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Full Length Research Paper

Phenolic profiles, antimicrobial and antioxidant activity of the various extracts of *Crocus* species in Anatolia

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The phenolic profile and quantitative composition of methanol extracts of *Crocus baytopiorum* which was endemic species in Denizli, Turkey was detected by high performance liquid chromatography (HPLC-DAD). The HPLC analysis of phenolic compounds in methanol extract of *C. baytopiorum* showed that p-Coumaric acid, apigenin-glucoside, rosmarinic acid, quercetin and kampferol were present. Also, the methanol, ethyl acetate and hexane extracts from *Crocus biflorus*, *C. baytopiorum* and *Crocus flavus* subp. *dissectus* were investigated for their *in vitro* antimicrobial and antioxidant activities in the present study. Ethyl acetate and methanol extracts have demonstrated significant antimicrobial activities against tested micro organisms *Escherichia coli* ATCC 35218, *Pseudomonas aeruginosa* NRRL B-23, *Klebsiella pneumoniae* ATCC 27736, *Yersinia enterecolitica* RSKK 1501, *Proteus vulgaris* RSKK 96026, *Bacillus cereus* RSKK 863, *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 25923, *Micrococcus luteus* NRRL B-4375 and *Candida albicans* (clinical isolate). The methanol extract of *C. flavus* subsp. *dissectus* had maximum activities on *Yersinia enterocolitica* RSKK 1501. Minimum inhibition concentrations of plant extracts have investigated on *Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* ATCC 6633 and *Bacillus cereus* RSKK 863. The Minimum inhibition concentration (MIC) of samples ranged from 0.10 to 20.48 mg/ml. In terms of radical scavenging activity and antioxidant activity, in the concentration of 2 mg/ml of methanol extract of *C. flavus* (92.67 and 89.32% respectively) displayed inhibition equal to in the concentration of 0.8 mg/ml butylated hydroxytoluene (BHA) used as standard antioxidant (91.45 and 89.78%, respectively). Positive correlations were found between total phenolic content in the *Crocus* extracts and their antioxidant activities. Thus, it is envisaged that *Crocus* species may have potential for acting as natural antioxidants.

Key words: *Crocus*, antimicrobial activity, antioxidant activity, HPLC, phenolic compounds.

INTRODUCTION

Crocus is the largest genus of Iridaceae. This genus is represented in Turkish flora by 70 taxa (Guner et al., 2000; Davis, 1984). The species *Crocus baytopiorum* is endemic to Denizli which is the province of Turkey. It is distributed Honaz Mountain, 2000 m. Saffron plant (*Crocus sativus* L.) is a vegetative propagated crop and is currently used as a source of food additives, colorants and as a component of traditional medicines (Escribano et al., 1999). Saffron, which is obtained from the dried stigmas of *Crocus sativus* is the most expensive spice used in industry with a wide range of uses from medicine, to textile dye and to culinary adjunct (Lozano et al., 1999). Studies have shown that crocin which is the compound of *C. sativus* has antioxidant effects (Zheng et al., 2007) and may have cardio protective effect (Goyal et al., 2010). During the last few years, the anti-tumoural properties of crude saffron stigma extracts, both *in vitro* and *in vivo*, have also been demonstrated (Escribano et al., 1999). Due to the large production of this plant and the use of its stigma only, it is very important to find some other uses of saffron (Vahidi et al., 2002). In addition, researchers have been interested in isolating biologically active compounds isolated from plant species for the elimination of pathogenic micro-organisms (Essawi and Srour, 2000). In this respect, the antimicrobial potential of

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Jatropha podagrica (hook) (Euphorbiaceae) was investigated in 2008. The researchers were found to be 15 mg/ml of minimum inhibitory concentration (MIC) value. At this concentration stem bark extract showed remarkable antibacterial activity as compared to stem extract and their zone of inhibition compared with standard antibiotics (Bhaskarwar et al., 2008). Also, a lot of plants used in traditional medicine today antimicrobial activity have been identified (Zampini et al., 2009; Oke et al., 2009; Mboss et al., 2010).

Antibiotics have been used for the treatment of infectious diseases for a long time. But, antimicrobial resistance, among pathogen bacteria, against drugs used in the treatment of human infection is increasing. This situation has forced scientists to search for new antimicrobial substances from various plants which are the good sources of novel antimicrobial chemotherapeutic agents (Karaman et al., 2003). Plant products have also been known to possess potential for food preservation (Baratta et al., 1998). Oxidation is essential to many living organisms for the production of energy to fuel biological processes. However, oxygen free radicals and other reactive oxygen species that are continuously produced *in vivo*, result in cell deaths and tissue damage. Oxidation-related damage caused by free radicals may be related to aging and diseases, such as atherosclerosis, diabetes, cancer and cirrhosis (Halliwell and Gutteridge, 1984). Although almost all organisms possess antioxidant defence and repair systems, which have evolved to protect them against oxidative damage, these systems are insufficient to prevent the damage entirely (Simic, 1988). However, antioxidant supplements or foods containing antioxidants may be used to help the human body reduce oxidation-related damage (Yanga et al., 2002). Synthetic antioxidants have been used in stabilisation of foods. The most commonly used synthetic antioxidants are butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and tert-butylated hydroxyquinone (TBHQ), which are applied in fat and oily foods to prevent oxidative deterioration (Löliger, 1991). Originally, BHA appeared to have tumour-initiating as well as tumour promoting action. Recently, it has been established that tumour formation appears to involve only tumour promotion caused by BHA and BHT (Botterweck et al., 2000). For this reason, governmental authorities and consumers are concerned about the safety of their food and about the potential effects of synthetic additives on health (Reische et al., 1998). Antimicrobial agents, including food preservatives have been used to inhibit food borne bacteria and extend the shelf life of processed food. Many naturally occurring extracts like essential oils, herbs and spices have been shown to possess antimicrobial functions and could serve as a source for antimicrobial agents against food spoilage and pathogens (Oussalah et al., 2006). The aim of the investigation presented in this paper is to evaluate the antimicrobial and antioxidant activities of various extracts of *Crocus biflorus* Miller, *C. baytopiorum* Mathew, *C. flavus* Weston subp. dissectus *T. Baytop* and Mathew on several pathogen micro-organisms, as there is a significant lack of information on such activities in literature.

MATERIALS AND METHODS

Plant materials

Plant samples were collected in an area around Denizli, particularly on Mount Honaz at 2000 m height by the authors of this paper in March and April. Dr. Ali CELIK further identified all of the collected plants. Specimens of the plants were preserved in the herbarium at Pamukkale University. Plants were dried in the shade and ground into a powder material using an appropriate seed mill.

Preparation of the crude extract

Extracts of plant materials were prepared using solvents of varying polarity. About 200 g of dry powdered plant material was extracted with hexane (HE), followed by ethyl acetate (ETA) and methanol (MeOH) in a soxhlet apparatus (6 h for each solvent). All solvents were purchased from Merck. The extracts were evaporated under reduced pressure and dried using rotary evaporator. Dried extracts were stored in labelled sterile screw capped bottles at -20°C.

Micro-organisms

The following nine strains of bacteria and one strain of yeast were used as test micro-organisms respectively: *Escherichia coli* ATCC 35218, *Pseudomonas aeruginosa* NRRL B-23, *Klebsiella pneumoniae* ATCC 27736, *Yersinia enterecolitica* RSKK 1501, *Proteus vulgaris* RSKK 96026, *Bacillus cereus* RSKK 863, *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 25923, *Micrococcus luteus* NRRL B-4375 and *Candida albicans* (clinical isolate). These micro-organisms were obtained from Microbiology Laboratory of Pamukkale University Biology Department.

Antimicrobial screening

Two different methods were employed for determining the antimicrobial activities of *Crocus* species: Agar-well diffusion method for the extracts and (MIC) analysis.

Agar-well diffusion method

The antimicrobial activity of the samples was assayed by the Agar-well diffusion method (Perez et al., 1990). All the aforementioned micro-organisms were incubated at 37 ± 0.1°C (30 ± 0.1°C for only *M. luteus* NRRL B-4375) for 24 h by inoculation into Nutrient broth. *C. albicans* was incubated YEPD in broth at 28 ± 0.1°C for 48 h. The culture suspensions were prepared and adjusted by using 0.5 Mc Farland turbidity standard tubes. Nutrient Agar (NA; g/L: beef extract 1, peptone 5, yeast extract 2, NaCl 5, agar 17) and YEPD Agar (g/L: peptone 20, yeast extract 10, dextrose 20) were poured into each sterilised Petri dish (10 x 100 mm diameter) after injecting cultures (100 µl) of bacteria and yeast and distributing medium in Petri dishes homogeneously. For the investigation of the antibacterial and anticandidal activity, the dried plant extracts were dissolved in dimethylsulfoxide (DMSO) to a final concentration of 30% (Tepe et al., 2005; Ali Shtayeh et al., 1998). Each sample (100 µl) was filled into the wells of agar plates directly. Plates injected with the yeast cultures were incubated at 28°C for 48 h and the

bacteria were incubated at 37°C (30°C for only *M. luteus* NRRL B-4375) for 24 h. At the end of the incubation period, inhibition zones formed on the medium were evaluated in mm. The experiment was repeated seven times and the inhibition zones were compared with those of reference discs. Inhibitory activity of DMSO was also tested. Reference discs used for control were as follows: Nystatin (100 U), ketoconazole (50 µg), tetracycline (30 µg), ampicillin (10 µg), penicillin (10 U), oxacillin (1 µg), tetracycline (30 µg) and gentamycin (10 µg).

Determination of minimal inhibition concentration (MIC)

The Minimal Inhibition Concentration method was applied on extracts that proved their high efficacy against micro-organisms by the disc diffusion method. *S. aureus* ATCC 25923, *B. subtilis* ATCC 6633 and *B. cereus* RSKK 863 were used. A stock solution of each selected plant extract was prepared in 90% dimethylsulfoxide (DMSO) and then serial dilutions of extracts were made in a concentration range from 0.1 to 25 µg/ml. The MIC was defined as the lowest concentrations of the plant extracts at which no bacterial growth was observed after incubation.

Antioxidant activity

Chemicals

β-Carotene, linoleic acid, 1,1-Diphenly-2-picrylhydrazyl (DPPH)) and buthylated hydroxyanisol (BHA) were purchased from Sigma (Sigma, Aldrich GmbH, Sternheim, Germany). Pyrocatechole, Tween-20, Folin-ciocalteu's phenol reagent (FCR), sodium carbonate, ethanol, chloroform and the other chemicals as well as the reagents were purchased from Merck (Darmstat, Germany). All other unlabeled chemicals and reagents were analytical grade.

DPPH assay

The hydrogen atom or electron donation abilities of the corresponding extracts and some pure compounds were measured from the bleaching of the purple-coloured methanol solution of 1,1-diphenyl-2-picrylhydrazyl (DPPH). This spectrophotometric assay uses the stable radical DPPH as a reagent (Burits and Bucar, 2000; Cuendet et al., 1997). One thousand micro litres of various concentrations of the extracts in methanol were added to 4 ml of 0.004% methanol solution of DPPH. After a 30 min incubation period at room temperature, the absorbance was read against a blank at 517 nm. The percentage of inhibition of free radical by DPPH in percent (I %) was calculated in the following way:

$$I\% = (A_{blank} - A_{sample} / A_{blank}) \times 100$$

Where Ablank is the absorbance of the control reaction (containing all reagents except the test compound) and Asample is the absorbance of the test compound.

β-Carotene-linoleic acid assay

In this assay, antioxidant capacity was determined by measuring the inhibition of the volatile organic compounds and the conjugated diene hydroperoxides arising from linoleic acid oxidation (Dapkevicius et al., 1998). A stock solution of β-carotene-linoleic acid mixture was prepared as follows: 0.5 mg β-carotene was dissolved in 1 ml of chloroform (HPLC grade) and 25 µl linoleic acid and 200 mg Tween 40 were added. Chloroform was completely evaporated using a vacuum evaporator. Then, 100 ml distilled water saturated with oxygen (30 min 100 ml/min) were added with vigorous shaking. Four thousand micro litres of this reaction mixture were dispensed into test tubes and 200 µl portions of the extracts, prepared at 2 mg/L concentrations, were added and the emulsion system was incubated for 2 h at 50°C temperature. The same procedure was repeated with synthetic antioxidant, BHA, as positive control and a blank, respectively. After this incubation period, the absorbance of the mixtures was measured at 490 nm. Antioxidant capacities of the extracts were compared with those of BHA and blank.

Determination of total phenolic compounds

Total soluble phenolics in all of the *Crocus* extracts were determined with Folin-Ciocalteu reagent according to the method of Slinkard and Singleton (1977) using pyrocatechol as a standard. Briefly, 1 ml of extract solution (contains 2000 µg) in a volumetric flask was diluted with glass-distilled water (46 ml). Folin-Ciocalteu reagent (1 ml) was added and the contents of the flask were mixed thoroughly. After 3 min, 3 ml of Na_2CO_3 (2%) was added, then the mixture was allowed to stand for 2 h with intermittent shaking. The absorbance was measured at 760 nm. The concentration of phenolic compounds was calculated according to the following equation that was obtained from standard pyrocatechol graph:

Absorbance = 0.00246 µg pyrocatechol + 0.00325 (R2: 0.9996)

Determination of total flavonoid concentration

Flavonoid concentration was determined as follows: *Crocus* extracts solution (1 ml) was diluted with 4.3 ml of 80% aqueous methanol and test tubes were added into 0.1 ml of 10% aluminum nitrate and 0.1 ml of 1 M aqueous potassium acetate solutions. After a 40 min incubation period at room temperature, the absorbance was determined spectrophotometrically at 415 nm. Total flavonoid concentration was calculated using quercetin as standard (Park et al., 1997).

Absorbance = 0.002108 µg quercetin – 0.01089 (R2: 0.9999)

High performance liquid chromatography

HPLC analyze of methanol extract of *C. baytopiorum* was performed by Suleyman Demirel University. The properties of HPLC equipment which was used: Detector: DAD detector (λmax=278), Auto sampler: SIL–10AD vp, System controller: SCL-10Avp, Pump: LC-10ADvp, Degasser: DGU- 14A, Column oven: CTO-10Avp, Column: Agilent Eclipse XDB C-18 (250 x 4, 6 mm) 5 µ, Mobil fase, A: 2% acetic acid, B: Methanol, Running speed: 0.8 ml/minute, Column temperature: 30°C

Statistical analysis

All data on antioxidant activity tests are the average of triplicate analyses. The data were recorded as mean ± SD. Analysis of variance was performed by ANOVA procedures. Significant differences between means were determined by student's t test, p - values < 0.05 were regarded as significant, p - values < 0.01 were regarded as very significant. Also, all antimicrobial experiments were done in seven times. Statistical analysis was performed on the data by ANOVA general linear model.

Table 1. Antimicrobial activities of hexane, ethyl acetate and methanol extracts of *C. flavus*, *C. biflorus* and *C. baytopiorum* by using agar well diffusion method[a].

	E. c.	*P. a.*	*K. p.*	*Y. e.*	*P. v.*	*B. c.*	*B. s.*	*S. a.*	*M. l.*	*C. a.*
Control	-	-	-	-	-	-	-	-	-	-
C. flavus										
Hexane	-	4 ± 0	-	-	-	8 ± 0	5 ± 1	-	-	-
Ethyl acetate	6 ± 0	6 ± 0	6 ± 0	10 ± 0	6 ± 0	10 ± 0	10 ± 0	11 ± 1	13 ± 1	6 ± 0
Methanol	-	6 ± 0	-	37 ± 1	6 ± 0	9 ± 1	6.5 ± 0.5	7 ± 1	15 ± 1	8 ± 0
C. biflorus										
Hexane	-	2 ± 2	-	-	-	8 ± 0	6 ± 0	-	-	-
Ethyl acetate	9 ± 1	8 ± 0	6 ± 0	12 ± 0	9 ± 1	12 ± 0	12 ± 0	9 ± 1	19 ± 1	7 ± 1
Methanol	-	6 ± 0	-	27 ±1	7.5 ± 0.5	14 ± 1	10 ± 0	7 ± 1	19 ± 3	6 ± 0
C. baytopiorum										
Hexane	-	-	-	-	-	4 ± 0	4 ± 0	4 ± 0	-	4 ± 0
Ethyl acetate	7 ± 1	14 ± 0	6 ± 0	10 ± 0	10 ± 0	10 ± 0	14 ± 0	10 ± 0	20 ± 0	4 ± 0
Methanol	-	6 ± 0	-	3 ± 0	-	8 ± 0	6 ± 0	4 ± 0	9 ± 1	4 ± 0
Reference antibiotics										
Ampicillin	10	ND	-	20	-	ND	ND	ND	30	ND
Penicillin	11	ND	ND	18	ND	22	12	31	31	ND
Gentamicin	ND	16	ND	ND	ND	ND	ND	ND	ND	ND
Tetracycline	8	8	5	7	16	19	17	20	19	ND

[a]Diameter in mm of the zone of inhibition, (-)= negative, ND: Not determined, E. c.= *E. coli* ATCC 35218, P. a.= *P. aeruginosa* NRRL B 23, K. p.= *K. pneumoniae* ATCC 27736, Y. e.= *Yersinia enterocolitica* RSKK 1501, P. v.= *P. vulgaris* RSKK 96026, B. c.= *B. cereus* RSKK 863, B. s.= *B. subtilis* ATCC 6633, S. a.= *S. aureus* ATCC 25923, M. l.= *M. luteus* NRRL B-4375, C. a.= *C. albicans*.

RESULTS AND DISCUSSION

In the present study, the antimicrobial effects of three species of *Crocus* genus were tested against five species of gram-negative bacteria, four species of gram-positive bacteria and one species of yeast. The crude extracts of *Crocus* genus were inhibitory to the growth of all species of the tested bacteria and yeast. These findings have been summarised in (Table 1). The antimicrobial activities of the extracts and their potency were quantitatively assessed by the presence or absence of inhibition zone and zone diameter. In general, plant extracts had a narrow antibacterial spectrum against gram-negative bacteria and strongly inhibited the growth of the gram-positive bacteria. In all plant samples, it was found that the ethyl acetate extracts against test micro-organisms were more influential than hexane and methanol extracts. Similar result was reported with *C. sativus* previously and shown that ethyl acetate extracts had the strongest antimicrobial activity (Vahidi et al., 2002).

Maximum inhibition zone diameter of ethyl acetate fractions were measured as 13 mm in *C. flavus*, 19 mm in *C. biflorus* and 20 mm in *C. baytopiorum* against *M. luteus* (Table 1). When comparing *C. flavus* and *C. biflorus* extracts, maximum activity was recorded against *P. aeruginosa* in ethyl acetate extracts of *C. baytopiorum*. Interestingly, similar activity was observed against *Y. enterocolitica* in methanol extracts of *C. flavus* and *C. biflorus* (respectively, 37 and 27 mm inhibition zone). The plant species were found of different significantly in their activity against test micro-organisms ($F=11.48$, $P< 0.0001$).

S. aureus and *Bacillus* species, particularly *B. cereus* are agents of food poisoning. In the study presented in this paper, *B. cereus*, *B. subtilis* and *S. aureus* were tested for MIC determination. Because most of the *Crocus* extracts are effective on these micro-organisms. The MIC of the plant samples is shown in (Table 2). The MIC of samples ranged from 0.10 to 20.48 mg/ml. The final concentration of DMSO in the assays did not interfere with the microbial growth. Thus, we may conclude that the antibacterial activity in this assay is exclusively due to plant extracts. As seen in the (Table 2), the most effective MIC values were methanol extracts of *C. flavus* (MIC=0.10 mg/ml) and *C. baytopiorum* (MIC=0.64 mg/ml) for *B. subtilis*. The hexane extract of *C. baytopiorum* showed a weak activity profile in *B. cereus*

Table 2. Minimum inhibition concentrations of various extracts of *C. flavus*, *C. biflorus*, *C. baytopiorum* (mg/ml).

	Extracts	Microorganisms		
		B. cereus	***B. subtilis***	***S. aureus***
C. flavus	HE	1.28	ND	ND
	ETA	ND	ND	ND
	MeOH	0.64	0.10	2.56
C. biflorus	HE	5.12	5.12	5.12
	ETA	ND	ND	ND
	MeOH	5.12	1.28	2.56
C. baytopiorum	HE	10.24	2.56	20.48
	ETA	2.56	2.56	5.12
	MeOH	5.12	0.64	5.12

ND: Not determined, HE: Hexane, ETA: Ethyl acetate, MeOH: Methanol.

and *S. aureus* (10.24 and 20.48 mg/ml concentrations, respectively). In a previous paper Vahidi et al. (2002), ethyl acetate extract of *C. sativus* was found to possess the strongest effect on *S. aureus* with 12.5 mg/ml concentration. In our study, the methanol extracts of *C. flavus* and *C. biflorus* showed a similar strong activity profile on *S. aureus* in 2.56 mg/ml concentration. Antibiotics have been used for the treatment of infectious diseases for a long time and unfortunately micro-organisms gain resistance to these antibiotics when prolonged uses take place. This has led the scientists to find alternative ways for treatment. Earlier it has been demonstrated that plant products show antimicrobial effects (Ahmad and Beg, 2001; Ali-Shtayeh, 1998). When the antimicrobial properties of plant species of *Crocus* genus are compared with those of widely used drugs against tested bacteria, it was found that some of them were more active than commercial antibiotics (Table 1).

The results showed that plant extracts inhibited the growth of micro-organisms like *E. coli*, *P. aeruginosa*, *S. aureus*, *Y. enterocolitica* and *C. albicans* which cause diarrhoea, urinary infection, wound infection and bactericidal meningitis. Especially, *Klebsiella pneumoniae*, which is known as a medically important pathogen, is resistant against ampicillin while it is susceptible to ethyl acetate extracts. While *P. vulgaris* was resistant against ampicillin, this bacterium is sensitive to the ethyl acetate extracts. Furthermore, the ethyl acetate extract of *C. baytopiorum* had higher effect than tetracycline on *P. aeruginosa* which was the strongest pathogen microorganism. In addition to this, the methanol extracts of *C. flavus* and *C. biflorus* had more inhibitory effect on *Y. enterocolitica* compared to all commercial antibiotics that are in use. The extracts were subjected to screening for their possible antioxidant activities. Four complementary test systems, namely 1,1, diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging, β-carotene/linoleic acid systems, total phenolic compounds and total flavonoid concentration were used for the analysis.

DPPH, a stable free radical with a characteristic absorption at 517 nm, was used to study the radical scavenging effects of the extracts. As antioxidants donate protons to these radicals, the absorption decreases and this decrease was taken as a measure of the extent of radical scavenging. Free radical scavenging capacities of the extracts, measured by DPPH assay, are shown in (Figure 1). All of the studied concentrations showed free radical scavenging activities. DPPH free radical scavenging activities of three different extractions (that is hexane, ethyl acetate and methanol) of three different *Crocus* species were studied. Among the extracts, the highest free radical scavenging activity was observed for methanol. Inhibition values of methanol extracts of *C. baytopiorum*, *C. flavus* and *C. biflorus* in the concentrations of 1.6 mg/ml were 78.21, 90.51 and 76.51%, respectively. However, inhibition of BHA used as standard antioxidants was 94.45%. Methanol extract of *C. flavus* showed higher activity than other species of *Crocus* we studied. While methanol extract of *C. flavus* in the concentration of 2.0 mg/ml was showing inhibition of 92.67%, it showed higher inhibition than BHA used as standard antioxidant (91.45%). In addition to this, ethyl acetate extract of *C. flavus* showed higher inhibition (87.70%) than other extract of it. It was observed that in line with the increase seen in the amount of extracts, an increase in DPPH free radical scavenging occurred.

Total antioxidant activities of *Crocus* species were measured using the β-carotene method. It was found that total antioxidant activities increased with concentration. These values are given in (Figure 2). The highest inhibition value was determined at 0.8 mg/ml concentration of *C. flavus* methanol extract, which showed 89.32% inhibitions. As methanol extract of *C. flavus* was

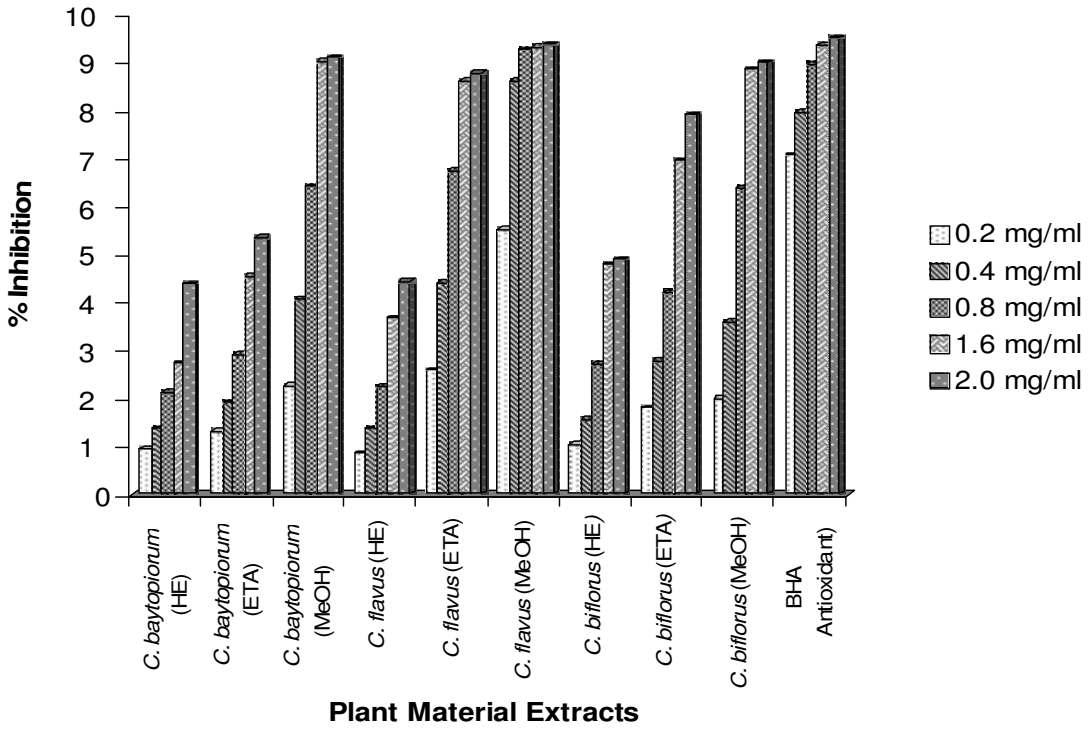

Figure 1. Free radical scavenging capacities of the extracts measured in DPPH assay.

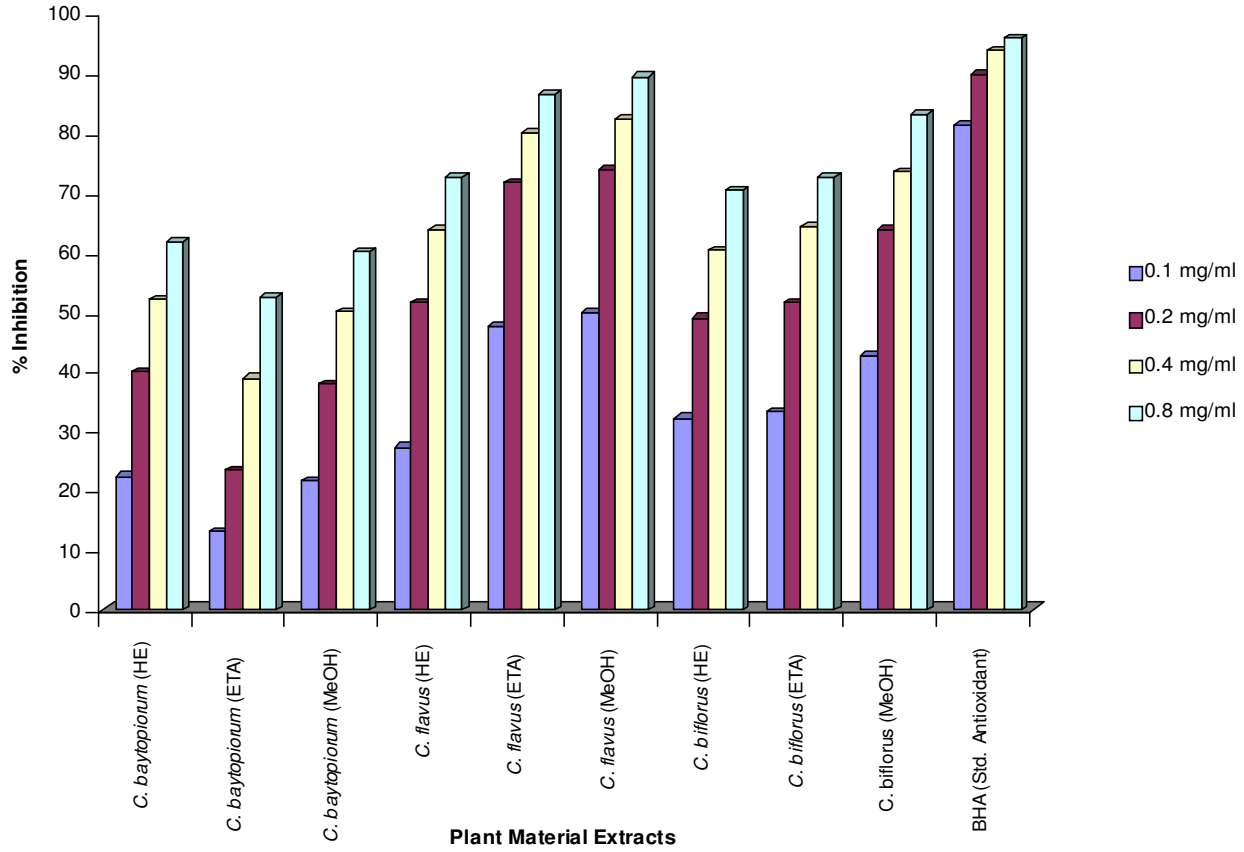

Figure 2. Total antioxidant activities of BHA and different doses of various *Crocus* extracts in the linoleic acid emulsion.

Table 3. Amounts of total flavonoid and total phenolic compounds in *Crocus* extracts.

Plant materials	Extracts	Total flavonoid content [quercetin equivalent (µg mg^{-1})]	Total phenolic compounds [pyrocatechol equivalent (µg mg^{-1})]
	HE	14 ± 0.08	25 ± 0.12
C. baytopiorum	ETA	12 ± 0.03	27 ± 0.09
	MeOH	36 ± 0.11	32 ± 0.07
	HE	13 ± 0.02	32 ± 0.11
C. flavus	ETA	40 ± 0.13	58 ± 0.16
	MeOH	71 ± 0.09	50 ± 0.19
	HE	16 ± 0.11	38 ± 0.04
C. biflorus	ETA	18 ± 0.07	36 ± 0.07
	MeOH	32 ± 0.16	20 ± 0.03

Data expressed as mean ± S.E.M. of three samples analyzed separately, HE: Hexane, ETA: Ethyl acetate, MeOH: Methanol.

Table 4. Chemical composition of methanol extracts of endemic *C. baytopiorum* .

Chemical compounds	Microgram/gram
p-coumaric acid ppm	25.36 ± 1.74
Naringin ppm	-
Hesperidin ppm	-
Apigenin-glucoside ppm	33,97 ± 1,97
Rosmarinic acid ppm	82,66 ± 0,31
Quercetin ppm	56,36 ± 2,17
Kampferol ppm	35,06 ± 0,61

showing higher activity than other extract of it in both radical scavenging activity and total antioxidant activity and competed with BHA standard antioxidant. According to this, it is possible that the high inhibition value of all *Crocus* extracts is due to the high concentration of phenolic compounds. Also, as seen in (Table 3), it was observed that methanol extract of *C. flavus* containing high phenolic and flavonoid materials also presented good results from the view of other activities as well.

The key role of phenolic compounds as scavengers of free radicals is emphasised in several reports (Komali et al., 1999; Moller et al., 1999). Polyphenolic compounds have an important role in stabilising lipid oxidation and are associated with antioxidant activity (Gülçin et al., 2003; Yen et al., 1993). The phenolic compounds may contribute directly to antioxidative action (Tepe et al., 2005; Duh et al., 1999). It is suggested that polyphenolic compounds have inhibitory effects on mutagenesis and carcinogenesis in humans when up to 10 g is ingested daily from a diet rich in fruits and vegetables (Tanaka et al., 1998). P-Coumaric acid, apigenin-glucoside, rosmarinic acid, quercetin and kampferol are detected by HPLC-DAD in methanol extracts of *C. baytopiorum* which was an endemic species in Anatolia (Table 4). From *Crocus laevigatus*, *C. heuffelianus* and *C. aureus* some flavone and flavonol glycosides based on 6-hydroxyluteolin, scutellarein, scutellarein 7-methyl ether and kaempferol have been isolated, in addition the aglycones acacetin and tricin have been identifed (Harborne and Williams, 1984). From *Crocus* species and cultivars, nine anthociyanins have been isolated by Nørbæk and Kondo (2002). The researches reported that the malonated anthociyanins were identified as 3,7-di-O-, 3,5-di-O-glucosides or 3-O-rutinosides of delphinidin and petunidin, 3,7-di-O-malonyl-glucosides of petunidin, malvidin and delphinidin 3-O-glucoside-5-O-malonylglucoside. Emergence of multi-drug resistance in human and animal pathogenic bacteria as well as undesirable side effects of certain antibiotics have triggered immense interest in the search for new antimicrobial drugs of plant origin (Ahmad and Beg, 2001). When comparing the antimicrobial activity of the tested samples to that of reference antibiotics, the inhibitory potency of tested extracts could mostly be considered as important. This is due to the fact that medicinal plants are of natural origin, which means more safety for consumers and are considered that they are being low risk for resistance development by pathogenic micro-organisms. To the best of our knowledge, this study is the first report on the antimicrobial and antioxidant activities of *Crocus* species. The extracts of *Crocus* can be used as a natural preservative in food because of their antioxidant activities. The antimicrobial activities of *Crocus* extracts against different strains of bacteria and fungi, which are known to be responsible for causing various diseases, could also be tested in future studies.

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