spinosa leaves extract: Source of bioantioxidants with nephroprotective and hepatoprotective effects. *Biomed. Pharmacother.* 2017; 87: 171–179.
- [15] Benzidane N, Charef N, Krache I, Baghiani A, Arrar L. *In vitro* bronchorelaxant effects of capparis spinosa aqueous extracts on rat trachea. *J. Appl. Pharm. Sci.* 2013; 3:85–88.
- [16] Al-Anazi KM, Al-Mareed AA, Farah MA, Ali MA, Hailan WAQ, Al-Hemaid FM. Protective Effect of Capparis spinosa Extract against Potassium Bromate Induced Oxidative Stress and Genotoxicity in Mice. Evidence-based Complement. *Altern. Med.* 2021.
- [17] Moghadamnia Y, Kani SNM, Ghasemi-Kasman M, Kani MTK, Kazemi S. The anti-cancer effects of capparis spinosa hydroalcoholic extract. *Avicenna J. Med. Biotechnol.* 2019; 11: 43–47.
- [18] Mollica A, Stefanucci A, Macedonio G, Locatelli M, LuisiG, Novellino E, Zengin G. Chemical composition and biological activity of Capparis spinosa L. from Lipari Island. *South African J. Bot.* 2019; 120: 135–140.
- [19] Merouani N, Boukhebt H, Belhattab R. Ethnobotanical Study, Anatomical Study and Phytochemical Screening of Aristolochia longa L. *J. Drug Deliv. Ther.* 2020; 10: 112–124.
- [20] Miliauskas G, Venskutonis PR, Van Beek TA. Screening of radical scavenging activity of some medicinal and aromatic plant extracts. *Food Chem.* 2004; 85:231–237.
- [21] Yakoubi R, Megateli S, Hadj Sadok T, Gali L. Photoprotective, antioxidant, anticholinesterase activities and phenolic contents of different Algerian Mentha pulegium extracts. *Biocatal. Agric. Biotechnol.* 2021; 34:102038.
- [22] Apak R, Güçlü K, Özyürek M, Karademir SE. Novel total antioxidant capacity index for dietary polyphenols and vitamins C and E, using their cupric ion reducing capability in the presence of neocuproine: CUPRAC method. *J. Agric. Food Chem.* 2004; 52: 7970–7981.
- [23] Boussadia A, Beghidja A, Gali L, Beghidja C, Elhabiri M, Rabu P, Rogez G. Coordination properties of two new Schiff-base phenoxy-carboxylates and comparative study of their antioxidant activities. *Inorganica Chim. Acta.* 2020; 508.
- [24] Mazouz W, Haouli NEH, Gali L, Vezza T, Bensouici C, Mebrek S, Hamel T, Galvez J, Djeddi S. Antioxidant, anti-alzheimer, anti-diabetic, and anti-inflammatory activities of the endemic halophyte Limonium spathulatum (Desf.) kuntze on LPS-stimulated RAW264 macrophages. *South African J. Bot.* 2020; 135: 101–108.
- [25] Rukayadi Y, Shim JS, Hwang JK. Screening of Thai medicinal plants for anticandidal activity. *Mycoses.* 2008; 51: 308–312.
- [26] Benachour H, Ramdani M, Lograda T, Chalard P, Figueredo G. Chemical composition and antibacterial activities of capparis spinosa essential oils from Algeria. *Biodiversitas.* 2020; 21:161–169.
- [27] Chedraoui S, Abi-Rizk A, El-Beyrouthy M, Chalak L, Ouaini N, Rajjou L. Capparis spinosa L. in A systematic review: A xerophilous species of multi values and promising potentialities for agrosystems under the threat of global warming. *Front. Plant Sci.* 2017; 8: 1–18.
- [28] Fadili K. Etude phytochimique et évaluation de l'activité antioxydante des feuilles et des fruits du Capparis spinosa L. phytochemical study and evaluation of antioxidant activity of leaves and fruits of Capparis spinosa L. *Am. J. Innov. Res. Appl. Sci.* 2017; 12:108–118.
- [29] Akkari H, B'chir F, Hajaji S, Rekik M, Sebai E, Hamza H, Darghouth MA, Gharbi M. Potential anthelmintic effect of Capparis

- spinosa (Capparidaceae) as related to its polyphenolic content and antioxidant activity. Vet. Med. (Praha).2016; 61: 308–316.
- [30] Fu XP, Wu T, Abdurahim M, Su Z, Hou XL, Aisa HA, Wu H. New spermidine alkaloids from *Capparis spinosa* roots. Phytochem. Lett.2008; 1: 59–62.
- [31] Muraih JK, Arian AG, Abdulabass HT. Phytochemical and antibacterial activity of *capparis spinosa* roots extracts against some pathogenic bacteria. Ann. Trop. Med. Public Heal. 2020; 23.
- [32] Rajhi I, Dhia MTB, Abderrabba M, Ayadi S. Phytochemical screening, *in vitro* antioxidant and antibacterial activities of methanolic extracts of *Capparis spinosa* L. different parts from Tunisia. J. Mater. Environ. Sci. 2019; 10: 234–243.
- [33] Khojasteh Rad M, Ghani A, Ghani E. *In vitro* effects of *Capparis spinosa* L. extract on human sperm function, DNA fragmentation, and oxidative stress. J. Ethnopharmacol.2021; 269: 113702.
- [34] Bakhouché I, Aliat T, Boubellouta T, Gali L, Send A, Bellik B. South African Journal of Botany Phenolic contents and *in vitro* antioxidant, anti-tyrosinase, and anti-inflammatory effects of leaves and roots extracts of the halophyte *Limonium delicatulum*. South African J. Bot.2021; 139.
- [35] Perron NR, Brumaghim JL. A review of the antioxidant mechanisms of polyphenol compounds related to iron binding. Cell Biochem. Biophys.2009; 53: 75–100.
- [36] Dragović-Uzelac V, Levaj B, Bursać D, Pedisić S, Radojčić I, Biško A. Total phenolics and antioxidant capacity assays of selected fruits. Agric. Conspec. Sci. 2007; 72: 279–284.
- [37] Rupasinghe HPV, Jayasankar S, Lay W. Variation in total phenolics and antioxidant capacity among European plum genotypes. Sci. Hortic. (Amsterdam). 2006; 108: 243–246.
- [38] Stefanucci A, Zengin G, Locatelli M, Macedonio G, Wang CK, Novellino E, Mahomoodally MF, Mollica A. Impact of different geographical locations on varying profile of bioactives and associated functionalities of caper (*Capparis spinosa* L.). Food Chem. Toxicol.2018; 118: 181–189.
- [39] Saleem H, Khurshid U, Sarfraz M, Ahmad I, Alamri A, Anwar S, Alamri AS, Locatelli M, Tartaglia A, Mahomoodally MF, Zainal Abidin SA, Ahemad N. Investigation into the biological properties, secondary metabolites composition, and toxicity of aerial and root parts of *Capparis spinosa* L.: An important medicinal food plant. Food Chem. Toxicol. 2021; 155: 112404.
- [40] Proestos C, Komaitis M. Application of microwave-assisted extraction to the fast extraction of plant phenolic compounds. LWT - Food Sci. Technol.2008; 41: 652–659.
- [41] AL-Azawi AH, Ghaima KK, Salih HH. Phytochemical, antibacterial and antioxidant activities of *Capparis spinosa* L. Cultivated in Iraq. Biosci. Res. 2018; 15, 2611–2618.
- [42] Gull T, Sultana B, Bhatti IA, Jamil A. Antibacterial potential of *Capparis spinosa* and *Capparis decidua* extracts. Int. J. Agric. Biol.2015; 17: 727–733.
- [43] Balouiri M, Sadiki M, Ibensouda SK. Methods for *in vitro* evaluating antimicrobial activity: A review. J. Pharm. Anal. 2016; 6: 71–79.
- [44] Fazeli MR, Amin G, Attari MMA, Ashtiani H, Jamalifar H, Samadi N. Antimicrobial activities of Iranian sumac and avishan-e shirazi (*Zataria multiflora*) against some food-borne bacteria. Food Control. 2007; 18: 646–649.