AcademicPres

Available online: www.notulaebotanicae.ro

Print ISSN 0255-965X; Electronic 1842-4309

Not Bot Horti Agrobo, 2015, 43(2):343-348. DOI:10.15835/nbha43210089

Notulae Botanicae Horti Agrobotanici Cluj-Napoca

Preliminary Phytochemical Analysis and Antioxidant, Antibacterial Activities of *Crocus alatavicus* from Kazakhstan

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Abstract

Phytochemical analysis of *C. alatavicus* revealed the presence of phenols, flavonoids, anthocyanins, carotenoids, amino acids and carbohydrates. The flavonoid, amino acids and carotenoid contents were higher in aerial part (1.50%, 7.49% and 9.78 mg%, respectively) than in bulb (0.43%, 3.88% and 0.91 mg%, respectively). Total phenolic content (TPC), total antioxidant (TAA), 2.2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging and antibacterial activities of water, methanol, ethanol and dichloromethane extracts from aerial part and bulb were tested. TPC ranged from 13.63 to 72.29 mg gallic acid equivalents (GAE)/g extract. The maximum TAA were observed in ethanol (61.34%) and methanol extracts (46.13%) from aerial part with a high TPC (72.29 and 62.37 mgGAE/g extract, respectively). Ethanol extracts from aerial part and bulb had good scavenger of DPPH radicals (65.5% and 54.08%, respectively) with an IC_{50} 387 and 447 µg/ml. Ethanol extract from aerial part was most effective against gram-positive bacterial strains *S. aureus, B. subtilis* and *B. cereus*. Biological activities of the extracts were correlated with the TPC. It can be deduced that ethanol and methnol extracts of *C. alatavicus* contains useful potent bioactive compounds with antioxidant and antimicrobial activities.

Keywords: bulb, DPPH-radical, extract, inhibition, phenolic

Introduction

The genus *Crocus* L. (Iridaceae) includes above 80 species occurring from Western Europe to western China (Harpke *et al*., 2013). *Crocus* species are highly valued as ornamental plants of their colourful flowers, horticultural varieties and industrial application (Saxena, 2015). Among the different species of *Crocus*, various biological activities and phytochemical constituents of *C. sativus* have been studied extensively because of saffron is widely used as a spice, food additive and as a textile dye or cosmetic ingredients and for treatment of some disease in many countries (Bhargava, 2011; Hosseinzadeh and Nassiri-Asl, 2013; Rezaee and Hosseinzadeh, 2013; Agnihotri, 2015). Antibacterial (Pintado *et al*., 2011; Agnihotri, 2015; Monte *et al*., 2015; Parray *et al*., 2015), antioxidant (Ferrara *et al*., 2014; Golmohammadi, 2014; Baba *et al*., 2015; Parray *et al*., 2015; Rahaiee *et al*., 2015), anti-mycobacterial (Hussain *et al*., 2014), cancer-suppressing (Samarghandian and Borji, 2014), anti-mutagenic, immunomodulating, antidepressant, antifungal (Golmohammadi, 2014), antiparasitic activities (Monte *et al*., 2015) and analgesic effects (Simbar *et al*., 2015) of *C. sativus* have been reported. Anticancer (Chryssanthi *et al*., 2007), antimicrobial, antioxidant (Acar *et al*., 2010), cytotoxic (Tokgun *et al*., 2012) properties of the other *Crocus* species also have been reported.

C. alatavicus is an early spring ephemeral and geophytic-geocarpic species that grows in subalpine areas of the northern and western Tianshan Mountains (Baytenov, 2001; Zhang and Tan, 2009). It is an endemic of Kazakhstan flora belonging to the group of bulbous and tuberous plant. Wild habitat of this species is the Kora river middle flow, Koppal district, Taldykurgan region and South-East Kazakhstan. It widely distributed in Xinjiang that bordered by Mongolia, Kazakhstan, Uzbekistan and Kyrgyzstan (Saxena, 2015). The species mainly has potential in horticulture; it is the beautiful herb, highly rated for early blooming and easy cultivation (Ivaschenko, 2005).

Received: 03 Sept 2015. Received in revised form: 19 Nov 2015. Accepted: 23 Nov 2015. Published online: 10 Dec 2015.

C. alatavicus is also interested as a source of naturally active substances that have many useful biological properties. Accumulation of the bioactive compounds is associated with the geophytic life-form of the plant and its ephemeral development cycle. Formation of underground storage corms contributes plant to survive adverse environment and proceeds the growth and reproducing of vegetative and generative organs (Kamenetsky and Okubo, 2013).

The species was used as spasmolytic, anti-inflammatory, bactericidal and antiviral agents in traditional Chinese medicine (Duke and Ayensu, 1985). The flowers were used as diuretic, for treatment of abdominal illness and to improve hormonal regulation of women (Egamberdieva *et al*., 2012). Dried stigmas are applied for colouring food products and impart flavour for them (Golovkyn *et al*., 2001).

Little is known about the phytochemical constituents of this species. The leaves contain ascorbic acid and the stigmas of flowers contain yellow pigment available for food colorant (Ivaschenko, 2005). Anthocyanins such as delphinidin 3-*O*-β-rutinoside and petunidin 3-*O*-β-rutinoside and flavonoids such as myricetin, quercetin, kaempferol have been investigated in flowers (Nørbæk*et al*., 2002). It was reported that content of biological active substances in bulb of *C. alatavicus* depens on the vegetation period and weather conditions and it contributes the resistance of the plant to hostility and microflora during the growth (Kukushkina and Sedelnikova, 2010; Sedelnikova and Kukushkina, 2014). Studies addressing its biological activities are currently nonexistent. Hence, the aim of this study was preliminary phytochemical screening of different plant part and estimation of *in vitro* antioxidant, antimicrobial activities and total phenolic content of various extracts from aerial part and bulb of *C. alatavicus*.

Materials and Methods

Plant material

Different parts (aerial part and bulbs) of *C. alatavicus* were collected from the natural environment of Almaty (Kazakhstan) in March, 2014. The Voucher specimen was deposited in the herbarium of Institute of Plant Biology and Biotechnology (Almaty, Kazakhstan). Aerial part and bulbs were cleaned, air dried in the shade at room temperature.

Preparations of the extracts

Powdered samples (10 g) were extracted with 100 ml of different solvents (distilled water, 96% ethanol, 99% methanol and 99% dichloromethane) at 60 °C (methanol at 35 °C) for 6 hours in a water bath shaker. After the filtration with Watman No:1 filter paper the solvents were concentrated under vacuum (48-49 °C) by evaporating to dryness. All the extracts were stored at -20 °C until use.

Phytochemical analysis

Preliminary qualitative tests for screening of major biological active compounds in powdered aerial part and bulb were carried out according to the Harborne (1984). Standard quantitative analysis of flavonoid, carotenoids, carbohydrates and amino acid was used. For the determination of the total flavonoid content of the samples the aluminum chloride colorimetric method was used with quercetin as a standard. Free amino acid content by ninhydrin reaction was analysed. The phenol-sulfuric acid method was used for determination of carbohydrates in the samples. The total carotenoid content was determined by the standard spectrophotometric method.

Determination of total phenolic content

Total phenolic content (TPC) of the different extracts of *C. alatavicus* was determined with the Folin-Ciocalteu reagent according to the procedure reported by Türkoğlu *et al.* (2010), using gallic acid as a standard. The standard gallic acid curve was produced within the concentration range from 0.01 to 0.2 mg. The results were calculated by the equation based on the standard curve: $y = 1.1592x - 0.0013$, $R^2 = 0.9986$. TPC was expressed as mg of gallic acid equivalents (GAE) per g of dry extract.

Determination of total antioxidant activity

Total antioxidant activity (TAA) of the extracts was estimated by the β-carotene bleaching test following the procedure described by Wettasinghe and Shahidi (1999) with a slight modification. A stock solution of β-carotene-linoleic acid mixture was prepared as follows: 0.2 mg β-carotene was dissolved in 1 ml of chloroform (HPLC grade), 20 µl linoleic acid and 200 µl Tween 20 were added. Chloroform is completely evaporated at 40°C for 10 min. Then, 50 ml distilled water was added with a vigorous shaking. 4.8 ml of this reaction mixture was dispensed to test tubes and 200 µl portions of the extracts, prepared at 2 mg/ml concentrations were added. The mixture is then gently mixed and incubated at 50°C for 2 h. Absorbance of the sample was measured every 30 min for 2 h at 470 nm using a spectrophotometer. The same procedure was repeated with synthetic antioxidant butylated hydroxytoluene (BHT) as a positive control. TAA was calculated using the following equation: $AA = (1 - A_0 - A_t / A_o^o - A_t^o) \times 100$,

AA is an antioxidant activity, A_0 and A_0^0 are the absorbance values measured at the initial incubation time of samples and control, respectively. While A_t and A_t^0 are the absorbance values measured in the samples and control at t = 120 min.

DPPH radical scavenging assay

The hydrogen atoms or electron-donation ability of the extracts is measured from the bleaching of a purple-coloured methanol solution of DPPH according to the method Sanja *et al*. (2009). Stock solutions of the extracts were prepared at 1 mg/ml concentration by dissolving in methanol and various concentrations (100-500 µg/ml) of the extracts were tested. Ascorbic acid (10, 15, 20, 25, 50 µg/ml) and Trolox (10, 25, 50, 75 µg/ml) were used as positive control. DPPH radical scavenging activity (% antiradical activity) was calculated using the following equation: % antiradical activity = $(A_0 - A_1) / A_0 \times 100$, A_0 is the absorbance of the control and A_1 is the absorbance of the extract/standard. A percent inhibition versus concentration curve was plotted and the amount of antioxidant necessary to decrease the initial DPPH concentration by 50% (IC_{50} value) was calculated.

Antimicrobial activity test

Antibacterial activity of some extracts was determined by the disk diffusion method (Singh *et al*., 2002). Among the extracts obtained by polar solvents only water and ethanol extracts were tested for antibacterial activity. The extracts obtained by

relatively nonpolar dichloromethane also were tested. Four strains of bacteria including gram-positive *Staphylococcus aureus, Bacillus subtilis, Bacillus cereus* and gram-negative *Escherichia coli* were used for tests. Sample solution were prepared by dissolving the dried extracts in their solvents (water, ethanol and dichloromethane) to give concentration of 1 mg/ml. 25 and 50 µl of the extract was impregnated into standard disks. Antibacterial agent kanamycin (1 mg/ml; 20 µl/disk,) was used as a positive control and antimicrobial activity of each solvent used for extraction (ethanol and dichloromethane at 50 µl/disk) was tested**.**

Statistical analysis

All experiments were carried out in triplicate and expressed as average of three analyses ± standard deviation and Excel 2013 was used.

Results and Discussion

Phytochemical screening

Qualitative analysis of the main biological active compounds revealed the occurrence of flavonoids, anthocyanins, phenols, carotenoids, amino acids and carbohydrates in aerial part of *C. alatavicus* (Table 1). The presence of flavonoids, phenols, carotenoids, carbohydrates and amine-containing compounds were found in bulb.

The results of quantitative analysis of some determined biological active compounds in different parts of *C. alatavicus* are given in Table 2.

The plant material of *C. alatavicus* has abundant presence of secondary metabolites such flavonoids, phenols, carotenoids. The carotenoids content was greater in aerial part (9.8 mg%) than in bulbs (0,9 mg%). Flavonoids are 1.50% in the powdered aerial part and 0.43% in the powdered bulbs. The results revealed that aerial part of plant contains more biological active substances that may have high biological activities.

Phytochemical constituents of this species insufficiently explored. Aerial part contains ascorbic acid and some glycosides. Presence and quantitative content of saponin, ascorbic acid, catechin, pectin and protopectin in bulb of *C. alatavicus* depends on vegetation period of the plant (Kukushkina and Sedelnikova, 2010). It was reported that high content of ascorbic acid in aerial part and in bulb is observed in summer and it twice decreases in autumn (Sedelnikova and Kukushkina, 2014). Some flavonoids such as derivatives of myricetin, quercetin and kaempferol have been identified by HPLC in flowers of *C. alatavicus* (Nørbæk *et al*., 2002).

Total phenolic content

The antioxidant activity, radical scavenging capacity and TPC of *C. alatavicus* has not previously been published. The reason to determine TPC in the plant extracts are directed that phenolics are the main group of compounds acting as antioxidants or free radical scavengers.

Methods for assessment of total phenolic content and determination of their antioxidant capacity are mostly based on oxidizing-reducing properties, possibility of phenolic compounds functioning as reduction agents and offering hydrogen radical or electron (Stratil *et al*., 2007). The results on TPC of *C. alatavicus* extracts obtained using different solvents are presented in Table 3.

The different solvents showed different content of phenolic. The highest phenolic contents were obtained in ethanol and methanol extracts from aerial part (72.29; 62.37 mg GAE/g extract, respectively). Ethanol and methanol extracts from bulb have shown higher amount of phenolic (43.65 and 36.06 mg GAE/g extract, respectively) than water and dichloromethane extracts from aerial part and bulb (23.55 and 24.26; 14.49 and 13.69 mg GAE/g extract, respectively). Rahaiee *et al*. (2015) reported that solvent type has significant effects on the TPC of the saffron extracts and 80% ethanol extract of saffron had the highest amount of TPC than aqueous and methanol (50%, 80%) extracts.

The different polarity of organic solvents influences to the efficiency of the extraction and the solubility of phenolic compounds. Previous study has shown that the phenolic compounds more extracted by ethanol and methanol than water and dichloromethane. Therefore, the lowest TPC was observed in water and dichloromethane extracts.

Table 1. Preliminary phytochemical screening of *C. alatavicus*

Metabolites	Aerial part	Bulb
Carbohydrates	+	+
Tannins	-	-
Saponins	-	-
Amino acids	+	+
Alkaloids	-	-
Phenols	+	+
Carotenoids	+	+
Flavonoids	+	+
Anthocyanins	+	-
Coumarins	-	-

Note: + Presence; – Absence.

Table 2. Quantitative contents of some biological active compounds of *C. alatavicus*

Metabolites	Aerial part	Bulb
Flavonoids	1.50 ±0.09	0.43 ±0.07
Carbohydrates	0.05 ±0.01	0.06 ±0.02
Amino acids	7.49 ±1.20	3.88 ±0.56
Carotenoids	9.78±1.09	0.91±0.28

Note: Values are expressed as g of the dry material (%) except for carotenoids (mg%), and all data are means ± SD; (n=3).

Table 3. Total phenolic content of the extracts from different parts of *C. alatavicus*

Extracts	Aerial part	Bulb
Water	24.26 ± 0.43^{c}	14.49 ± 0.29^{c}
Methanol	62.37 ± 0.49^{b}	43.65 ± 0.76^{a}
Ethanol	72.29 ± 2.16^{a}	36.06 ± 0.43^{b}
Dichloremethane	23.55 ± 2.15^{c}	13.63 ± 0.43^{c}

Note: Values are expressed as mg GAE/g extract; different superscript letters within column are significantly different at $p < 0.01$ (values are from high to low).

Table 4. Total antioxidant activity (% ± SD) of *C. alatavicus* extracts and standard by the β-carotene bleaching method

Extracts/Standard	Aerial part	Bulb
Water	33.57 ± 1.17^{c}	$27,38 \pm 0.16^{c}$
Methanol	46.13 ± 0.25^{b}	$37,55 \pm 1.51^{a}$
Ethanol	$61.34 \pm 0,58^{a}$	$30.03 \pm 0,62^{b}$
Dichloromethane	$26.66 \pm 3,12^{d}$	$17.21 \pm 0,48^{d}$
BHT	91.4 ± 1.19	

Note: Different superscript letters within column are significantly different at $p < 0.05$ (values are from high to low).

Total antioxidant activity

The β-carotene bleaching test is based on the loss of the yellow colour of β-carotene due to its reaction with radicals which are formed by linoleic acid oxidation in an emulsion (Kulisic *et al*., 2004). Table 4 shows the potential of *C. alatavicus* extracts and standard (BHT) to inhibit lipid peroxidation as evaluated by the bleaching of β-carotene.

The highest TAA was determined in ethanol extract from aerial part (61.34%). Methanol, water and dichloromethane extracts have shown middle and low TAA (46.13%; 33.57% and 26.66%). It is statistically significant ($p < 0.05$). Bulb methanol extract showed higher AA (37.55%) than water and dichloromethane extracts (27.38% and 17.21%). All the extracts showed low TAA against synthetic standard BHT (91.4%).

Radical scavenging activity

The DPPH test is based on the ability of the stable DPPH free radical to react with hydrogen donors. In this test, a solution of radical is decolourized after reduction with an antioxidant or a radical (Parejo *et al*., 2000).

The results of the experiments also indicated that ethanol extracts of *C. alatavicus* have the high free radical scavenging activity. The comparable free radical scavenging rates of the extracts at the concentration of 500 μg/ml are shown in Fig. 1.

The highest scavenging activity was observed in ethanol extracts from aerial part and bulb (65.5% and 54.08%). Methanol extracts showed lower inhibitory activity (48.73% and 35.04%), but higher than activity of dichloromethane extracts (17.9% and 13.9%, respectively).

The IC_{50} value is one of the important parameter in determination of antioxidant capacity. IC_{50} value was determined from the plotted graph of scavenging activity against various concentrations of the extracts. IC_{50} values of *C. alatavicus* extracts were calculated to compare the antioxidant activity and listed in Table 5.

The lowest IC_{50} indicated the strongest ability of the extracts to act as DPPH radicals scavengers. Out of the all extracts minimum IC_{50} (387 μg/ml) was determined in ethanol extract from aerial part. Dichloromethane and water extracts showed the high IC_{50} values (from 801 to 3,221 μg/ml). Antiradical activities of commercial antioxidants of vitamin C and Trolox were higher (IC_{50} 26.35 and 37.16 μg/ml).

The results obtained in this study show that DPPH scavenging activity of *C. alatavicus* is less than *C. sativus*, but higher than other species of *Crocus* as compared to the published experimental data. So, IC_{50} value of *C. sativus* ranged from 231.75 to 210.79 μg/ml for methanol extract, 255.44 μg/ml for water extract and 299.4 μg/ml for ethanol extracts (Karimi *et al*., 2010; Sariri *et al*., 2011). Whereas methanol extracts of *C. baytopiorum*, *C. flavus*, *C. biflorus* showed 78.21%, 90.51% and 76.52% radical scavenging activity at 1.6 mg/ml concentration (Acar *et. al*., 2010). Trice less concentration of ethanol extracts from different part of *C. alatavicus* initiate activity about 65.5% and 54.98% in this study.

Table 5. IC_{50} values of *C. alatavicus* extracts and standards

Part of plant	Extracts	IC_{50} (μg/ml)
Aerial part	Water	865
	Methanol	510
	Ethanol	387
	Dichloromethane	926
Bulb	Water	801
	Methanol	853
	Ethanol	443
	Dichloromethane	3,221
Vitamin C	Standards	26.35
Trolox		37.16

Antibacterial activity

The various extracts from *C. alatavicus* showed selective and varying degree of activity against different bacteria. The results of the zone inhibition determined by the disk diffusion method are presented in Table 6.

It is clear from the results that all tested bacterial strains were susceptible at 20 μl concentration of antibiotic kanamycin. Among the solvents, ethanol at 50 μl showed antibacterial activity against strains of *E. coli, B. subtilis* and *B. cereus,* but did not show activity against stains of *St. aureus*. Dichloromethane was low effective only against *E. coli* with 13.08 mm diameter zone of inhibition. Plant extracts exhibited selective activity against different bacteria; did not show activity against gram-negative bacteria *E.coli*, but were effective against gram-positive *S. aureus*, *B. subtilis* and *B. cereus.* Ethanol extracts with a high phenolic content showed a high inhibition activity. In addition, ethanol extract from aerial part at 50 μl concentration showed antibacterial activity against *B. subtilis, B. cereus* and *S. aureus.* Diameter of zone inhibition ranged from 17.32-21.72 mm, compared to control (70% ethanol) 10 mm. Minimal concentration of the extract (25 μl) did not show activity against all test-cultures. Ethanol extract from bulb was effective against *B. subtilis* and *B. cereus* at all tested concentrations. Diameter of zone inhibition ranged from 15.66 to 20.67 mm. Water and dichloromethane extracts with a low phenolic content were not effective against all the tested bacteria. Dichloromethane extract from bulb showed antibacterial activity against only *B. cereus* at 25 μl concentration (zone of inhibition – 20.03 mm). It has been reported that aqueous extracts of saffron onion at different concentration also have not antibacterial activity against *B. anthracis, S. enteritidis, S. aureus, Proteus, E. coli* by the disk diffusion test (Soureshjan and Heidari, 2014). Aqueous extract of *C. sativus* also showed least activity, but hexane and methanol extracts showed a promising antibacterial activity (Hussain *et al*., 2014).

The results of this study reveal selective antibacterial activity of ethanol extracts of *C. alatavicus* with a high phenolic content against gram-positive bacteria *S. aureus*, *B. subtilis* and *B. cereus*.

Conclusions

The results obtained by the phytochemical screening of *C. alatavicus* revealed the predominant content of secondary metabolites in aerial part of the plant. The highest TPC and DPPH-radical scavenging activity and TAA were detected in ethanol and methanol extracts. These results indicated that antioxidant activities *C. alatavicus* are correlated with the amount of phenolic in the plant extracts. Ethanol extract from aerial part compared to the other extracts is the most effective against tested gram-positive strains. Hence, *C. alatavicus* growing in Kazakhstan area may be considered for accessible source of natural active compounds with antioxidant properties as well as antimicrobial activity. Further studies will be done to develop the effective methods of *in vitro* propagation of the valuable endemic species of crocus.

Table 6. Antimicrobial activity of the various extracts from *C. alatavicus*

Plant material	Extracts	Zone of inhibition (mm) ± SD (n=3)							
		E. coli		*B. subtilis*		*B. cereus*		*S. aureus*	
		Concentration (µl)							
		25	50	25	50	25	50	25	50
Aerial part	Water	-	-	-	-	-	-	-	-
	Ethanol	-	-	-	20.03 ± 0.19	-	17.32 ± 0.04	-	21.72 ± 0.17
	Dichloromethane	-	-	-	-	-	-	-	-
Bulb	Water	-	-	-	-	-	-	-	-
	Ethanol	-	-	15.66 ± 0.51	18.01 ± 0.18	18.44 ± 0.07	20.67 ± 0.46	-	18.71 ± 0.12
	Dichloromethane	-	-	-	-	20.03 ± 0.01	-	-	-
Control	70% Ethanol	10.04 ± 0.11		10.33 ± 0,32		14.86 ± 0.89			-
	Dichloromethane	13.08 ± 0,09			-		-		-
	Kanamycin, 20 µl	25.02 ± 0,08		32.91 ± 0,42		14.53 ± 0,61		26.03 ± 0,63	

Note: – = no activity; data are mean of three replications

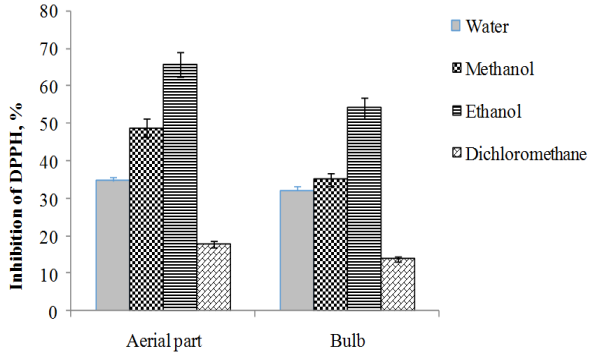

Fig. 1. Scavenging effect of *C. alatavicus* extracts at 500 µg/ml on DPPH radicals

Acknowledgments

This work was supported by the Institute of Plant Biology and Biotechnology (grant number N0113 PK00272) and Al-Farabi KazNU as a part of training programs for doctoral students.

References

Acar G, Dogan NM, Duru ME, Kivrak I (2010). Phenolic profiles, antimicrobial and antioxidant activity of various extracts of *Crocus species* in Anatolia. African Journal of Microbiology Research 4(11):1154-1161.

Agnihotri VK (2015). *Crocus sativus* Linn: An Informative Review. Aperito Journal of Advanced Plant Biology 1(1):1-29.

Baba SA, Malik AH, Wani ZA, Mohiuddin T, Shah Z, Abbas N, Ashraf N (2015). Phytochemical analysis and antioxidant activity of different tissue types of *Crocus sativus* and oxidative stress alleviating potential of saffron extract in plants, bacteria, and yeast. South African Journal of Botany 99:80-87.

Baytenov MS (2001). Flora Kazakhstana [Flora of Kazakhstan]. Gylym, Alma-Ata.

Bhargava VK (2011). Medicinal uses and pharmacological properties of *Crocus sativus* Linn (Saffron). International Journal of Pharmacy and Pharmaceutical Sciences 3(3):22-26.

Chryssanthi DG, Lamari FN, Iatrou G, Pylara A, Karamanos NK, Cordopatis P (2007). Inhibition of breast cancer cell proliferation by style constituents of different *Crocus* species. Anticancer Research 27:357-362.

Duke JA, Ayensu EE (1985). Medicinal plants of China. Reference Publications, Algona.

Egamberdieva D, Mamadalieva N, Khodjimatov O, Tiezzi A (2012). Medicinal Plants from Chatkal Biosphere Reserve Used for Folk Medicine in Uzbekistan. Medicinal and Aromatic Plant Science and Biotechnology 7(1):56-64.

Ferrara L, Naviglio D, Gallo M (2014). Extraction of Bioactive Compounds of Saffron (*Crocus sativus* L.) by Ultrasound Assisted Extraction (UAE) and by Rapid Solid-Liquid Dynamic Extraction (RSLDE). European Scientific Journal 10(3):1-13.

Golmohammadi F (2014). Saffron and its Farming, Economic Importance, Export, Medicinal characteristics and Various Uses in South Khorasan Province – East of Iran. International Journal of Farming and Allied Sciences 3(5):566-596.

Golovkin BN, Rudenskaya RN, Trofimova IA, Shreter AI (2001). Biologicheskie aktivnye veshestva rastitelnogo proiskhozhdeniya [Biologically active substances of plant origin]. Nauka (2nd Ed), Moscow.

Harborne JB (1984). Phytochemical methods. Chapman and Hall (2nd Ed), London, NewYork.

Harpke D, Meng S, Kerndorff H, Rutten T, Blattner FR (2013). Phylogeny of *Crocus* (Iridaceae) based on one chloroplast and two nuclear loci: ancient hybridization and chromosome number evolution. Molecular Phylogenetics and Evolution 66(3):617-627.

Hosseinzadeh H, Nassiri-Asl M (2013). Avicenna's (Ibn Sina) the canon of medicine and saffron (*Crocus sativus*): A review. Phytotherapy Research 27:475-483.

Hussain S, Haq A, Nisar M, Ahmad T, Bhardwaj P (2014). Evaluation of *In-vitro* Anti-Mycobacterial Activity and Isolation of Active Constituents from *Crocus sativus* L. (Iridaceae). Asian Journal of Medical and Pharmaceutical Researches 4(2):130-135.

Ivaschenko AA (2005). Tulips and other bulbs plants of Kazakhstan. Printing house "Two Capitals", Almaty.

Kamenetsky R, Okubo H (2013). Ornamental geophytes: from basic science and to sustainable production. CRC Press, New York.

Karimi E, Oskoueian E, Hendra R, Hawa ZE Jaafar (2010). Evaluation of *Crocus sativus* L. stigma phenolic and flavonoid compounds and its antioxidant activity. Molecules 15:6244-6256.

Kukushkina TA, Sedelnikova LL (2010). Dinamika nakopleniya zapasnykh veshestv v klubnelukovisakh *Crocus alatavicus* i *Gladiolus hybridus* [Dynamic of accumulation of reserve substances in bulbs of *Crocus alatavicus* and *Gladiolus hybridus*]. Chemistry of Plant Raw Materials 2:123-126.

Kulisic T, Radonic A, Katalinic V, Milos M (2004). Use of different methods for testing antioxidative activity of oregano essential oil. Food Chemistry 85:633-640.

Monte CD, Bizzarri B, Gidaro MC, Carradori S, Mollica A, Luisi G, Granese A, Alcaro S, Costa G, Basilico N, Parapini S, Scaltrito MM, Masia C, Sisto F (2015). Bioactive compounds of *Crocus sativus* L. and their semi-synthetic derivatives as promising anti-Helicobacter pylori, anti-malarial and antileishmanial agents. Journal of Enzyme Inhibition and Medicinal Chemistry 30(6):1027-1033.

Nørbæk R, Brandt K, Nielsen JK, Ørgaard M, Jacobsen N (2002). Flower pigment composition of *Crocus* species and cultivars used for a chemotaxonomic Investigation, Biochemical Systematics and Ecology 30:763-791.

Parejo L, Codina C, Petrakis C, Kefalas P (2000). Evaluation of scavenging activity assessed by Co (II)/EDTA-induced luminol chemiluminescence and DPPH center dot (2.2-diphenyl-1-picrylhydrazyl) free radical assay. Journal of Pharmacological and Toxicological Methods 44:507-512.

Parray JA, Kamili AN, Hamid R, Reshi ZA, Qadri RA (2015). Antibacterial and antioxidant activity of methanol extracts of *Crocus sativus* L. c.v. Kashmirianus. Frontiers in Life Science 8(1):40-46.

Pintado C, Alicia de Miguel, Acevedo O, Nozal L, Novella JL, Rotger R (2011). Bactericidal effect of saffron (*Crocus sativus* L.) on *Salmonella enterica* during storage. Food Control 22:638-642.

Rahaiee S, Hashemi M, Moini S, Abbas Shojaosadati S, Razavi SH (2015). Comparison of phytochemical constituents and antioxidant activities of aqueous and alcoholic extracts of saffron. Quality Assurance and Safety Crops and Foods 7(4):521-529.

Rezaee R, Hosseinzadeh H (2013). Safranal: from an aromatic natural product to a rewarding pharmacological agent. Iranian Journal of Basic Medical Sciences 16(1):12-26.

Samarghandian S, Borji A (2014). Anticarcinogenic effect of saffron (*Crocus sativus L.*) and its ingredients. Pharmacognosy Research 6(2):99-107.

Sanja SD, Sheth NR, Patel NK, Patel D, Patel B (2009). Characterization and evaluation of antioxidant activity of *Portulaca oleracea*. International Journal of Pharmacy and Pharmaceutical Sciences 1(1):74-84.

Sariri R, Sabbaghzadeh R, Poumohamad F (2011). *In-vitro* antioxidant and anti-tyrosinase activity of methanol extracts from *Crocus sativus* flowers. Pharmacologyonline 2:1205-1215.

Saxena RB (2015). Botany, Taxonomy and cytology of *Crocus orientales*-series. Asian Journal of Science and Technology 6(5):1406-1410.

Sedelnikova LL, Kukushkina TA (2014). O soderzhanii askorbinovoi kisloty i saponinov u dekorativnykh rastenii [Ascorbic acid and saponin contents of ornamental plants]. In: Bazarnova NG, Markyna BI (Eds). Proceedings of the 6th All-Russian Conference on new advances in chemistry and chemical technology of plant raw materials. Publishing house of the Altai State University, Barnaul, Russia pp 287-239.

Simbar M, Shadipour M, Salamzadeh J, Ramezani-Tehrani F, Nasiri N (2015). The combination of "*Pimpinella anisum, Apium graveolens* and *Crocus sativus* (PAC)" is more effective than "mefenamic acid" on postpartum after-pain. Journal of herbal medicine 5:20-25.

Singh B, Sahu PM, Sharma MK (2002). Anti-inflammatory and antimicrobial activities of triterpenoids from *Strobilanthes callosus* Nees. Phytomedicine 9(4):355-359.

Soureshjan EH, Heidari M (2014). *In vitro* variation in antibacterial activity plant extracts on *Glaucium elegans* and saffron (*Crocus sativus*). Bangladesh Journal of Pharmacology 9:275-278.

Stratil P, Klejdus B, Kuban V (2007). Determination of phenolic compounds and their antioxidant activity in fruits and cereals. Talanta 71:1741-1751.

Tokgun O, Akca H, Mammadov R, Aykurt C, Deniz G (2012). *Convolvulus galaticus*, *Crocus antalyensis* and *Lilium candidum* extracts show their Antitumor activity through induction of p53-mediated apoptosis on human breast cancer cell line MCF-7 Cells. Journal of Medicinal Food 15(11):1000-1005.

Türkoğlu S, Çelik S, Türkoğlu I, Çakılcıoğlu U, Bahşi M (2010). Determination of the antioxidant properties of ethanol and water extracts from different parts of *Teucrium parviflorum* Schreber. African Journal of Biotechnology 9(40): 6797-6805.

Wettasinghe M, Shahidi F (1999). Antioxidant and free radical scavenging properties of ethanolic extracts of defatted borage (*Borago officinalis* L.) seeds. Food Chemistry 67:399-414.

Zhang Y, Tan D (2009). Breeding system and pollination biology of *Crocus alatavicus* (Iridaceae) geocarpic subalpine plant of the western Tianshan Mountain. Biodiversity Science 17(5):468-475.