AMERICAN JOURNAL OF BOTANY AND BIOENGINEERING
https://biojournals.us/index.php/AJBB
ISSN: 2997-9331

Article

Chemical Studies on Capparis Spinosa Leaves

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Citation: Hussein D. S., Ghoneim G. A., Raya M. A. A., Khalil M. M. Chemical Studies on Capparis Spinosa Leaves. American Journal Of Botany And Bioengineering 2026, 3(3), 38-45.

Received: 10th Dec 2025
Revised: 16th Jan 2026
Accepted: 20th Feb 2026
Published: 06th Mar 2026

Abstract: This study was to determine the chemical, mineral content the total phenolic, flavonoid content and the antioxidant capacities of Capparis spinosa leaves in the Al-Ramadi area of the Anbar governorate. The chemical composition of capper leaves revealed that the moisture content of capper leaves was 10.42%, protein 13.76%, fat 2.48%, ash 18.47% fiber 29.59%, and carbohydrates, 54.87%. Capper leaves included high levels of calcium (Ca), magnesium (Mg), sodium (Na), iron (Fe), boron (B), zinc (Zn), manganese (Mn), and potassium (K). The corresponding values were 12380, 11196, 6003, 233.935, 195.77, 70.188, 53.979, and 28547 ppm. Furthermore, very low levels of copper (Cu), chromium (Cr), barium (Ba), cadmium (Cd), and cobalt (Co) were found in the samples (8.307, 8.056, 5.926, 2.058, 1.333, and 0.004 ppm, respectively). Total phenolic content and total flavonoid content were evaluated according to the Folin-Ciocalteu procedure, and a colorimetric method, respectively. Extracts capper leaves content was determined by using a high-performance liquid chromatography (HPLC)-UV method. Capper leaves had a total phenolic content of 44.79 mg gallic acid equivalent/gm. The flavonoid content was 20.06 mg QE/gm. Capper leaves had an antioxidant activity of 82.76%.

Keywords: Capparis Spinosa L., Chemical Properties, Mineral Composition, Bioactive Composition, Antioxidant Activity.

Introduction

One of the most widely consumed edible herbs in the Capparidaceae family is Capparis spinosa L. (C. spinosa), [1]. The Capparis genus, which includes Capparis Spinosa L., known as "Kabar" in Arabic, is considered one of the most economically important relatives of the Caparidaceae family, showing significant diversity with 700 to 900 species. It is resident in the Mediterranean region and is widespread from Morocco to Crimea, Armenia, Iraq and Iran, [2]. The caper bush is a resilient, valuable crop that is well-known for its abilities to grow in difficult, dry conditions and adjust to the challenges posed by climate change, [3]. Capers have an important role in the food industry; For example, the flower buds are stored in brine and have become an expensive product in recent years, [4]. It contains carbohydrate 5 %, fat 0.9 %, dietary fiber 3 %, sugar 0.4 %, protein 2 % and vitamin C 4 % mg. and Energy 20 %, [5]. According to Shahrajabian, et al., capers are a medicinal plant whose therapeutic effect is based on the richness of their numerous bioactive components [6]. The strong cytotoxic action of Capparis spinosa is attributed to its abundance of sulfur compounds as well as phenolic and flavonoid glycosides. The strong cytotoxic action of Capparis spinosa is attributed to its abundance of sulfur compounds as well as phenolic and flavonoid glycosides [7]. Numerous biological effects, including

antioxidant activity, immune system regulation, and health advantages, have been demonstrated by pharmacological studies. [8]. Traditional medicine used roots, leaves, buds, fruit, bark, and seeds to treat a variety of diseases, including kidney disease, headaches, and toothaches, [9]. Its leaves are an underappreciated plant portion with promising phytochemical richness, in addition to its well-researched flower buds and fruits. According to phytochemical investigations, C. spinosa leaves are exceptionally rich in polyphenolic chemicals, particularly flavonoids like catechin, rutin, and quercetin-3-glucoside, which have been shown to have potent anti-inflammatory and antioxidant properties, [10]. The aim of the research is to evaluate the chemical, mineralogical

composition, total phenol content, total flavonoid content and antioxidant activity as well as the profile of phenolic acids and flavonoids of the methanol extract of Capparis spinosa L. leaves using the HPLC technique.

Materials and Methods

Plant materials:

Fresh aerial parts leaves of *Capparis spinosa* were collected for the growing season 2023-2024 from different regions of the desert and areas of Iraq's Mosul and Anbar Governorates. The plant material was washed under running tap water, dried in the shade and then ground to powder using a mechanical grinder and airtight container with proper labeling for future use as shown in **Figure (1).**

Figure 1. Capparis spinosa plant.

Chemical properties of Capparis spinosa leaves

1. Moisture Determination

The percentage of moisture in the samples was estimated using the method outlined in **AOAC,** dried in an air -drying oven at 105 ° C to a constant weight [11].

2. Protein Determination

The percentage of protein was estimated by the official Kjeldahl method outlined in **AOAC,** and the percentage of total nitrogen was multiplied by a factor of 6.25 to find the percentage of protein [11].

3. Ash Determination

The ash percentage was estimated using the basic method the ash was calcinated at a constant weight at a temperature of 600 ° C in a sound oven that was outlined in **AOAC [11].**

4. Fat Determination

The percentage of fat in the samples was estimated by the use of Soxhlet (intermittent method) and solvent hexane (boiling point 40–60 ° C) according to the method outlined in **AOAC [11].**

5. Fiber Determination

The percentage of raw fiber in the samples was estimated based on those outlined in **AOAC [11].** The fiber content was calculated by pulling the ash content from the weight of the digested sample. The percentage of raw fiber content was then calculated.

6. Carbohydrates

Determination It was determined according to the method of **AOAC** and in the following equation: % carbohydrate = 100 - (%moisture content + % protein + % ash + % fat + % fiber) [11].

7. Determination of minerals

Inductance connected plasma was used to determine the mineral concentrations of capper leaves, according to the method proposed by. **Bettinelli, et al. [12].**

8. Determination of free amino acids

Amino acid contents of capper leaves were determined according to the method described by **Karpyuk, et al.** using an HPLC instruments, at the National Research Center, Dokki, Cairo, Egypt [13].

9. Determination of antioxidant activity free radical scavenging procedure

This method was performed based on the described procedure by **Fenshyur, et al. [14].** The DPPH test was used to measure the ability to scavenge free radicals. Methanol was used to create a 7.5 µmol/L DPPH• reactive. After adding 3.9 mL of DPPH• solution to 0.1 mL of the sample, the absorbance at 515 nm was measured at t = 10 minutes. After adding 3.9 mL of DPPH• solution to 0.1 mL of methanol, the absorbance was measured at t=0 min. The antioxidant activity was computed as follows: DPPH radical scavenging activity (%) = [(Abs Control – Abs Sample)]/ (Abs Control)] × 100 where; Abs Control is the absorbance of DPPH radical methanol; Abs Sample is the absorbance of DPPH radical+ sample extract/standard.

10. Determination of bioactive compounds

Total phenolic content (TPC) The Folin-Ciocalteu reagent **Chang, et al.** was used to measure the total phenolic content (TPC) of the (Capparis spinosa L. leaves) samples, which were carried out in the bio-science lab at Madinat Nasr, Egypt. 2.5 mL of 10% Folin-Ciocalteu's reagent and 0.5 mL of specimen extract solutions were combined to create the reaction blend. 0.5 mL of methanol, 2.5 mL of 10% Folin-Ciocalteu's reagent dissolved in water, and 2.5 mL of 7.5% NaHCO3 were used as a blank at the same time [15]. The specimens were then brooded in a thermostat at 30 oC for 90 minutes. The absorbance was measured at λmax = 765 nm using a spectrophotometer. The phenolic content of extracts was determined using gallic acid equivalent (mg of GAE/g of extract). TPC is computed using the average phenolic content of methanol extract, as shown in Table (1), and the linear regression equation of the standard Gallic Acid curve **(Figure 2).**

Table 1. Total Phenolic Contents Gallic Acid Equivalent (GAE) of methanol Extracts of capper plant.

St (phenolic)	500	250	125	62.5	31.25	15.62
Ab	1.219	0.699	0.401	0.248	0.11	0.071

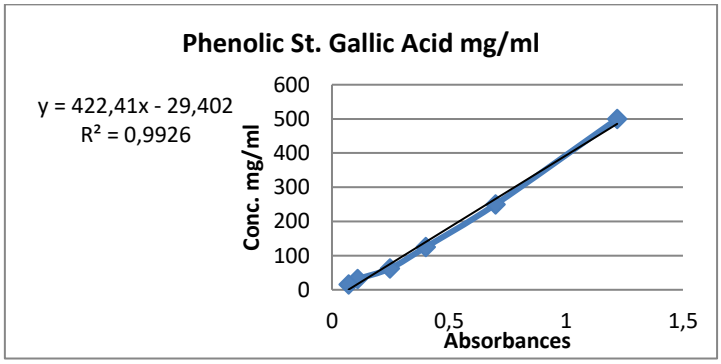

Figure 2. Standard curve of Gallic acid.

Total flavonoid contents (TFC) were calculated according to Kuntic, et al. as follows [16]: The mobile phase consisted of a binary mixture of methanol /water (1:1 v/v) adjusted to pH 2.8 with phosphoric acid, at isocratic flow rate of 1.0 mL min– 1 .The absorbance was measured at λ = 360 nm after an hour at room temperature The presence of flavonoids was indicated by a yellow color. Quercetin (mg/100g dry weight) was used to determine TFC. The average methanol extract flavonoid concentration, as shown in Table (2), and the linear regression equation of the typical quercetin curve (Figure 3) were used to determine TFC.

Table 2. Total Flavonoid Contents Quercetin Equivalent (QE) of methanol Extracts of capper plant.

Flav. St. Qu. mg/ml	2.544	1.327	0.841	0.515	0.325	0.239
Ab	2.544	1.327	0.841	0.515	0.325	0.239

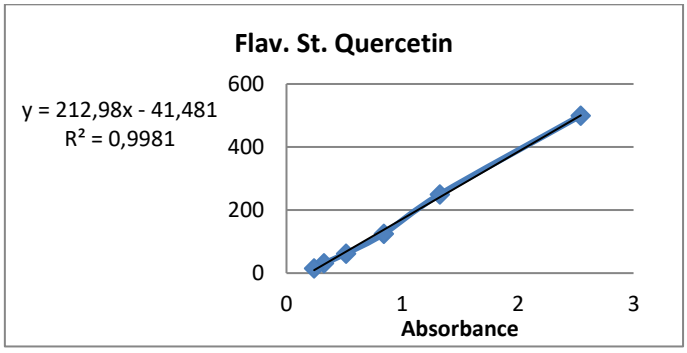

Figure 3. Standard curve of Quercetin

Determination of phenolic acids and flavonoids using HPLC

The samples' phenolic acid and flavonoid components were extracted using the procedure outlined by Mattila, et al. [17]. An Agilent 1200 chromatograph (Agilent Technologies Inc., USA) with column C18 Zorbax ODS (with particle size 5µm, 4.60mm × 250mm) was used for the HPLC analysis. A UV detector set at 280 nm for phenolic acids (gallic, syringic, vanillic, and caffeic acid) and 330 nm for flavonoids (quercetin, luteolin, rutin, apigenin, and kaempferol) was used to monitor the elutes. Methanol and acetonitrile were used as a mobile phase in the separation process, with a flow rate of 1 ml/min. By comparing the retention periods with the corresponding retention times of recognized standard reference material, chromatographic peaks were found.

Results and Discussion

Chemical composition of raw capper leaves

The chemical composition of capper leaves is presented in (Table 3). Capper leaves has higher content of raw fiber, ash, protein and fat and lower total carbohydrate content. Capper leaves has moisture content was 10.42%, protein 13.76%, fat 2.48%, ash 18.47% fiber 29.59%, and carbohydrates, 54.87%.

Table 3. Chemical composition of raw Capparis spinosa L. leaves.

Composition	**Capparis spinosa L leaves**
Moisture content, %	10.42
Ash, %	18.47
Fiber, %	29.59

Fat, %	2.48
Protein, %	13.76
Carbohydrates, %	54.87

Mineral of raw Capparis spinosa L. leaves, (ppm)

The Macro and microelement sources in Capparis Spinosa L. were identified and recorded in the data **(Table 4).** The highest percentages of calcium (Ca), magnesium (Mg), sodium (Na), iron (Fe), boron (B), zinc (Zn), manganese (Mn), and potassium (K) were discovered in the leaves of Capparis spinosa L. The corresponding values were 12380, 11196, 6003, 233.935, 195.77, 70.188, 53.979, and 28547 ppm. Furthermore, very low levels of copper (Cu), bismuth (Bi), chromium (Cr), barium (Ba), cadmium (Cd), and cobalt (Co) were detected in the samples (8.307, 8.056, 5.926, 2.058, 1.333, and 0.004 ppm, respectively).

Table 4. Mineral contents of *Capparis spinosa L* leaves (ppm).

Potentially toxic elements	*Capparis spinosa L* leaves Concentration, ppm
Boron (B)	195.775
Barium (Ba)	2.058
Calcium (Ca)	12380
Cadmium (Cd)	1.333
Cobalt (Co)	0.004
chromium (Cr)	5.926
Copper (Cu)	8.307
Iron (Fe)	233.935
Magnesium (Mg)	11196
Manganese (Mn)	53.979
Nickel (Ni)	N. D
Potassium (K)	28547
Zinc (Zn)	70.188
Sodium (Na)	6003
Bismuth (Bi)	8.056

Amino acids of *Capparis spinosa L*. leaves

The amount of amino acids in Capparis Spinosa L. leaves was measured in milligrams per milligram. The highest concentration of amino acids in Capparis spinosa L. leaves was found in glutamic acid**,** followed by alanine (**16.02 mg/g** and **15.87 mg/g,** respectively). Amino acids of Capparis spinosa L. were found to have a low content of methionine **(2.29µg/mg).**

Antioxidant scavenging activity in *Capparis spinosa* L leaves

DPPH% was used to evaluate the plant extracts' antioxidant properties. According to the results, Capparis spinosa L. leaves had (82.76%), as indicated in Table (6). These results were similar to those reported for the leaves that shown stronger antioxidant activity, with lower IC50 values in DPPH (36µg/mL) testing. C. spinosa leaves offer better antioxidant potential, making them ideal for medicinal, cosmetic, and nutritional uses, [18].

Total phenolic and flavonoid content in *Capparis spinosa L* leaves

Table 5 shows the total phenolic and flavonoid contents of capper leaves. The TFC was 20.06 mg QE/gm and the TPC content was 44.79 mg (GAE)/gm. Phenolic and flavonoid chemicals are abundant in plants. According to a study by Hazrati et al., the TPC and TFC in leaves at the mature fruit stage were only 13.39 mg GAE/g for total phenols and 2.23 mg QE/g for total flavonoids [19], [20].

Table 5. Total phenolic, flavonoid content, and Antioxidant scavenging activity in *Capparis spinosa L* leaves.

Treatment	Total phenolic Content, mg (GAE)/gm	Total Flavonoid content, mg (QE)/gm	Antioxidant Activity, %
***Capparis spinosa L.* leaves**	44.79	20.06	82.76

Identification of phenolic compounds in *Capparis spinosa L* leaves using HPLC

A combination of various phenolic and flavonoid compounds, including Naringin, Rutin, Quercetin, Kampferol, Luteolin, Apegenin, Catechin, 7-OH Flavone, Chlorogenic acid, catechol, syringic acid, caffeic acid, Pyrogallol, ferulic acid, Ellagic, Unknown, vanillin, and Hisperetin, was identified in Capparis spinosa L. leaves are shown in Figures 4 and 5. Vanillin acid had low amounts (3.44) µg/gm for phenolic compounds, but caffeic acid had the highest concentration (17.36) µg/gm, according to Table 6. Kampferol and quercetin had the largest quantities of flavonoids (14.56 and 11.02 µg/gm), while 7-OH flavone had the lowest values (1.45 µg/gm). According to a study by Shahrajabian et al., rutin and quercetin were found in high concentrations in Capparis leaves, with rutin levels of 16,939.2±0.01 and quercetin levels of 908.93 ±0.01 µg/g fresh weight [6].

Table 6. Identification of Phenolic and Flavonoid compounds in *Capparis spinosa L.* leaves, HPLC analysis (mg/gm).

Phenolic compounds		Flavonoid compounds	
Compound	**Concentration µg/gm**	**Compound**	**Concentration µg/gm**
Chlorogenic acid	15.54	Naringin	7.96
Catechol	10.21	Rutin	10.96
Syringic acid	8.47	Quercetin	11.02
Caffeic acid	17.36	Kampferol	14.56
Pyrogallol	10.11	Luteolin	5.17
Ferulic acid	2.44	Apegenin	9.39
Ellagic	6.23	Catechin	6.65
Un Known	--	7-OH Flavone	1.45
Vanillin	3.44	Hisperetin	2.16

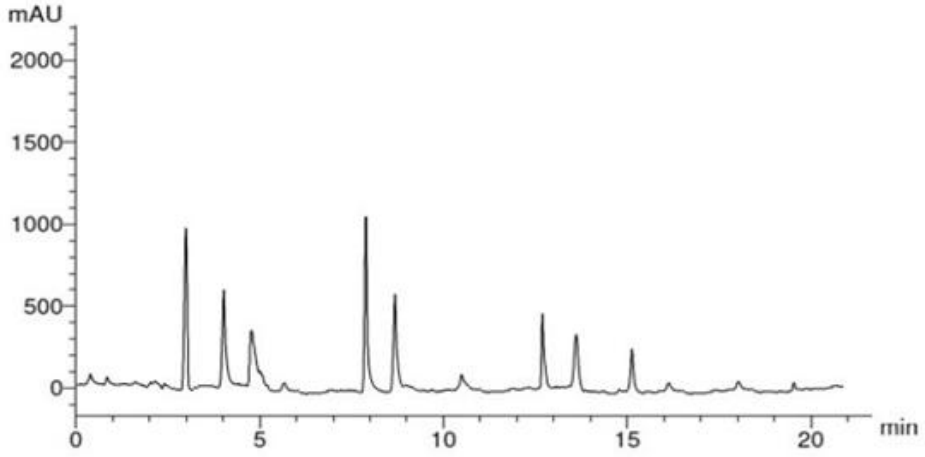

Figure 4. A HPLC reagent of phenolic acids in *Capparis spinosa L.* leaves: chlorogenic acid; catechol; syringic acid; caffeic acid; ferulic acid; Pyrogallol; Ellagic; Unknown; vanillin.

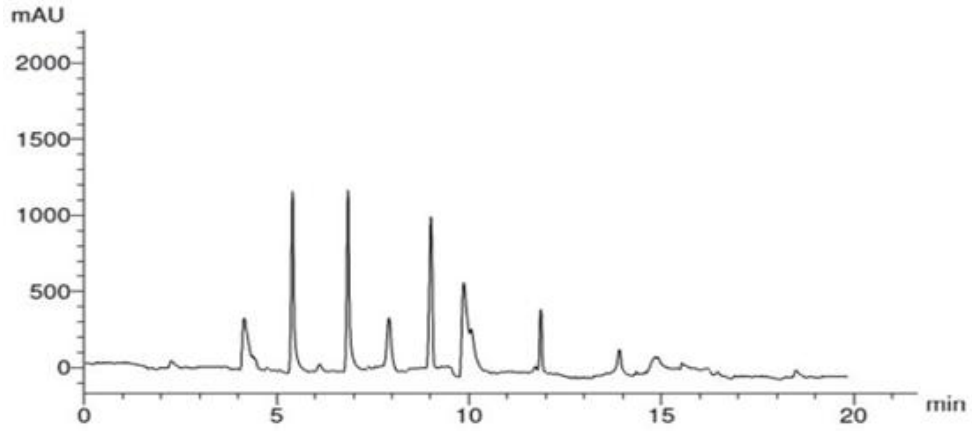

Figure 5. HPLC reagents of flavonoid components of *Capparis spinosa* L. leaves: Naringin; Rutin; Quercetin; Kampferol; Luteolin; Apigenin; Catechin; 7-OH Flavone and Hisperetin.

Conclusion

In addition to being a rich source of chemical compounds, Capparis spinosa also contains phenolic, flavonoid, and amino acids, all of which contribute to its potent cytotoxic effect. Furthermore, caper leaves are an excellent source of macro and microminerals and can be utilized as a dietary item to offer nourishment. Because C. spinosa is a natural medicine with a long history, it is seen to be a suitable candidate for additional study and development as a plant-based medicinal or functional food ingredient.

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