Caspian J. Environ. Sci. 2016, Vol. 14 No.4 pp. 331~338

CJES
Caspian Journal of Environmental Sciences

[Research]

Antioxidant and antimicrobial activities in the different extracts of Caspian saffron, *Crocus caspius* Fisch & C. A. Mey. ex Hohen.

M. Asadi

Department of Biology, Tonekabon Branch, Islamic Azad University, Tonekabon, Iran
E-mail: Mahmoudasadi50@yahoo.com

(Received: June. 1.2016 Accepted: Nov. 14.2016)

ABSTRACT

Nowadays it is very desirable to investigate and discover new antibacterial and antioxidant agents from natural products and medicinal plants. The current study was conducted to examine the antimicrobial and antioxidant properties of an endemic plant named Caspian saffron, *Crocus caspius*. After collecting *C. caspius* and drying them in the shade, ethanol, methanol and hydroalcohol extracts were prepared using maceration method. The amount of phenols and flavonoids measured in this study 2, 2_diphenyl-1_picrylhdrazyl (DPPH) test and disc diffusion method were used to evaluate antioxidant and antimicrobial activities, respectively. According to the results, phenols and free radical scavenging capacity were at highest level in the hydroalcoholic extract and flavonoids at highest level in the ethanol extract. More antioxidant activity of extracts was obtained in the higher concentrations. The highest amount of inhibitor for antimicrobial activity was in methanol extract using *Candida albicans*. The results indicated the potentiality of *C. caspius* extract to use as bio-preservatives and antimicrobial agents. However, further investigations are needed in the future in this regard.

Key words: Antimicrobial, Antioxidant, DPPH assay, Phenol, Flavonoid, *Crocus caspius.*

INTRODUCTION

Biochemical reactions in the body generate reactive oxygen species which can damage important bio-molecules, leading to several problems. The harmful action of the free radicals can be blocked by antioxidants which scavenge the free radicals and nullify their damaging effect on cellular constituents. Natural antioxidants from plants have been shown to increase the antioxidant capacity of the plasma and reduce the risk of certain diseases such as cancer, heart diseases and stroke (Prior & Cao 2000). Dietary antioxidants can stimulate cellular defenses and help to prevent cellular components against oxidative damage. In addition, they have been used in the food industry to prolong shelf life as they inhibit lipid oxidation. Majority of the antioxidants from plants are secondary metabolites like phenolics and flavonoids that have been reported to be potent free radical scavengers. They are found in different parts of the plants such as leaves, fruits, seeds, roots and barks (Mathew & Abraham 2006). Many of these phenolic compounds also possess other functional attributes like antimicrobial, anti-inflammatory, antimutagenic, hypocholestemic and antiplatelet aggregation properties (Riso *et al.* 2005).

Synthetic antioxidants and antimicrobials have been shown to have harmful side effects (Osawa & Namiki 1981; Gao *et al.* 1999; Williams *et al.* 1999). Therefore, there is a need for more effective, less toxic and cost - effective antioxidants and antimicrobials from natural sources. Several medicinal plants with ethno-botanical uses have been used traditionally for the treatment of diseases (Patel *et al.* 2010; Okoro *et al.* 2010; Lagnika *et al.* 2011). Consequently, there has been a growing interest to identify natural antioxidants and antimicrobials in the plants (Rice-Evans 2004;

Chanda & Dave 2009). Caspian saffron, *Crocus caspius* is an endemic perennial plant with white flowers and belongs to family Iridaceae (Mozaffarian 1996; Mazhary 1999). The aim of this research was to assess the antioxidant and antimicrobial activity of leaf and flower extracts in *C. caspius.*

MATERIALS AND METHODS

Plant Materials

C. caspius were collected from Dohezar with 1200 m elevation in Tonekabon City (Mazandaran Province, Iran). The plants were identified in the herbarium of the Faculty of Sciences, Islamic Azad University, Tonekabon Branch, Iran.

Antioxidant Activity

To examine the antioxidant activity, flower of *C. caspius* were cut into small pieces and shade dried at room temperature for fifteen days. Finely - powdered plant materials were successively extracted with organic - solvent methanol, ethanol and hydroalcohol using maceration method (Ahmed *et al.* 2006).

DPPH radical - scavenging activity assay

To determine the free radical - scavenging activity using DPPH method 2ml of 0.33% methanolic solution of DPPH was added in different concentration of methanol, ethanol and hydroalcohol *C. caspius* (100-500 µg.ml^{-1}) extract. After 30 minutes; absorbance was measured at 517 nm using UV-Visible spectrophotometer (Brand-Williams *et al.* 1995). All the tests were performed in triplicate and averaged. Ascorbic acid was used as standard. The percentage scavenging of the DPPH free radical was calculated using the following equation.

DPPH radical - scavenging activity (%) = (A control -A test)/A control × 100.

Determination of phenolic contents

Phenolic contents were determined by a Folin-Ciocalteau reagent using a method described by Spanos & Wrolstad (1990), so that 0.50 ml of each sample (three replicates), 2.5 ml of 1/10 dilution of Folin-Ciocalteau's reagent and 2 ml of Na_2CO_3 (7.5%, w/v) were added and incubated at 45°C for 15 min.

The absorbance of all samples was measured at 765 nm. The values were expressed as milligrams of Gallic acid equivalent per gram of dry weight (mg GAE/g dry weight).

Determination of flavonoids contents

1 g of potassium acetate (KOH) + 10 ml distilled water, 1 g of aluminum chloride ($AlCl_3$), 10 ml of distilled water was added in the flask. Then 7 g of sodium carbonate and 100 ml of distilled water were added. We choose 3 tubes for three replicates for each samples, then the 5 ml of the extract was infused in each tube.

Then, 1.5 ml of methanol and 0.1 ml aluminum chloride ($AlCl_3$), 0.1 ml potassium acetate and 2.8 ml distilled water mixture. After 30 min the absorbance at 510 nm was measured (Chung *et al.* 2002). The results were expressed as milligrams of Quercetin equivalents per gram dry weight (mg of QUE per g dw).

Antimicrobial activity

The aerial parts of the selected plants were dried in room temperature (27 ± 3°C) in the dark, and powdered.

Methanol, ethanol and hydroalcohol extracts were obtained by maceration of the crude plant powder with methanol/water (90/10 for) 2 days (26 ± 3°C) in the dark.

A Disk Diffusion Method was used to determine antimicrobial activity of the extracts (Bauer *et al.* 1966; Cruickshank 1968), and the microorganisms were cultured at 37°C for 16–24 h, prepared in a turbidity equivalent to McFarland standard No. 0.5 and consequently the suspensions were spread on the test plates (nutrient agar). Sterile discs were impregnated with 0.5, 1, 2 & 4 mg of the plants extract, and placed on the surface of test plate. Positive control discs with Gentamicin, (10 µg.disc^{-1}) for bacteria, each extract and control was tested in triplicate and the experiments were repeated three times.

Following microbes were selected for this study: *Staphylococcus aureus* (ATCC 9144), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (NCIMB 1081), *Bacillus subtilis*

(ATCC 1156), *Candida glabrata* (DSM 11226) and *Candida albicans* (ATCC 10231).

Statistical analyses

The analysis of variance (ANOVA) was performed using the SPSS 16.0 software, and the means were compared by Duncan's test. The values are reported as mean ± SD.

RESULTS AND DISCUSSION

The radical - scavenging activity of the extracts of *C. caspius* at different concentrations is shown in Table 1. ANOVA test showed that the differences in extract, concentration and extract × concentration interactions were significant at $p<0.05$ (Table 1). The results showed that at 1000 µg.ml^{-1}, the radical scavenging activity was highest for hydroalcohol extract (77.84 ± 1.6%), while it was least for methanol extract (14.83 ± 1.32 %) at 125µg.ml^{-1}. The scavenging activity of the extracts was dose - dependent. The DPPH radical - scavenging activity of the plant extracts was in the order of hydroalcohol > ethanol> methanol.

The DPPH radical is a widely used model to evaluate the antioxidant property of plant extracts (Ebrahimzadeh *et al.* 2008). DPPH is a stable nitrogen-centered free radical, the color of which changes from violet to yellow upon reduction by either the process of hydrogen or electron donation. Substances which are able to perform this reaction can be considered as antioxidants and therefore radical scavengers (Dehpour *et al.* 2009).

Table 1. Effects of extract, concentration and interaction of extract and concentration on the inhibition (%) in the *C. caspius* on DPPH model.

Extract	Concentration (µg.ml^{-1})	Inhibition (%)
Hydroalcohol	125	17.75 ± 0.55^{d}
	250	28.10 ± 0.54^{c}
	500	44.77 ± 1.43^{b}
	1000	77.84 ± 1.61^{a}
Methanol	125	14.83 ± 1.32^{d}
	250	22.25 ± 0.71^{c}
	500	33.10 ± 1.81^{bc}
	1000	55.17 ± 1.14^{ab}
Ethanol	125	17.13 ± 1.70^{d}
	250	26.89 ± 0.91^{c}
	500	40.26 ± 4.35^{b}
	1000	66.62 ± 2.88^{a}
ANOVA	Extract	*
	concentration	*
	Extract × concentration	*

The data are expressed as means ± SD (n = 3). The means marked with different letter in the same column are significantly ($P<0.05$) different. Significant levels; **significant at $p<0.01$; *significant at $p<0.05$.

Total phenol content of different extracts of *C. caspius* is presented in Fig. 1. Results showed that the differences in ethanol, methanol and hydroalcohol extracts were significant ($P < 0.05$). The highest total phenol content (243.45 mg GAE.g dry weight^{-1}) was found in hydroalcohol extract, while the lowest content

(197.1 mg GAE.g dry weight^{-1}) in methanol extract. The total phenol content of the plant extracts was in the order of hydroalcohol > ethanol > methanol. Total flavonoid content of different extracts of *C. caspius* is presented in Fig. 2. The results showed that the differences in the ethanol, methanol and hydroalcohol extracts were significant (P<0.05). The highest total phenol content (289.50 mg GAE.g dry weight^{-1}) was observed at ethanol extract while the lowest content (214.49 mg GAE.g dry weight^{-1}) at methanol extract. The total phenol content of the plant extracts was in the order of ethanol > hydroalcohol > methanol. An adequate intake of natural antioxidants can protect macromolecules against oxidative damage in cells (Riso *et al.* 2005). The term antioxidant refers to free radical scavengers, inhibitors of lipid peroxidation and chelating agent (Lee *et al.* 2003). Phenolic compounds possess a wide spectrum of biological effects including antioxidant and free radical - scavenging (Pellati *et al.* 2004). It has been reported that there is a significant relationship between the presence of total phenol and flavonoid content and antioxidant activity in many species. Phenolic compounds show significant antioxidant activity (Matkowski & Piotrowska 2006; Wei & Shibamoto 2007; Ghasemnezhad & Javaherdashti 2008).

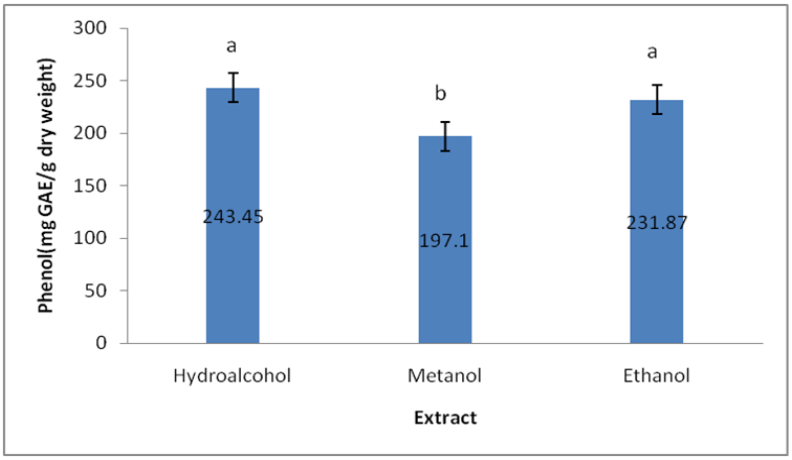

Fig. 1. The total phenol contents in different extract of *C. caspius*.

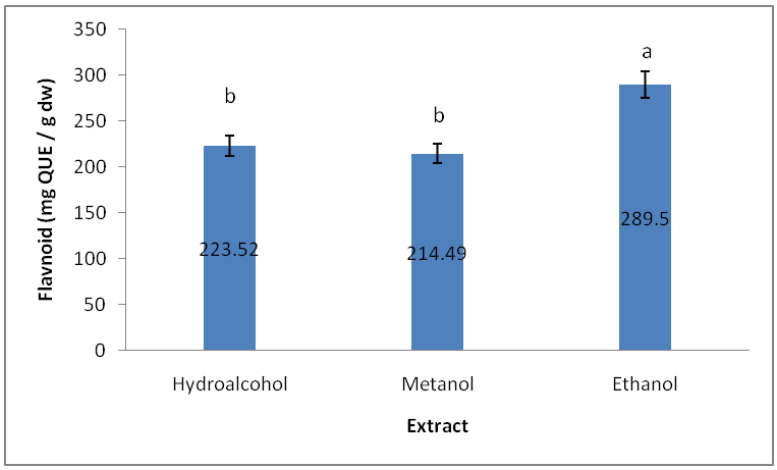

Fig. 2. The total flavonoid contents in different extract of *C. caspius*.

Antibacterial activity of different extracts of *C. caspius* was presented in Table 2. According to ANOVA test, the differences in diameter of inhibition zone (DIZ) of extract, microorganism and extract × microorganism interactions were significant at $p < 0.01$ (Table 2). Results showed that the highest antimicrobial activity of *C. caspius* was against *Candida albicans* with DIZ of

12.50 ± 0.82 mm (Table 2). Microbial resistance is a growing-problem worldwide (WHO 2001). One of the measures to combat the increasing rate of resistance in the long run is to have continuous investigation for new, safe and effective antimicrobials as alternative agents to substitute with no effective ones. Over the past 20 years, there has been a lot of interest in the investigation of natural materials as sources of new antimicrobial agents. Different extracts from traditional medicinal plants were tested and some natural products were approved as new antimicrobial drugs. However, there is still an urgent need to identify novel substances to be active against pathogens with higher resistance (Malika *et al*. 2004).

Table 2. Effects of extract, microorganism and interaction of extract and microorganism on the diameter of inhibition zone (DIZ) in the *C. caspius* on disc diffusion method.

Extract	Microorganism	DIZ
Hydroalcohol	*Staphylococcus aureus*	8.17 ± 0.55^{c}
	Bacillus subtilis	6.25 ± 0.54^{d}
	Escherichia coli	0.00^{e}
	Pseudomonas aeruginosa	0.00^{e}
	Candida albicans	12.50 ± 0.82^{a}
	Candida glabrata	11.33 ± 0.71^{ab}
Methanol	*Staphylococcus aureus*	9.25 ± 0.90^{c}
	Bacillus subtilis	6.42 ± 0.44^{d}
	Escherichia coli	0.00^{e}
	Pseudomonas aeruginosa	9.17 ± 0.91^{c}
	Candida albicans	11.67 ± 1.35^{ab}
	Candida glabrata	11.42 ± 1.88^{ab}
Ethanol	*Staphylococcus aureus*	6.75 ± 0.55^{d}
	Bacillus subtilis	8.58 ± 0.54^{c}
	Escherichia coli	0.00^{e}
	Pseudomonas aeruginosa	0.00^{e}
	Candida albicans	10.92 ± 1.32^{b}
	Candida glabrata	10.58 ± 0.71^{b}
ANOVA	Extract	**
	Microorganism	**
	Extract × Microorganism	**

The data are expressed as means ± SD (n = 3). The means marked with the different letter in the same column are significantly (P<0.05) different. Significant levels; **significant at p<0.01; *significant at p<0.05.

Lots of works have been indicated the antimicrobial and phytochemical constituents of medicinal plants and their use for the treatment of microbial infections (both topical and systemic applications) as possible alternatives to chemically synthetic drugs to

which many infectious microorganisms have become resistant.

During the last ten years, the pace of development of new antimicrobial drugs has slowed down, while the prevalence of resistance (especially multiple) has increased a lot (Hugo & Russell 1984). Literature reports and ethnobotanical records suggest that plants are the sleeping giants of pharmaceutical industry (Hostettmann & Hamburger 1991) and provide natural source of antimicrobial drugs that provides novel compounds that may be employed in controlling some infections globally.

As mentioned earlier, *C. caspius* has major medicinal effects and used traditionally. Therefore the potency of these extracts could provide a chemical basis for some of the health benefits claimed for *C. caspius* in folk medicine. Further studies are necessary to assess the potential components of *C. caspius* as effective natural medicine.

ACKNOWLEDGMENTS

This research (number: 27225) funded by the Islamic Azad University, Tonekabon Branch, Tonekabon, Iran.

REFERENCES

Ahmed, SU, Ali, MS, Begum, F & Alimuzzaman, M 2006, Analgesic activity of methanolic extract of *Nerium indicum* Mill. *Dhaka University Journal of Pharmaceutical Sciences*, 5: 85-87.

Assimopoulou, AN, Sinakos, Z & Papageorgiou, VP 2005, Radical scavenging activity of *Crocus sativus* L. extract and its bioactive constituents. *Phytotherapy Research*, 19: 997-1000.

Bathaie, SZ & Mousavi, SZ 2010, New applications and mechanisms of action of saffron and its important ingredients. *Critical Reviews in Food Science and Nnutrition*, 50: 761-786.

Berahou, A, Auhmani, A, Fdil, N, Benharref, A, Jana, M & Gadhi, CA 2007, Antibacterial activity of *Quercus ilex* bark's extracts. *Journal of Ethnopharmacology*, 112: 426-429.

Bibak, B, Khakshor, A, Kamali, H, Ahmadzadeh Ghavidel R & Amini Moghadamfarouj, N 2012, Evaluation of antibacterial properties, phytochemical contents and antioxidant capacities of leaf and stem barks of *Uvaria grandiflora* roxb. *Journal of North Khorasan University of Medical Sciences* , 4(1): 113-119 (in Persian).

Brand – Williams, W, Cuvelier, ME & Berset, C 1995, Use of a free radical method to evaluate antioxidant activity. LWT – *Food Science and Technology,* 28: 25-30.

Chimi, H, Cillard, J, Cillard, P & Rahmani, M 1991, Peroxyl and hydroxyl radical scavenging activity of some natural phenolic anti-oxidants. *Journal of the American Oil Chemists' Society*, 68: 307-311.

Chung , YC, Chien, CT, Teng, KY & Chou, ST 2006, Antioxidative and mutagenic properties of *Zanthoxylum ailanthoides* Sieb & Zucc. *Food Chemistry*, 97: 418-425.

Cowan MM 1999, Plant Products as antimicrobial agents. *Clinical Microbiology Reviews*, 12: 564-582.

Dorman, HJD, & Deans SG 2000, Antimicrobial agents from plants: Antibacterial activity of plant volatile oils. *Journal of Applied Microbiology*, 88: 308–316.

Ebrahimzadeh, MA, Nabavi, SF, Nabavi, SM & Eslami, B 2010a, Antioxidant activity of *Hibiscus esculentus* seeds. *Grasas Aceites*, 61: 30-36.

Ghasemnezhad, M & Javaherdashti, M 2008, Effect of methyl jasmonate treatment on antioxidant capacity, internal quality and postharvest life of raspberry fruit. *Caspian Journal of Environmental Sciences*, 6: 73-78.

Gil, MI, Tomás-Barberán, FA, Hess-Pierce, B & Kader, AA 2002, Antioxidant capacities, phenolic compounds, carotenoids, and vitamin C contents of nectarine, peach, and plum cultivars from California. *Journal of Agricultural and Food Chemistry*, 50: 4976-4982.

Hashemi, M, Azizi, IG, Rezai, Z & Rouhi, S 2012, Mycological survey and total

aflatoxin analyze in silage from Qaemshahr City (Northern Iran). *Journal of Chemical Health Risks*, 2: 51-56.

Kartal N, Sokmen, M, Tepe, B, Daferera, D, Polissiou, M & Sokmen, A 2007, Investigation of the antioxidant properties of *Ferula orientalis* L. using a suitable extraction procedure, *Food Chemistry*, 100: 584 -589.

Krittika, N, Natta, L & Orapin, K 2007, Antibacterial effect of five Zingiberanceae essential oil. *Molecule*, 12: 2047-2060.

Mazhary, N 1999, *Flora of Iran*. Research Institute of Forests and Rangelands Publishers, No. 31 (In Persian).

Miliaskas, G, Venskutonis, PR & Beek, TA 2004, Screening of radical scavenging activity of some medicinal and aromatic plant extracts, *Food Chemistry*, 85: 231-237.

Mozaffarian, V 1996, *A Dictionary of Iranian Plant Names*. Farhang Moaser Publication, Tehran, Iran, 522p. (In Persian).

Riso P, Visioli F, Gardana C, & Grande S 2005, Effect of blood orange juice intake on antioxidant bioavailability and on different markers related to oxidative stress. *Journal of Agricultural and Food Chemistry*, 53: 941-947.

Sagdic O 2003, Sensitivity of four pathogenic bacteria to Turkish thyme and oregano hydrosols, *Lebensmittel-Wissenschaft Und-Technologie-Food Science and Technology*, 36: 467-473.

Zakaria, M 1991, Isolation and characterization of active compounds from medicinal plants. *Asia Pacific Journal of Pharmacology*, 6: 15-20.

فعالیت آنتی اکسیدانی و ضد میکروبی عصاره‌های مختلف گیاه زعفران خزری (*Crocus caspius* Fisch & C. A. May.)

م. اسدی

گروه زیست شناسی، واحدتنکابن، دانشگاه آزاد اسلامی ، تنکابن، ایران

(تاریخ دریافت: ۹۵/۰۳/۱۲ تاریخ پذیرش: ۹۵/۰۸/۲۴)

چکیده

امروزه استفاده از فرآورده‌های طبیعی و گیاهان دارویی در یافتن ترکیبات نوین با خواص ضد میکروبی و ضد اکسیدانی مورد توجه است. در این مطالعه اثرات ضد اکسیدانی و ضد میکروبی گیاه منحصر زعفران خزر مورد بررسی قرار گرفت. پس از تهیه و جمع‌آوری زعفران خزر و خشک نمودن آن‌ها در سایه، نمونه‌ها پودر شده و عصاره‌های متانولی، اتانولی و هیدروالکلی به روش ماسراسیون تهیه شد. برای اندازه‌گیری ترکیبات آنتی اکسیدانی از آزمون فنل تام و فلاونوئید استفاده شد. همچنین در این مطالعه از روش (DPPH) 2, 2_diphenyl-1_picrylhdrazyl برای سنجش ظرفیت مهارکنندگی رادیکال آزاد عصاره-های مختلف بهره گرفته شد. اثر ضد میکروبی نیز با به کارگیری روش Disc Diffusion مورد ارزیابی قرار گرفت. نتایج نشان داد که بیشترین مقدار ترکیبات فنل و ظرفیت مهارکنندگی رادیکال آزاد برای حلال هیدروالکل و بیشترین مقدار ترکیبات فلاونوئید برای حلال اتانول به دست آمد. در روش دیسک، بیشترین قطر هاله عدم رشد در حلال متانول مشاهده شد. بیشترین قطر هاله عدم رشد توسط قارچ کاندیدا آلبیکنس در زعفران خزری به دست آمد. همچنین بهترین فعالیت آنتی اکسیدانی و ضد میکروبی در غلظت‌های بالاتر عصاره‌ها حاصل شد. یافته‌ها، پتانسیل عصاره زعفران خزری را برای استفاده به عنوان نگهدارنده زیستی و ترکیبات ضد میکروبی نشان دادند، اگرچه انجام تحقیقات بیشتر در این زمینه ضروری است.