

Full Length Research Paper

Phenolic components, antioxidant activity, and mineral analysis of *Capparis spinosa* L

Rezzan Aliyazicioglu^{1*}, Ozan Emre Eyupoglu², Huseyin Sahin², Oktay Yildiz³, Nimet Baltas⁴

¹Department of Biochemistry, Faculty of Pharmacy, Karadeniz Technical University, 61080 Trabzon, Turkey.

²Department of Chemistry, Faculty of Science, Karadeniz Technical University, 61080 Trabzon, Turkey.

³Maçka Vocational School, Karadeniz Technical University, 61080 Trabzon, Turkey.

⁴Department of Chemistry, Faculty of Science, Recep Tayyip Erdoğan University, 53100 Rize, Turkey.

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In addition to being consumed as food, caper (*Capparis spinosa* L.) fruits are also used in folk medicine to treat inflammatory disorders, such as rheumatism. *C. spinosa* L. is rich in phenolic compounds, making it increasingly popular because of its components' potential benefits to human health. We analyzed a number of individual phenolic compounds and investigated *in vitro* biological activities of *C. spinosa* L. Sixteen phenolic constituents were identified using reverse phase-high performance liquid chromatography (RP-HPLC). Total phenolic compounds (TPCs), ferric reducing antioxidant power (FRAP) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity were used as determinants of antioxidant capacity. *C. spinosa* L. exhibited strong antioxidant activity and contained high levels of antioxidant compounds. Gentisic, sinapic and benzoic acid were detected in *C. spinosa* L. No gallic acid, *proto*-catechuic acid, *proto*-catechuic aldehyde, chlorogenic acid, *p*-OH benzoic acid, vanillic acid, caffeic acid, syringic acid, vanillin, syringaldehyde, *p*-coumaric acid, ferulic acid or rosmarinic acid were identified. Iron and zinc were present at high levels in samples. *C. spinosa* L. appears to be a good source of antioxidants and minerals that might serve to protect health and combat several diseases.

Key words: Antioxidant, capers, *Capparis spinosa* L., phenolics.

INTRODUCTION

Reactive oxygen species (ROS) generation in excess of a biological system's antioxidant capacity results in oxidative stress (Zima et al., 2001). Free radical oxidative stress is involved in the pathogenesis of a range of human diseases. Cells and tissue generally possess antioxidant defense mechanisms to ensure the removal of ROS; while some are controlled endogenously (superoxide dismutase), others are supplied by diet and other means (ascorbic acid, α -tocopherol and β -carotene) (Haslam, 1996). In terms of cellular pro-oxidant states

and lipid peroxidation, the consumption of simple plant components in regular diet, apart from supplying traditional nutrients, may provide benefits in the treatment, improvement or prevention of numerous chronic diseases, such as cancer, and cardiovascular and inflammatory damage including aging-related cellular degeneration (Steinmetz and Potter, 1991).

Capparis spinosa L. (Capparidaceae) is a particularly common aromatic in the Mediterranean area, and is also important in the commercial preparation of frozen food.

*Corresponding author. E-mail: rezzanaoglu@mynet.com. Tel: +905335113364.

Abbreviations: DW, Dry weight; RP-HPLC, reverse phase-high performance liquid chromatography; TPC, total phenolic compounds; FRAP, ferric-reducing/antioxidant power; DPPH, 2,2-Diphenyl-1-picrylhydrazyl; GAE, gallic acid equivalents; TPTZ, 2,4,6-tripyridyl-s-triazine; BHT, butylated hydroxytoluene; ROS, reactive oxygen species.

The aromatic part of the caper consists of the floral bud, collected immediately prior to blossoming. The plant is not generally cultivated, with wild buds being picked by seasonal workers. These are then stored in salt before packaging takes place. Earlier studies involving *C. spinosa* L. have identified alkaloids, lipids, flavonoids and glucosinolates (Brevard et al., 1992), naturally occurring products from the order Capparales, also known as flavor compounds, anticarcinogenic agents and biopesticides (Mikkelsen et al., 2000). Also, Bonina et al. (2002) had reported that methanolic extract of *C. spinosa* L. was shown to possess strong antioxidant/free radical scavenging effectiveness in different *in vitro* tests. Besides this information, *C. spinosa* L. is a source of phenolic compounds. According to the study of Argentieri et al. (2012), rutin was the dominant phenolic in it.

This study was designed to assess the phenolic composition, including phenolic acids and *in vitro* biological activities as antioxidant and mineral analysis of *C. spinosa* L., as well as to evaluate their nutritional and medicinal potentials.

MATERIALS AND METHODS

Chemicals and instrumentation

The phenolic standards (purity > 99.0%) gallic acid, protocatechuic acid, phydroxybenzoic acid, vanillic acid, caffeic acid, chlorogenic acid, syringic acid, gentisic acid, vanilline, protocatechualdehyde, rosmarinic acid, sinapic acid, syringaldehyde, *p*-coumaric acid, ferulic acid and benzoic acid, were purchased from Sigma-Aldrich (St. Louis, MO, USA) and Merck (Darmstadt, Germany). Methanol, acetic acid and acetonitrile were obtained from Merck (Darmstadt, Germany). Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), 2,4,6-tripyriddy-s-triazine (TPTZ) and Folin-Ciocalteu's phenol reagent were obtained from Fluka Chemie GmbH (Buchs, Switzerland) and polytetrafluoroethylene membranes (porosity 0.45 µm) for extract filtration from Sartorius (Goettingen, Germany). 2,2-Diphenyl-1-picrylhydrazyl (DPPH) was supplied from Sigma-Aldrich (St. Louis, MO, USA).

Reverse phase-high performance liquid chromatography (RP-HPLC) (Agilent 1100, DAD 1200 Agilent Technologies, Waldbronn, Germany) and reverse phase waters spherisorb ODS2-C18 column (Water Corporation, Milford, USA) were used for analysis of phenolics. A Spectro UV-Vis Double PC-8 auto cell spectrophotometer (Labomed Inc., Los Angeles, CA, USA) was used in all absorbance measurements. Water solutions were prepared using deionized water purified in an Elgacan® C114 Ultra Pure Water System Deioniser (The Elga Group, Buckinghamshire, England). Ultrasonic bath (Transsonic Digital S, Elma®, Germany) was used during sample extraction procedure and rotary evaporator system (IKA® RV 05 Basic, Werke, USA) for evaporation. Epsilon 5 EDXRF Spectrometer (PANalytical Inc., Westborough, MA USA) was used for mineral analysis

Preparation of extracts

C. spinosa L. samples were selected from herb markets in Gaziantep, Turkey, in June 2013. Approximately 5-10 g of dry *C. spinosa* L. sample was extracted with 30 mL methanol in a flask attached to the condenser, in a sonicator device at 60°C over 3 h. Ten milliliters was separated from methanolic extract in order to

determine antioxidant activities. The residual extracts of methanol were evaporated until dry and then concentrated in a rotary evaporator at 50°C. The crude extract was then dissolved in 10 mL distilled water, and liquid-liquid extractions were performed. The mixtures were extracted three times consecutively with 5 mL diethyl ether and 5 mL ethyl acetate. The organic moiety was picked up in the same flask and evaporated to dryness under reduced pressure in a rotary evaporator at 40°C. The residue was finally weighed and dissolved in methanol for high performance liquid chromatography (HPLC) analysis.

Reverse phase-high performance liquid chromatography (RP-HPLC) analysis of phenolic compounds

RP-HPLC analysis of phenolic compounds was conducted by using a reverse phase column (4.6 × 250 mm, 5 µm), on a gradient program with a two solvents system (A: 2% acetic acid in water; B: 0.5% acetic acid in acetonitrile: water [1:1]) at a constant solvent flow rate of 1.2 mL.min⁻¹. Injection volume was 20 µL. Signals were detected at 232, 246, 260, 272, 280, 290, 308, 328 nm by DAD and at 280 nm by UV detection. Column temperature was maintained at room temperature, 25°C.

Determination of antioxidant capacity

Total phenolic contents were analyzed with Folin-Ciocalteu's phenol reagent method, using gallic acid as the Standard (Singleton and Rossi, 1965; Akyuz et al., 2013). Briefly, 20 µL of various concentrations of gallic acid and 20 µL methanolic samples (1 mg.mL⁻¹), 400 µL of 0.5 Folin-Ciocalteu reagents and 680 µL of distilled water were mixed and the mixture was vortexed. Following 3 min incubation, 400 µL of Na₂CO₃ (10%) solution was added, and after vortexing the mixture was incubated for 2 h. After the incubation period at room temperature, absorbances of the mixtures were measured at 760 nm. The concentration of total phenolic compounds was calculated as mg of gallic acid equivalents per 100 g of dry weight (DW) sample, by using a standard curve for gallic acid in the concentration range between 0.015 and 0.5 mg.mL⁻¹ ($r^2 = 0.99$).

Ferric reducing antioxidant power (FRAP) assay was used to determine antioxidant activity of the sample. The technique is based on the measurement of ferric reducing ability. FRAP assay was performed following the method described by Benzie and Strain (1996), with minor modifications. Working FRAP reagent was prepared as required by mixing 25 mL of 0.3 M acetate buffer at pH 3.6 with 2.5 mL of 10 mM TPTZ solution in 40 mM HCl and 2.5 mL of 20 mM FeCl₃.6H₂O. Subsequently, 100 µL of sample was mixed with 3 mL of freshly prepared FRAP reagent. The reaction mixture was then incubated at 37°C for 4 min. Absorbance was determined at 593 nm against blank prepared using distilled water and incubated for 1 h rather than 4 min. A calibration curve was employed, using an aqueous solution of ferrous sulphate Trolox concentrations in the range of 100–1000 µM, $r^2=0.97$. For purposes of comparison, Trolox® was also tested under the same conditions as a standard antioxidant compound. FRAP values were expressed as µmol Trolox equivalent of 100 g DW sample. Radical scavenging activity of *C. spinosa* L. extracts against DPPH radical was spectrophotometrically studied at 517 nm (Molyneux, 2004). The assay is based on the color change of the DPPH solution from purple to yellow as the radical is deactivated by the antioxidants. Briefly, various concentrations 0.75 mL of parts of *C. spinosa* L. methanolic extracts were mixed with 0.75 mL of a 0.1 mM of DPPH in methanol. Radical scavenging activity was measured by using butylated hydroxytoluene (BHT) as standards and the values are expressed as SC₅₀ (mg sample per mL), the concentration of the samples that causes 50% scavenging of DPPH radical.

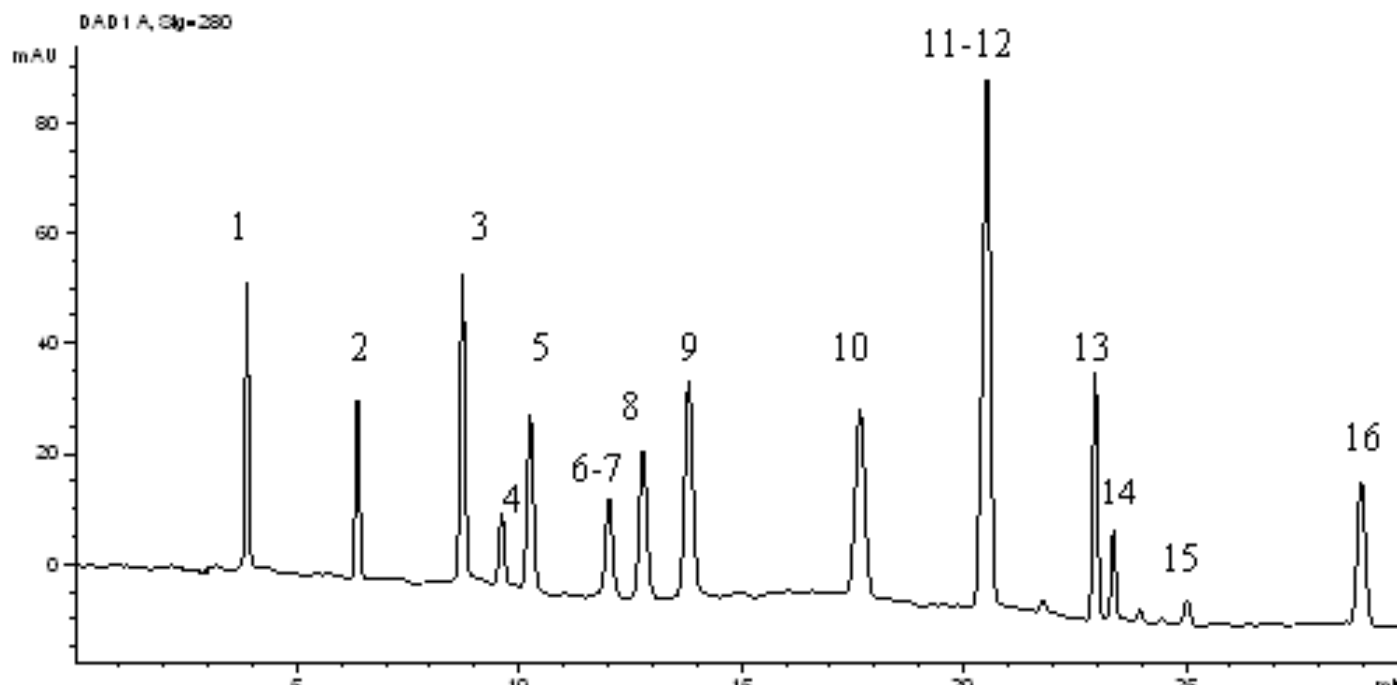


Figure 1. RP-HPLC chromatogram of phenolic standards at 280 nm. Peak identification: 1, Gallic acid; 2, *proto*-catechuic acid; 3, *proto*-catechuic aldehyde; 4, gentisic acid; 5, chlorogenic acid; 6, *p*-OH benzoic acid; 7, vanillic acid; 8, caffeic acid; 9, syringic acid; 10, vanillin; 11, syring aldehyde; 12, *p*-coumaric acid; 13, ferulic acid; 14, sinapic acid; 15, benzoic acid; 16, rosmarinic acid.

Sample preparation for mineral analysis

All the *C. spinosa* L. samples were dried in an incubator and then ground in a Spex mill. In order to minimize the effect of particle size, the resulting powder was sieved using a 400 mesh sieve and then stirred for 25 min to produce a well-mixed sample. The mixed samples were then pressed into 40 mm diameter pellets.

Mineral analysis

The measurement parameters were set up using the Epsilon 5 EDXRF system inbuilt software. Samples were irradiated by X-rays from a Gd tube under a vacuum equipped with a liquid nitrogen cooled PAN-32 Ge X-ray detector with a Be window thickness of 8 μ m. Instrument power, current and high voltage were 600W, 6 mA and 100 kV, respectively. The system's software (Epsilon 5 software) automatically analyzed the sample spectrum and determined the net intensities of element peaks once measurement was completed. When elements overlap one another, accuracy is essential for trace element analysis. A set of secondary standards, available from PANalytical, was used for the calibration of this application. The resulting samples were again measured three times.

RESULTS AND DISCUSSION

Identification of phenolic compounds using RP-HPLC

Plants and fruits contain biologically active products that protect them against a range of physical and chemical hazards, including diseases, parasites and bacteria (Kolayli et al., 2003, 2010). Due to their phenolic constituents they may also possess biologically active properties. Any

natural sample will contain numerous different phenolic compounds, making individual measurement difficult. This study was restricted to measuring only 16 phenolic substances using RP-HPLC. We also used RP-HPLC to analyze 16 phenolic compounds; gallic acid, *proto*-catechuic acid, *proto*-catechuic aldehyde, gentisic acid, chlorogenic acid, *p*-OH benzoic acid, vanillic acid, caffeic acid, syringic acid, vanillin, syring aldehyde, *p*-coumaric acid, ferulic acid, sinapic acid, benzoic acid, rosmarinic acid. The RP-HPLC chromatograms of the standard phenolic compounds are shown in Figure 1. When the individual phenolic compounds were compared with each standard, sinapic acid emerged as the main phenolic component in *C. spinosa* L (Table 1). Gentisic acid and benzoic acid were determined in very low concentrations, while no gallic, *proto*-catechuic, *p*-hydroxybenzoic, chlorogenic, vanillic, caffeic, syringic, *p*-coumaric, ferulic, rosmarinic, vanillin, syringaldehyde and *proto*-catechuic aldehyde were detected in *C. spinosa* L. (Figures 2 and 3). A previous study reported a methanolic extract of *C. spinosa* buds of 0.39% w/w of rutin as revealed by HPLC analysis (Germano et al., 2002).

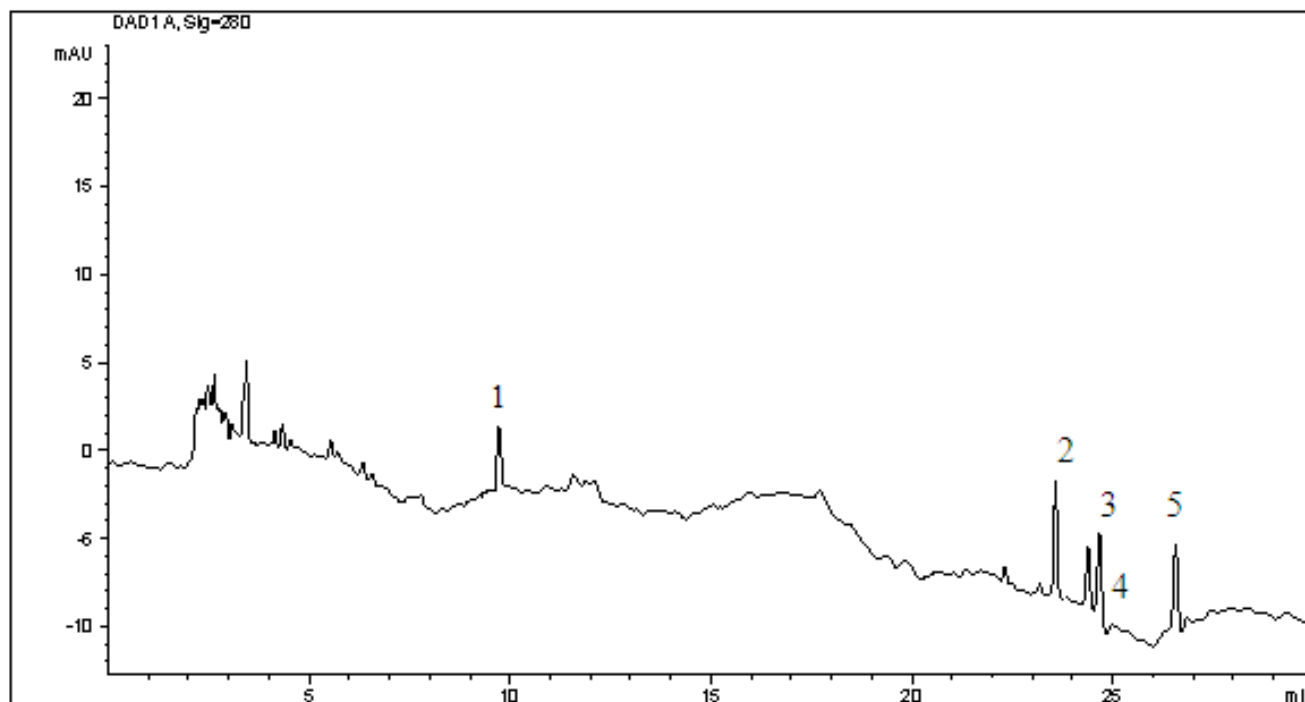
Total antioxidant capacity

Total phenolic compounds (TPC)

TPC was determined in comparison with standard gallic acid and calculated at 37.01 ± 0.03 mg GAE.100 g⁻¹ DW of

Table 1. Retention times and peak areas of phenolic component peaks of *Capparis spinosa* L.

Peak number	Phenolic's Name	Retention time (RT)
1	Gentisic acid	9.65
2	Sinapic acid	23.57
3	Unknown	24.66
4	Benzoic acid	24.97
5	Unknown	26.56

**Figure 2.** RP-HPLC chromatogram of *Capparis spinosa* L. (1 mg/mL) at 280 nm. Peak identification: 1, Gentisic acid; 2, sinapic acid; 3, unknown; 4, benzoic acid; 5, unknown.

C. spinosa L. using the Folin-Ciocalteu method (Table 2). Plants compounds are an important source of active natural products with a wide range of different structures and biological properties. Phenolic compounds are widely present in both edible and non-edible plants. Previous studies have reported that they exhibit multiple biological effects, including antioxidant activity (Yoshino and Murakami, 1998). Several studies have demonstrated a correlation between antioxidant activity and phenolic content (Nagai et al., 2003; Yang et al., 2002). The efficacy of natural antioxidants is closely associated with the chemical composition and structures of active extract components. It is therefore not possible to account for the antioxidant activity of an extract on the basis of its phenolic content, and characterization is also required (Heinonen et al., 1998). A high antioxidant activity may also be associated with compounds other than phenolics which are soluble in different solvents.

Ferric reducing antioxidant power (FRAP)

The FRAP test was used to measure the total antioxidant capacity of *C. spinosa* L. method is based on electron transfer and is regarded as accurate indicators of total antioxidant power, since total reducing power is defined as the sum of the reducing powers of the individual compounds contained in a particular sample (Tezcan et al., 2011). FRAP activity of sample is given in Table 2.

DPPH radical scavenging activity

The free radical scavenging activity of *C. spinosa* L. was determined using the DPPH test. When compared to reference antioxidants with the sample extract of *C. spinosa* L., it was found more efficient than BHT. This may be attributed to polyphenols in the samples varying

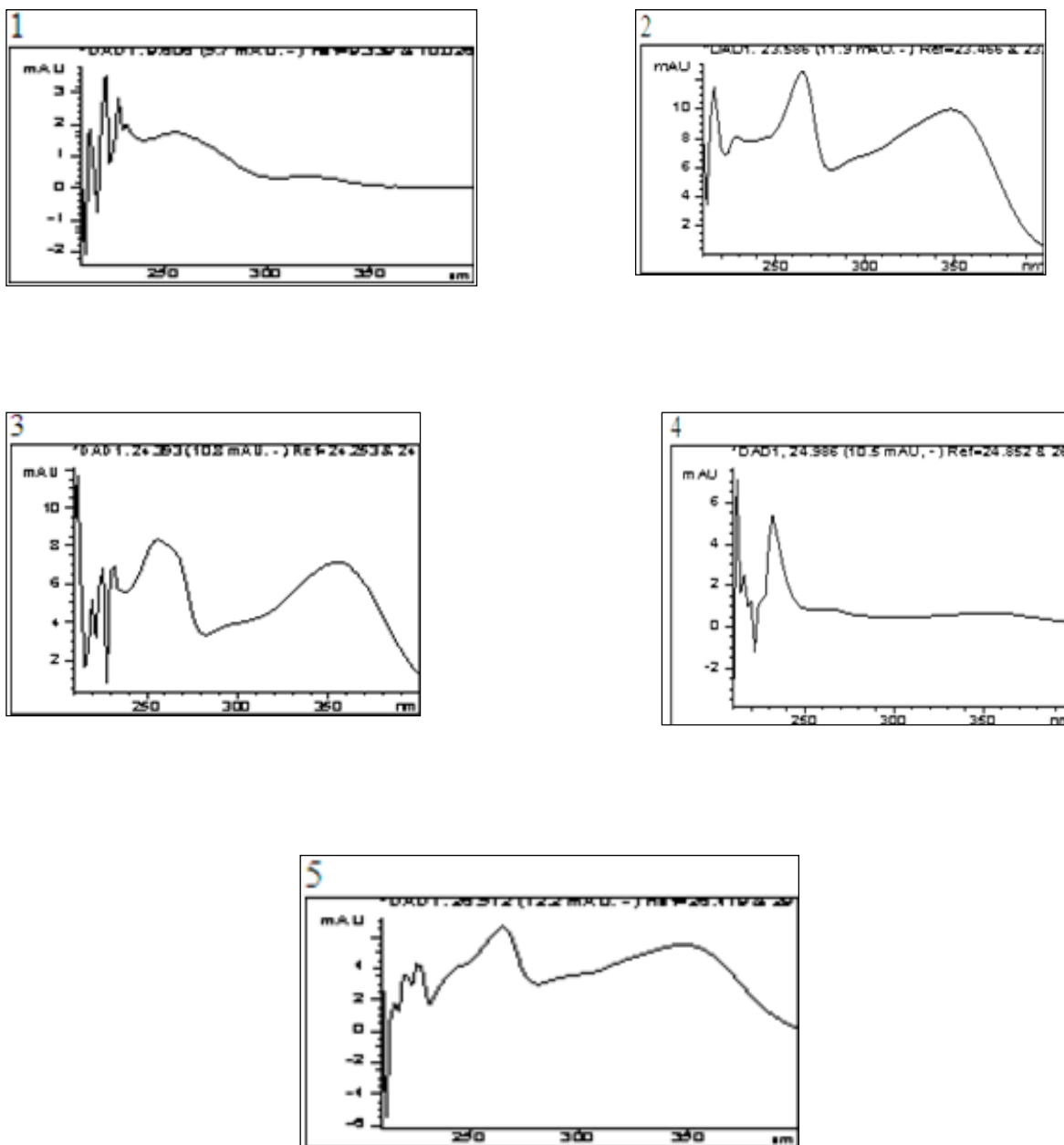


Figure 3. RP-HPLC-UV spectra of phenolic component peaks of *Capparis spinosa* L. (1 mg/mL). Peak identification: 1, Gentisic acid; 2, sinapic acid; 3, unknown; 4, benzoic acid; 5, unknown (200–400 nm).

Table 2. Antioxidant activities of *Capparis spinosa* L.

Plant	TPC (mg GAE.100 g ⁻¹ DW)	FRAP (μ mol Trolox.100 g ⁻¹ DW)	DPPH radical scavenging activity (SC ₅₀ : mg.mL ⁻¹)
<i>Capparis spinosa</i> L.	37.01±0.03	145.07 ± 0.04	0.32 ± 0.26

Values given are the mean and standard deviation of triplicate measurements. Standard antioxidants used were BHT (10 μ g mL⁻¹, 0.01 ± 0.25).

significantly, in the same way as their scavenging capacities. The phenolic compounds may thus have acted as free radical scavengers on the basis of their hydrogen-

donating ability (Molyneux, 2004). The sample extract possessed hydroxyl radical scavenging properties acting as donor for hydrogen atoms or electrons in the DPPH test.

Table 3. Elemental analysis values ($X \pm SD$) of *Capparis spinosa* L. using EDXRF system.

Mineral	Sample
Al ^a	0.48 ± 0.05
P ^a	1.15 ± 0.01
S ^a	4.00 ± 0.06
K ^a	4.54 ± 0.03
Ca ^a	1.18 ± 0.01
Cl ^b	94.86 ± 25.51
Ti ^b	55.24 ± 2.30
Mn ^b	70.04 ± 1.00
Fe ^b	520.72 ± 4.05
Ni ^b	24.10 ± 0.05
Cu ^b	88.27 ± 0.45
Zn ^b	250.75 ± 0.80
Br ^b	11.92 ± 0.07
Rb ^b	79.03 ± 0.19
Sr ^b	40.20 ± 0.69
Y ^b	2.48 ± 0.38
Hf ^b	27.32 ± 0.87
Pb ^b	5.34 ± 0.13

a, % ; b, ppm. Values given are the mean and standard deviation of triplicate measurements.

This also increased iron autoxidation, while significantly suppressing the accessibility of iron to oxygen molecules by oxidizing ferrous ion to a ferric state, in turn inhibiting hydroxyl radical production (Yoshino and Murakami, 1998). These findings suggest that the antioxidant activities of the sample extract of *C. spinosa* L. are associated with the high level of phenolic compounds.

The lyophilized and methanolic extract of *C. spinosa* L. exhibited a significant antioxidant effect (Germano et al., 2002). Extract concentrations of 100 and 1000 g.mL⁻¹ significantly inhibit ($p < 0.01$) lipid peroxidation by 71.50 and 90%, respectively. These antioxidant activities of methanolic extract are associated with the high phenolic compound levels (Tlili et al., 2010; Tlili et al., 2011). This protective effect may possibly be attributed to the richness of phenolic compounds, tocopherols and carotenoids. Many previous studies have suggested that these compounds possess very good antioxidative properties (Ihme et al., 1996; Burton et al., 1983; Mozaffarieh et al., 2003; Sommer and Davidson, 2002).

Mineral contents

The mineral contents of *C. spinosa* L. were shown to be excellent using the Epsilon 5 EDXRF system's inbuilt software (Table 3). Potassium, magnesium, manganese and sodium contents of *C. spinosa* L. buds as reported in Ozcan (2004) study were low compared to Ozcan and Akgul (1998) results. Some of our results concerning the

mineral contents of condiments differ from those in the literature (Akgul, 1993). These differences may be attributed to growth conditions, genetic factors, geographical variations or the analytical procedures involved (Guil et al., 1998; Ozcan, 2004).

Calcium is the major component of bone and also assists in teeth development (Brody, 1994). Magnesium, iron and phosphorus levels are also high. These elements are highly important since they are required as cofactors by many enzymes (Akpanabiatu et al., 1998). There are also inorganic elements which may be involved in biological processes, but which have not been shown to be essential (Macrae et al., 1993a). Absence of toxic element contents such as mercury and arsenic is highly advantageous. Zinc is essential for growth in animals, human beings and plants. It is also crucial to crop nutrition since it is required in various enzymatic reactions, metabolic processes and oxidation-reduction reactions. It is present in the enzyme system as a co-factor and mental activator of many enzymes. Zinc has been reported as potentially limiting the growth of bacteria at higher levels ($>13.60 \text{ mg.kg}^{-1}$) (Hafeez et al., 2013). In conclusion, *C. spinosa* L. possesses antioxidant activity and mineral content. The purpose of the study is to contribute to knowledge of the nutritional properties of *C. spinosa* L. knowledge of the mineral contents of condiments is also of great interest.

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