

Original Article

Phytochemical Analysis, Antioxidant Activity and Ecological Requirements of *Capparis spinosa* L. in Golestan and Semnan Provinces (North of Iran)

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Abstract

Natural antioxidants have an important role against damage by ROS. This research had been carried out about ecological characters, phytochemical and antioxidant activity of *Capparis spinosa* L. in Semnan and Golestan province. So in much field observation, different parts of *C. spinosa* were collected from two natural habitats in Golestan (200 m) and Semnan province (2100 m) during August to September 2011. Methanolic extracts were obtained by maceration, TP (total phenol) and TF (Total flavonoid) were determined by spectro photometrically method and the antioxidant capacity were obtained by TAC, RP and DPPH methods. Results showed that *Capparis spinosa* L. (Kabar) is an edible plant which was growing wild in clay loam to sandy clay loam soils. In both regions TF and TP content in buds and flowers extracts were highest, especially in 2100 m. Antioxidant activity of buds and flower extracts (IC₅₀) were the highest (1.27±0.1, 4.66±0.42 µg/ml) in 2100 m, especially in DPPH method and the lowest content belongs to fruit extract in 200m. These data will be confirmed the traditional uses of *C. spinosa* as an antioxidant and anti-inflammatory to treat of many current ailments.

Key words: Antioxidant capacity, *Capparis spinosa* L., Ecological requirements, Golestan and Semnan Provinces, Phytochemical (TF, TP)

Introduction

Capparis spinosa L., belongs to Capparidaceae family “Kabar” is a common medicine plant which growing wild in dry regions around the Mediterranean basin, originated from dry warm region in West or Central Asia. It is easily survive in higher than 40°C and well adapted to dry areas receiving less than 200 mm annual rainfall [1].

From ancient times, the floral buds of *Capparis spinosa* were employed as a flavoring in cooking and are also used in traditional medicine as diuretic, anti hypertensive and tonic to treat atherosclerosis, chronic renal failure, diabetes and immune dysfunction and aging [1-9].

Poly phenols, phenolics and flavonoides including several quercetin and kaempferol glycosides were

demonstrated to possess strong antioxidant/free radical scavenging effectiveness [3,4,10] and have received considerable attention to their pharmacological functions as antioxidant, anti-mutagenic and anti-tumor activities [7,11]. So in present study was carried on natural secondary metabolites sources (TF and TP contents) and the evaluate of their antioxidant activity which were collected from different habitats in North of Iran due to findings more overall value of the medicinal and natural antioxidant potential of the wild edible herbs [8-10].

Material and Methods

Plant materials

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In many field observation, most ecological requirements and plant parts (root, stem, leaf, flowers, buds and fruits) of *Capparis spinosa* L. were collected from Golestan (200m) and Semnan province (Khosh yelagh Mountainous region - 2100m) during August to September 2011.

The voucher specimen was identified and has been deposited in the Herbarium Museum of the RCMP of Islamic Azad University of Gorgan branch. The samples were separated, dried in the shade, grounded into fine powder and maintained at room temperature (21-23 °C). The prepared powder was kept in tight containers protected completely from light to perform the extraction of the secondary metabolites.

Extract preparation for phytochemical and antioxidant tests

One gram of different parts of plants (roots, stems, leaves, flowers, buds and fruits) samples with 100 ml (methanol 80%) were extracted by maceration. Extracts were filtered with Whatman No. 1 filter paper. The filtrates obtained from extracts were evaporated into dry rotary evaporator at 40 °C and were stored at 4 °C [12].

Chemicals

2,2'-diphenyl-1-picrylhydrazyl (DPPH) and quercetin (2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-chromen-4-one) were purchased from Sigma Chemical Co. (St., Louis, USA). Gallic acid, Folin-Ciocalteu reagent and methanol were purchased from Merck Co. (Germany).

Total antioxidant capacity

The spectrophotometric assay for the quantitative determination of antioxidant capacity was carried out [12]. The assay is based on reduction of Mo (VI) to Mo (V) by the sample analyst and subsequent formation of green Phosphate Mo (V) complex at acidic PH. The amount of TAC was expressed for samples in mM α -tocopherol/100 ml infusion.

Free radical scavenging capacity (DPPH-RSC)

Free radical scavenging capacity for plant infusions against stable DPPH' (2, 2-diphenyl-2-picrylhydrazyl hydrate) was determined spectrophotometrically [14]. When DPPH reacts with an antioxidant compound, that can donate hydrogen, it is reduced. The changes in colour (from deep-violet to light yellow) were measured at 515 nm on UV/visible light spectrophotometer.

H₂O₂ Reducing Power (H₂O₂-RP)

The reducing power assay was determined according to Arabshahi-Delouee and Urooj (2007) method [15]. At first, the dried extract (12.5 to 1000 μ g) in 1 ml of the corresponding solvent was combined with 2.5 ml of phosphate buffer (0.2 M, PH 6.6) and 2.5 ml of potassium ferricyanide (K₃Fe(CN)₆; 10 gL⁻¹), after the mixture was incubated at 50 °C for 30 min. Then, 2.5 ml of trichloroacetic acid (100 gL⁻¹) were added and the mixture centrifuged at 1650 g for 10 min. Then, 2.5 ml of the supernatant solution was mixed with 2.5 ml of distilled water and 0.5 ml of FeCl₃ (1 gL⁻¹), and the samples absorbance was measured at 700 nm.

Determination of total phenolic content

It was determined using the Folin-Ciocalteu Reagent. Total phenolic content was estimated by the Folin Ciocalteu method, based on the procedure suggested by Pourmorad *et al.* (2006) [12]. Then 0.5 ml of plant extracts or gallic acid (standard phenolic compound) was mixed with Folin Ciocalteu Reagent (5 ml) and aqueous Na₂CO₃ (4 ml, 1 M). The mixtures were allowed to stand for 15 min and the total phenols were determined by colorimetry at 765 nm. Gallic acid was used as a standard for calibration curve. Total phenol values were expressed in terms of mg equal gallic acid in 1 gr powder dry plant [12].

Determination of total flavonoid content

Total flavonoids content were determined by aluminum chloride method. Extract plants (0.5 ml) were separately mixed with 1.5 ml of solvent, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1 M potassium acetate and 2.8 ml of distilled water. They were kept at room temperature for 30 min; the absorbance of the reaction mixture was measured at 415 nm with a spectrophotometer. Total flavonoid values were expressed in terms of mg equal quercetin in one gram powder dry plant [12].

Results

Capparis spinosa L. 'Kabar' is one of the most wild edible herbs, which was growing wild in 200-2100 m, in temperate (414mm) to dry cold climate region (30 mm) and clay loam to sandy clay loam soils (Table 1) and which have been used in traditional medicines of Golestan and Semnan province as vegetable, spicy and tonic to treat cold, infections,

gastrointestinal problems, liver dysfunction, inflammation, rheumatism, hypertension and diabetic complications. As shown in Table 2, the results indicated that the TP contents of plant extracts ranging from 12.7 ± 0.5 to 89 ± 0.7 mg GAEg⁻¹ in dried weight, TF contents ranging from 3.5 ± 0.4 to 99.8 ± 0.3 mgQUEg⁻¹ and their antioxidant activities was varied in IC₅₀ = 3.5 ± 0.4

to 99.8 ± 0.3 µg/ml (Table 3). In both regions (200-2100m) TF and TP content in floral buds extract were (99.8 ± 0.3 , 71.7 ± 0.1 mgQUEg⁻¹ and 89 ± 0.7 , 80.4 ± 1.1 mgGAEg⁻¹), respectively. On the other hand floral buds and flower extract were the best plant parts which had high TF and TP results, (Table 2, Fig. 1 and 2) but additionally the stems and roots in both regions had lowest contents.

Table 1 Ecological requirements of *C. spinosa* L. in two localities

Characteristic Regions	Hight (m)	Rainfall (mm per year)	Temperature (°C per year)	Soil characters					
				Ec	PH%	TEXT.S	Clay%	Silt%	Sand%
Gorgan	200	414.8	17.8	0.73	8.1	Clay Loam	39	33.4	27.6
Khosh yelagh	2100	30.5.9	17.5	2.5	7.63	Sandy Clay Loam	23.8	32	53.6

Table 2 TF and TP contents in different parts of *C. spinosa* L. from two natural regions

Plant Parts	Total Flavonoid (mgQUEg ⁻¹)		Total Phenol (mgGAEg ⁻¹)	
	Khosh Yelagh Mountain (2100m)	Gorgan(200m)	Khosh Yelagh Mountain (2100m)	Gorgan(200m)
Floral buds	99.8±0.3	71.7±0.1	89±0.7	80.4± 1.1
Flowers	76.4±1.1	37.7±0.4	82.9±0.7	71±0.41
Leaves	68.2±0.2	35.4±0.6	62±1.2	33.9±0.7
Fruits	18.2±0.1	13±0.3	29±0.8	15.4±1.8
Stems	6.4±0.7	3.7±0.2	23.5±0.2	9.3±0.5
Roots	3.5±0.4	3.8±0.8	12.7±0.5	11±0.1

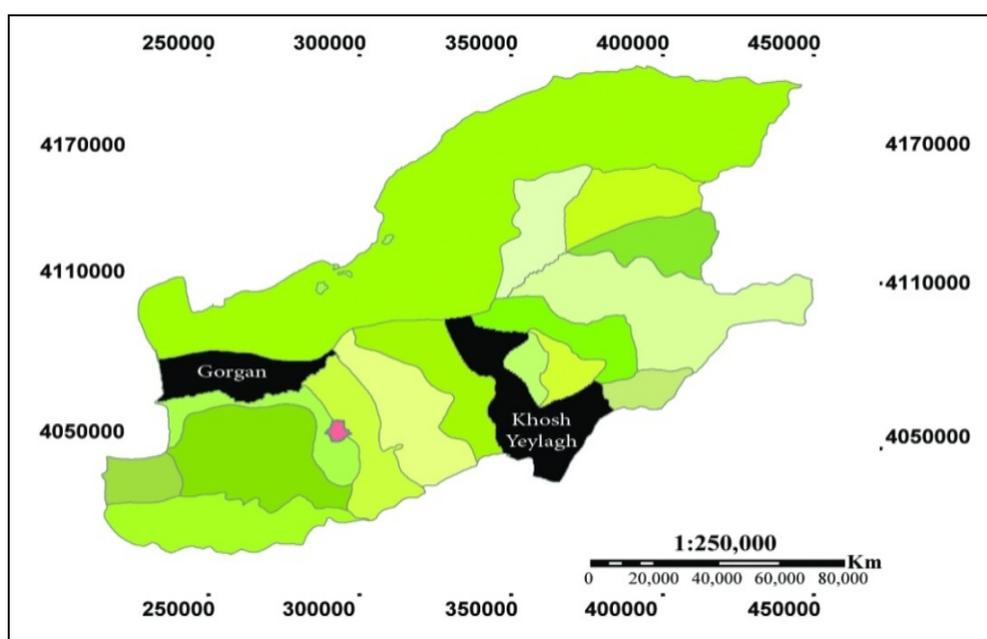
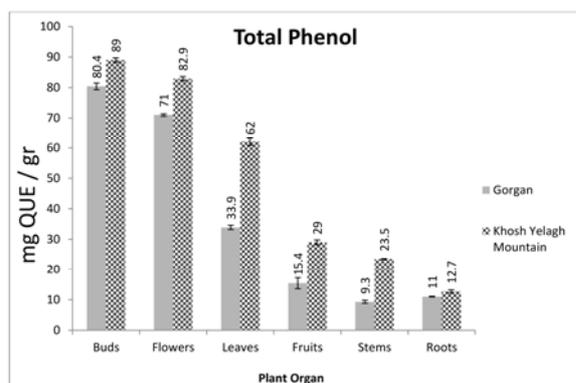
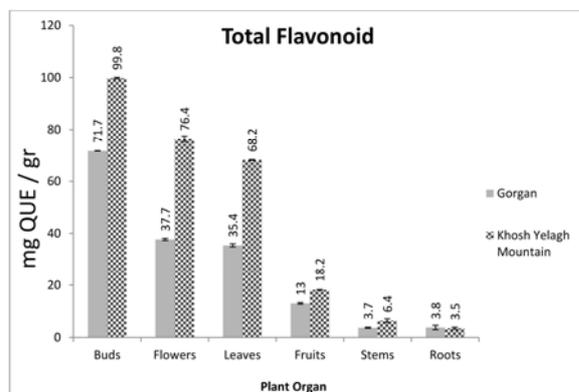
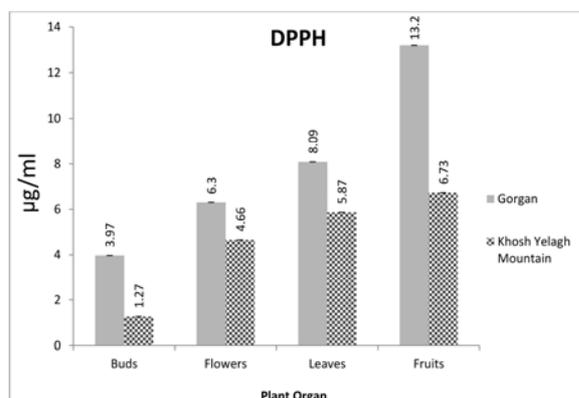
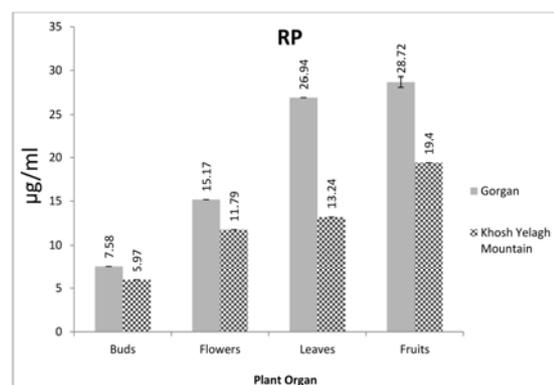
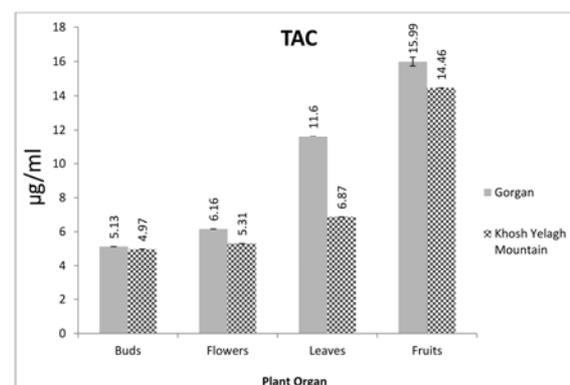


Fig. 1 Natural habitats of *C. spinosa* L. in Golestan and Semnan provinces

Table 3 Comparison of antioxidant activity in part extracts of *C. spinosa* extracts from different methods and regions ($\mu\text{g/ml}$)

Parts	IC50 RP		IC50 TAC		IC50 DPPH	
	Khosh Yelagh Mountain (2100m)	Gorgan(200m)	Khosh Yelagh Mountain (2100m)	Gorgan(200m)	Khosh Yelagh Mountain (2100m)	Gorgan(200m)
Floral bud	5.97±0.1	7.85±0.8	4.97±0.1	5.13±0.1	1.27±0.1	3.97±1.2
Flower	11.79±0.8	15.17±0.45	5.31±1.8	6.16±0.6	4.66±0.42	6.3±0.1
Leave	13.24±0.4	26.94±0.5	6.87±0.1	11.60±0.1	5.87±0.28	8.09±0.5
Fruit	19.40±0.4	28.72±0.6	14.46±0.31	15.99±0.26	6.73±0.6	13.20±0.21

**Fig. 2** Total phenol (mgQUEg^{-1}) contents in different parts of plant in two regions**Fig. 3** Total flavonoids (mgQUEg^{-1}) contents in different parts of plant in two regions**Fig. 4** Antioxidant activity of plant parts extracts of *C. spinosa* in DDPH method from different regions ($\mu\text{g/ml}$)**Fig. 5** Antioxidant activity of *C. spinosa* part extracts in RP method from different regions ($\mu\text{g/ml}$)**Fig. 6** Antioxidant activity of *C. spinosa* parts in TAC method from different regions ($\mu\text{g/ml}$)

According to Table 3 and Fig. 4,5,6, maximum antioxidant activity in DPPH, RP and TAC methods was observed from floral buds and flowers extracts, especially in 2100m and DPPH method with $\text{IC}_{50} = 1.27 \pm 0.1, 4.66 \pm 0.42 \mu\text{g/ml}$ (Fig. 4), because the floral buds extract have more TP and TF content ($89 \pm 0.7 \text{ mgQUEg}^{-1}$ and $99.8 \pm 0.3 \text{ mgQUEg}^{-1}$), than another parts.

Discussion

It has been recognized that secondary metabolites (phenols and flavonoids) have antioxidant agents through scavenging on human health [13], so the

same results in present study were suggested that the TP and TF compounds contributed significantly to antioxidant capacity of *C. spinosa* in both regions, especially in 2100m [12,14-15].

According to above, the extract of floral buds and flowers in 2100m has better potential antioxidant capacity against free radical, especially in DPPH test, due to their traditional uses as anti inflammation and anti diabetes against hypertension, diabetes, cold and related infections.

In many traditional medicine in the World, *C. spinosa* L. is well known established to economic medicinal plant[15], dried fruits were taken orally to treat hypertension and diabetic as antispasmodic, analgesic, anti elliptic, anti hemorrhoid and expectorant against cold ,infections, and externally to treat swollen joints, diarrhea , rheumatism skin rashes and dry skin[16-19] .

In previous research, rich in phenolics and flavonoides (quercetin and kaempferol) were demonstrated to possess strong antioxidant/free radical scavenging effectiveness, antiviral and immune stimulant affected in *in vitro* tests [9, 20]. These findings indicated that there was direct correlation between total phenol and flavonoid contents and their antioxidant activities [12,23-26]. Phenolic and flavonoids compounds may be involved in the antioxidant properties, which can play an important role in absorbing and neutralizing free radicals [13,21] and according to previous studies about antioxidant activity of *Onosma dichroanthum* Boiss.,[13] *Heracleum gorganicum* [24,27], *Peganum harmala* [24], *Nasturtium officinalis* [28], and *Artemisia annua* [23] were directly depended on amount of TP and TF.

These data demonstrated that *C. spinosa* L. especially which growing in Mountain area possess antioxidant activities against free radical produced. Some researches has been indicated that plant which lives in mountain area has a better antioxidant capacity and more flavonoids and poly phenols.

Conclusion

The results of the present study showed that the floral buds and leave extracts of *C. spinosa* L. with more content of TP and TF compounds have high potency in scavenging of free radical, which could provide natural sources of antioxidant compounds to treatment of disorders associated with free

radicals and also confirmed the traditional uses of *C. spinosa* buds as antiseptic, diuretic, anti-inflammatory and ant diabetic to treat of liver and gastrointestinal disease in north provinces of Iran.

More attention has been focused on the protective biochemical function of naturally occurring antioxidant in biological systems and on the mechanism of their action. Polyphenols are the most secondary antioxidant compounds in medicinal plants, which have important role in blocked activity of free radicals and so there was a positive correlation between total phenolic content and antioxidant activity.

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