Plant Archives Vol. 18 No. 1, 2018 pp. 357-360 *ISSN 0972-5210*

BIOACTIVITY OF CROCIN PIGMENT OF SAFFRON PLANT

Rajaa A. Hussein*, Nada A. Salih[1] and Eman Thabit N.

Department of Clinical-Laboratory Sciences, College of Pharmacy, Kufa University, Al-Najaf, Iraq.
[1]Department of Pharmaceutical Chemistry, College of Pharmacy, Kufa University, Al-Najaf, Iraq.

Abstract

The present study was carried ot to investigate bioactivity activity of Crocin carotenoid pigment, which obtained from the stigma of *Crocus sativus* L. plant. It characterized by thin layer chromatography TLC, in UV-visible and IR spectroscopy, antimicrobial activity detected by disc diffusion method and antioxidant activity using DDPH scavenging assay.

The results of Crocin extract showed significant inhibitory activity against selected strains of bacteria (*Staphylococcus aureus* and *Escherichia coli*) with inhibition zones values ranging from 20 to 36 mm at the concentration of 100 µg/ml as compared with standard antibiotics chloramphenicol and ciprofloxacin.

Crocin pigment exhibited appreciable *in vitr*o antioxidant activity (at a concentrations of 50-400 µg/ml)as assessed by 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) radical scavenging as compered with ascorbic acid. It had free radical scavenging capacity at 300 µg/ml (65.7%, IC50 202 µg/ml) close to that of the positive ascorbic acid drug (71%, IC50 178 µg/ml) in the DDPH assay.

Key words : Antimicrobial activity, DDPH antioxidant activity assay, crocin, saffron plant.

Introduction

Crocus sativus L., commonly known as saffron, is used in traditional medicine for various tenacities**.** The word "saffron" is derived from the Arabic word za'faran, which translates to "yellow." a name imitating the high concentration of carotenoid pigments existent in the saffron flowers' stigmas, which subsidize most to the color profile of this spice (Abdullaev, 2009; Heydari and Haghayegh, 2014).

The chemical structure of stigmas of *Crocus sativus* L. has been studied in several studies during the past two decades. Reportedly, stigma of *Crocus sativus* flower comprises three main metabolites; (1) Crocins which are the saffron-colored composites (unusual water-soluble carotenoids due to their high glycosyl contents); (2) Picrocrocins which are the chief substances responsible for saffron's bitter taste; and (3) Safranal which is the volatile oil responsible for the distinctive saffron aroma (Rios *et al*., 1996; Fernandez and Pandalai, 2004; Winterhalter and Straubinger, 2000). Crocin and picrocrocin are the major compounds in saffron. Crocin is responsible for its characteristic color, and picrocrocin is a precursor of safranal (Mayer *et al*., 2009; Nakayama *et al*., 2007). Fig. 1 explains the structure of the most important components of saffron.

The main constituent of saffron is crocin. It is a natural carotenoid and is also found in *Gardenia Jasmenoides*. It is the diester made from gentiobiose and the dicarboxylic acid crocetin. It has a deep red color and forms crystals with a melting point of 146°C. When dissolved in water, it forms an orange solution. It has also been revealed to have an anticarcinogenic action. Crocin (200 mg/kg, i, p.) did not show anticonvulsant activity10. It means crocin must be a good anti-depressant. Chemically it is (*β, β* 'digentiobiosyl 8, 8'diapocarotene 8, 8'oate or crocetin digentiobioside ester (Abdullaev, 2003; Xi *et al*., 2007).

Crocin is the chief pigment in the saffron (approx. 80%), with water as a stationary phase and butanol as mobile phase, Crocin(I) can be isolated in pure form from the saffron extract and directly crystallized. Crocin has shown numerous pharmacological activities such as anti-oxidant, anti-cancer, learning and memory enhancer in medicinal field (Singla and Bhat, 2000). As well its high anti-oxidant activity, the typical orange-red color of its pigment has made it also notable for various industrial presentations such as coloring matter in food and dying

Author for correspondence : E-mail: raja.hussein@uokufa.edu.iq

industry, preservative, bleaching agent for estimation of pro-oxidant action of foods by kinetic analysis and anti-aging agent in cosmetics (Akhondzadeh *et al*., 2008). Pharmacological studies have demonstrated antiepileptic, neuroprotective, anti-diabetic, antioxidant, anti-inflammatory and antinociceptive properties for crocin and safranal (Assimopolou and Papageorgiou, 2005; Nam *et al*., 2010; Omoigui, 2007; Suleyman *et al*., 2007; Tamaddonford *et al*., 2012, 2013; Tamaddonford and Farshid, 2012; Tamaddonford and Hamzeh-Gooshchi, 2010).

Many studies on medicinal properties of saffron had showed that saffron has a potent antioxidant activity which is frequently due to the occurrence of crocin as a unique carotenoid. The crocin bleaching test was also proposed according to this main property of crocin as a basic constituent for the antioxidant activity of saffron (Schmidt *et al*., 2007; Liu *et al*., 2005). It was shown that the antioxidant properties of both methanol extract and water-methanol (50:50 v/v) extract of *Crocus sativus* stigmas were higher than those of tomatoes and carrots (Akhondzadeh *et al*., 2008).

As a part of our current investigations about natural antioxidants from local medicinal plants, in this research, we analyzed carotenoid crocin compound present in *Crocus sativus stigmas* using crystallization extraction and estimated antibacterial and antioxidant activities *in vitro* by DPPH radical scavenging test in order to contribute to shift the balance from synthetic antioxidant towards plant–derived antioxidants.

Materials and Methods

Plant material

The sample of stigmas of *Crocus sativus* plant were obtained from local market was properly identified authenticated on the basis of literary description as well as modern literature.

Extraction of Crocin from Saffron stigmas

Crystallization procedure was carried out in two steps at different temperatures for the extraction of crocin from dried powder of saffron stigmas (Hosseinzadeh *et al*., 2012). Among the solvents 80% ethanol was the most convenient for crocin extraction from saffron stigmas. Saffron stigmas powders (10 g) were suspended in 25 mL ethanol (80%) at 0°C and shaked by vortex for 2 min. After centrifugation at 4000 rpm for 10 min the supernatant was separated. Twenty five millilitre of water was added to sediment and extraction was repeated again. This step was repeated 6 other times. The total volume of solvent consumption for 10 g saffron stigmas

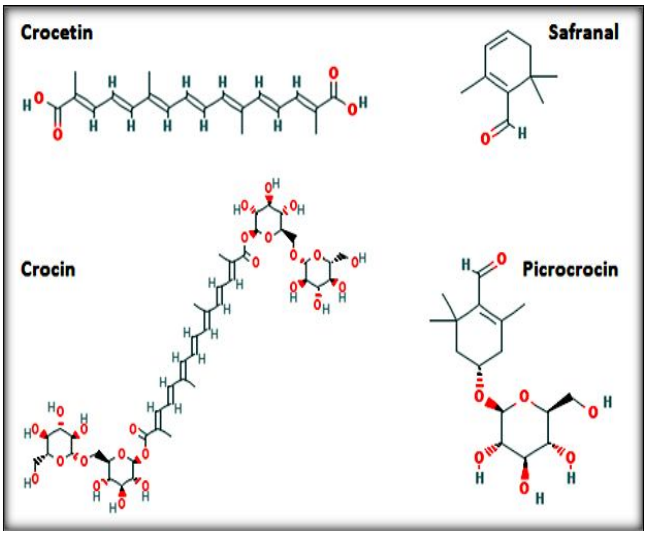

Fig. 1 : Chemical composition of the most active constituents of saffron.

in extraction process was 200 mL (8×25 mL). The resulting solution was kept in a thick walled glass container at -5°C for 24 days in darkness. The container was sealed in this period. The obtained crystals were separated from solution and washed with acetone to remove remaining water. The yielded amount of crystals was 1.66 gm. In the next step; the gained crystals were dissolved in 120 mL ethanol 80% and kept at -5°C in darkness for 20 extra days for re-crystallization.

Chemical identification

All measurements were determined in Central Laboratory/Kufa University.

I. Thin layer chromatography (TLC)

Chemical constituents of the extract was analyzed using aluminium-backed thin layer chromatography (TLC) plates, that were developed with of the eluent system: butanol: acetic acid: water: 4:1:1 v/v/v [BAW] (polar). Development of the chromatograms was under eluent-saturated conditions. Plant extract sample (100 µg) was applied on the TLC plates in and developed without delay to minimize the possibility of photo-oxidative change. The separated constituents were visualized under visible and ultraviolet light (254 and 360 nm, Camac Universal UV lamp TL-600).

II. Ultra-violet and visible spectroscopy

Plant extract was dissolved in ethanol (80%) to study the UV-visible in range of 200-600 nm, using a computerized thermospectronic.

III. Infrared spectroscopy

Infrared spectra were recorded in KBr pellets via a FT.I.R. Spectrophotometer, in the range 4000-200 cm^{-1}

to find the functional groups of extracted material.

IV. Melting point measurement

Melting point was determined using electro- thermal® melting point apparatus (Gallenhamp, England).

Standard and sample preparation

Stock standard solution (500µg/ml) of each crocin pigment and ascorbic acid drug were prepared in methanol by weighing out approximately 0.050 g of the analyte into 50 ml volumetric flask. The mixed standard solution was prepared by dilution the mixed stock standard solutions in methanol to give a concentration of 50-400 µg/ml for crocin extract and standard ascorbic acid drug. Saffron stigma extract solution was centrifuged at 13000 rpm and supernatant was kept at 20°C till further using .

Antimicrobial assay

Preparation of bacterial cultures

species of Gram-positive bacteria *Staphylococcus aureus* ATCC and Gram-negative bacteria (*Escherichia coli* ATCC 13883 were routinely used for the antimicrobial assay in our laboratory. These specific strains were recommended for antibacterial screening purpose (NCCLS, 1990). The bacteria were sub-cultured on nutrient agar at 37°C prior to overnight growth in nutrient broth. All overnight cultures were standardized using sterile saline to produce approximately 1.5×10^7 colony forming units (cfu) per ml.

Culture media

Muellar Hinton Agar (Himedia, India) was prepared according to the manufacturer's instructions, autoclaved at 15 lbs pressure and 121ºC for required time and dispensed into petridishes more than half. Set plates were incubated overnight at 37ºC to ensure sterility before use.

Disc diffusion method

The antibacterial activity of the extract was carried out by the disc agar diffusion method. Brief, Nutrient agar (MHA) plates were swabbed with the respective broth cultures of the organisms. Sterile filter paper discs (Whatman's No. 1, 6 mm in diameter) were saturated with the appropriate equivalent amount of crocin pigment dissolved in sterile methanol at concentrations of 100 µg/ disc. Control discs impregnated with 10 ml of the solvent methanol (negative control) and 100 µg/disc for each chloramphenicol and ciprofloxacin antibiotics (positive standard antibacterial drug) were used to determine the sensitivity of one strain in each experiment. The plates were incubated at 37°C for 24 h and the antimicrobial activity was estimated by measuring the inhibition zones expressed millimeters (mm) of inhibition against the tested organism (NCCLS, 1990).

Antioxidant activity test

Quantitative analysis for DPPH radical scavenging activity

The free radical scavenging capacity of the extracts was determined using DPPH (Alam *et al*., 2008; Verma and Bordia, 1998; Kamalipour and Akhondzadeh, 2011). DPPH solution was prepared in 95% methanol. Extract of *Crocus sativus* plant was mixed with 95% methanol to prepare the stock solution (500 µg/ml). Freshly prepared DPPH solution (0.004% w/v) was taken in test tubes and saffron extract was added followed by serial dilutions (50 µg/ml to 400 µg/ml) to every test tube so that the final volume was 3 mL and after 10 min, the absorbance was read at 515 nm using a spectrophotometer. Ascorbic acid was used as a reference standard and dissolved in methanol to make the stock solution with the same concentration (50-400 µg/ml). Control sample was prepared comprising the same volume without any extract and reference ascorbic acid. 95% methanol was served as blank. % scavenging of the DPPH free radical was measured using the following equation:

% Scavenging activity = [(Absorbance of the control – Absorbance of the test sample)/Absorbance of the control] × 100.

The inhibition curve was plotted for duplicate investigates and represented as % of mean inhibition ± standard deviation. A curve of percent inhibition or percent scavenging effect against samples concentrations was plotted and the concentration of the sample required for 50% inhibition was determined. The value for each of the test sample was presented as the inhibition curve at 50% or IC50.

Statistical analysis

All investigates were conducted in duplicate and values expressed as mean ± standard deviation. Calculations of antibacterial activity were determined by Standard Deviation and Mean of replicates. Statistical analysis was performed using one way ANOVA and results were compared using the Fisher's least significant difference (LSD) at a 5% significance level.

Results and Discussion

Crocin pigment extraction

The final amount of yielded crystals of ethanol extract from *C. sativus* plant (gm/100gm) was 15% (1.5 gm/ 10gm) with melting point of 146°C. This level was found to be similar to that found in the literatures.

Crocin extract is gained by solvent extraction of the

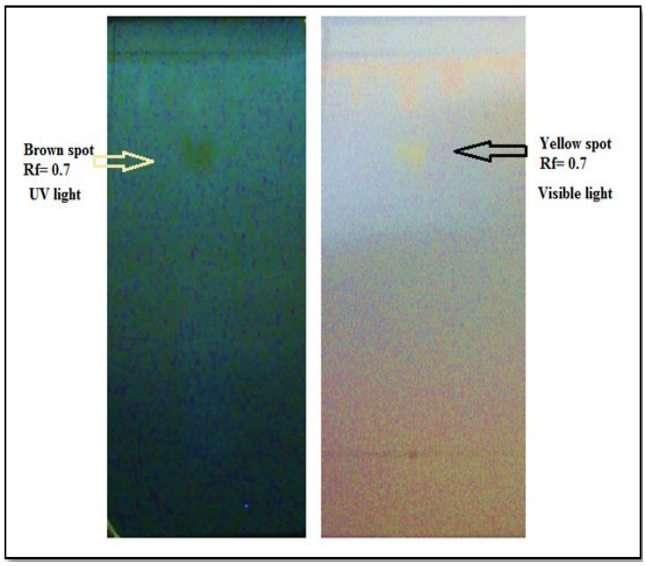

Fig. 2 : Thin layer chromatography plate of crocin under UV and Visible light.

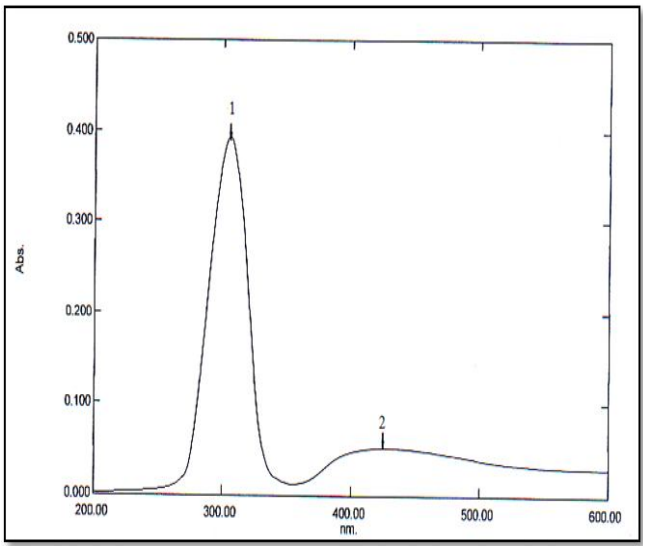

Fig. 3 : UV-Visible of crocin carotenoid pigment of saffron plant.

dried stigma of saffron using ethanol (80%)as a best solvent for crocin pigment extraction. Table 1 shows some chemical and physical properties of extracted pigment.

In earlier studies ethanol 80% have been used for extraction of crocins from saffron. Crocins are soluble carotenoid esters in this solvent a room temperature. Other compounds in saffron stigmas are insoluble or fully soluble at this condition. Therefore, ethanol 80% was applied for extraction of crocins. Insoluble compounds were parted from ethanolic extract by centrifugation. Different temperatures were tested. At lower temperatures than -5 C°, the nucleation and crystal growth happened faster but the purity and quality of product

Table 1: Chemical and physical properties of extracted pigment.

Tests	Properties
Description	***Orange-red***
Solubility	Highly soluble in water, methanol, ethanol, propanol, ethyl acetate and Dimethyl sulfoxide(DMSO), insoluble in hexan
Melting point	(145-146) °C
Weight of extract (Yield)	1.5 gm/ 10 gm dry matter stigmas
Total pigment (%) (Crocin)	15%

declined (Wang *et al*., 2009). The amount of obtained crocin was 15% from the initial stigmas powder, this quantity was nearly to that mentioned in the earlier literatures (Hadizadeh *et al*., 2010).

Thin Layer Chromatography (TLC)

Crocin was identified with the aid of TLC analytical process using (butanol :acetic acid: water) (4:1:1 v/v/v) as developing solvents and literature comparison.

Fig. 2 shows the results of thin layer chromatography of crocin pigment, the separation mode, nature and color in visible light and UV (360 nm) of formed band. TLC chromatogram display single separation spot, that has conjugated double bonds, which appeared as fluorescent spot under UV-lamp (360 nm) with R_f value (0.7). The separation spot was yellow in visible light and dark brown in UV lamp.

Ultraviolet spectroscopy

Fig. 3 shows the UV-visible spectrum of the pigment, the UV spectrum has shown two peaks one at λmax 314nm due to the presence of pairs of electrons types ($n \rightarrow \pi^*$) on O atom and one peak at λmax 425 nm due to the presence of conjugated double bond of C = C type ($\pi \rightarrow \pi^*$) within the structure of compounds found in the extracted pigment.

The chromatophore and thus the spectral characteristics of the compound differ depending on the extent of the system of conjugated double bonds and the various functional groups contained in the molecule, as a result, the color ranges from yellow to red (Britton, 1983; Britton, 1995).

Infrared spectrum

FT-IR spectrum results for the extracted pigment was shown in fig. 4 and table 2, the appearance of a single peak at 3415cm^{-1} related to the vibration stretching for (–OH) bond indicated the presence of alcoholic group,

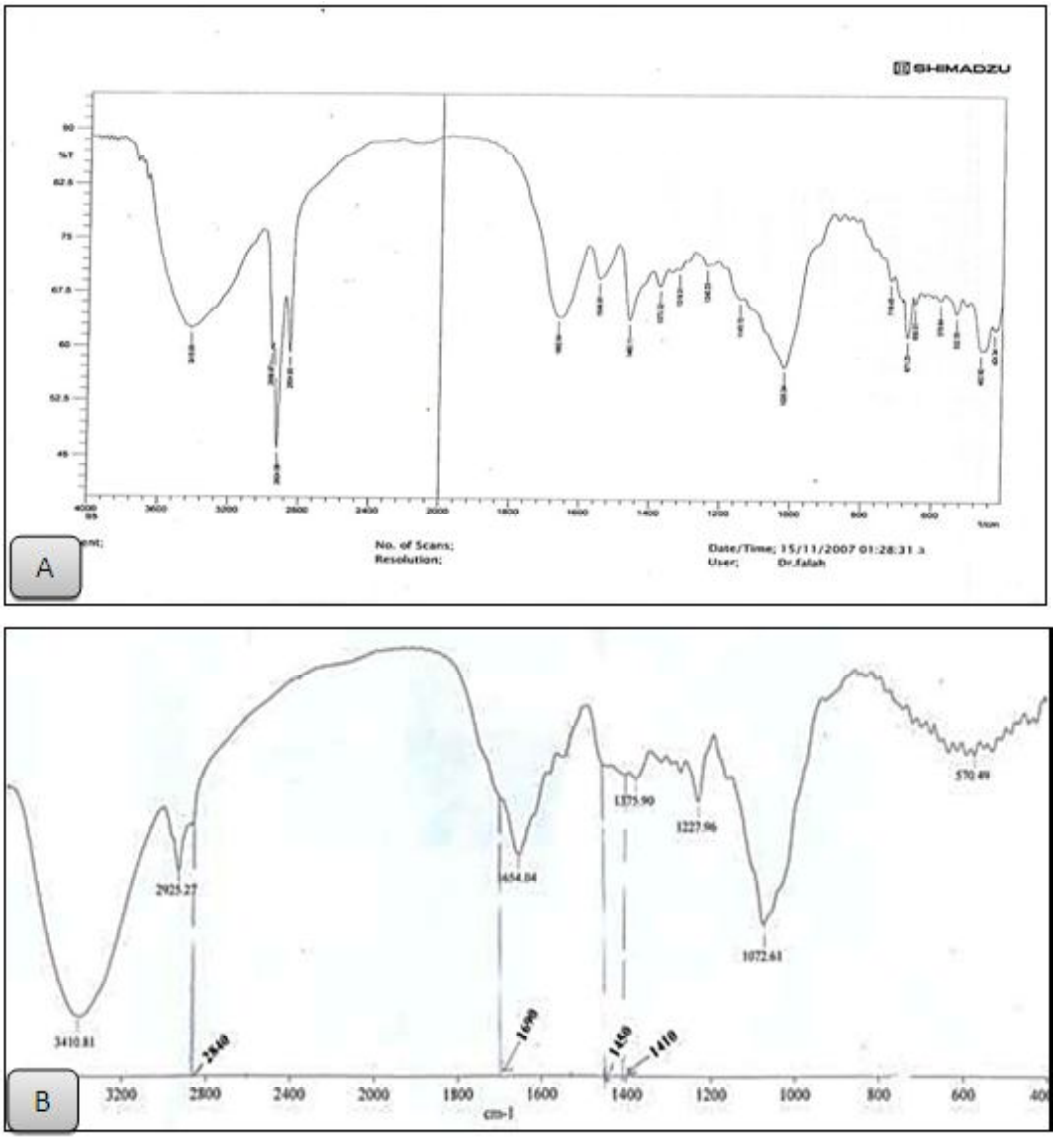

Fig. 4 : Infrared spectrum of the crocin pigment [A]crocin isolated from *C. sativus*, [B] Standard crocin.

The bands at 2924 cm^{-1} and 2854 cm^{-1} were related to the vibration stretching of (C-H) bond of methyl groups a symmetric and symmetric, respectively. Finally, the band at 1662 cm^{-1} is belonged to the vibration stretching for (C=O) bond of ester group due to the presence of carotenoid compound. According the chemical data obtained by IR spectroscopy, the isolation compound was found to be crocin pigment, especially when it compared with standard crocin (fig. 4).

Antibacterial activity

The results are listed in table 3 and fig. 5. Results obtained in the present study relieved that tested medicinal saffron extract possess potential antibacterial activity against all selected bacteria (agar well diffusion method) at the concentration of 100 µg/ml. Crocin pigment methanolic extract showed maximum antibacterial activity against all the bacterial strain used (*Escherichia coli* and *Staphylococcus aureus*) with a zone of inhibition ranges from (20-36) mm. The test results were compared with standard antibiotics Chloramphenicol (100µg) and Ciprofloxacin (100µg).

The zone of inhibition was measured for crocin extract from saffron plant and the results were depicted in table 3 and fig. 5.

It was found that Gram negative bacteria (*Escherichia coli*) were more susceptible (36mm) for the saffron extract than Gram positive *Staphylococcus aureus* (20 mm). The biological activity of present extract was due to crocin, which considered as one of the major carotenoides of saffron plant. The presence of alcoholic groups(-OH) in the structure of the crocin pigment increase the activity of the plant extract to inhibit the

Table 2 : Infrared absorption peaks and their related functional groups for crocin pigment.

Wave number (cm^{-1})	Functional groups assignment
3415	O-H stretching of alcohol(broad band)
2924	C-H stretching of $-CH_3$ and CH_2 groups
2854	C-H stretching of alkane
1662	C=O stretching
1546	Weak band of C=C stretching
1460	C-H blending of CH_2 or CH_3
1373	C-H blending of aldehydeic hydrogen or O-H blending of alcohol

Table 3 : The antibacterial activity of carotenoid pigment.

Compounds	Diameter of Inhibition zones (mm)	
	***Escherichia coli* (NCTC 25922)**	***Staphylococcus aureus* (NCTN 25923)**
Crocin 100 µg/ml	36	20
*Chloramphenicol 100 µg/ml	29	NA
*Ciprofloxacin 100 µg/ml	27	25
**Methanol	*NA*	NA

NA (No activity), *(positive control), **(negative control).

Table 4 : DDPH radical scavenging activity (inhibition %)of crocin pigment. Data was represented as Mean ± SD of duplicate experiments.

Concentrations µg/ml	Inhibition %	
	Crocin extract	Ascorbic acid
	25.5 ± 3.40	28.8 ± 3.55
100 µg/ml	35.7± 3.47	38.2± 4.71
150 µg/ml	41.9± 15.71	48± 16.78
200 µg/ml	49.7± 15.75	51.2± 17.22
250 µg/ml	55.7± 19.71	58.9± 20.50
300 µg/ml	65.7 ± 20.21	71± 27.22
350 µg/ml	71.0± 21.91	77.4±30.21
400 µg/ml	82.5± 32.71	89.7± 40.77
IC 50	202 ± 66.48 µg/ml	178± 43.04µg/ml

microbial growth, so, the alcoholic compounds and their derivatives are considered as antiseptic agents (Dey and Harborne, 1997), which are altering the cell protein nature and increase the permeability of the cell membranes (Feeny, 1998).

Antioxidant activity

In the present study, we have estimated the free radical scavenger activity of ethanolic extract of saffron stigmas *C. sativus* using the DPPH method at the concentrations of 50, 100, 150, 200, 250, 300, 350 and 400 µg/ml as shown in table 4 and fig. 6 and compared with standard ascorbic acid.

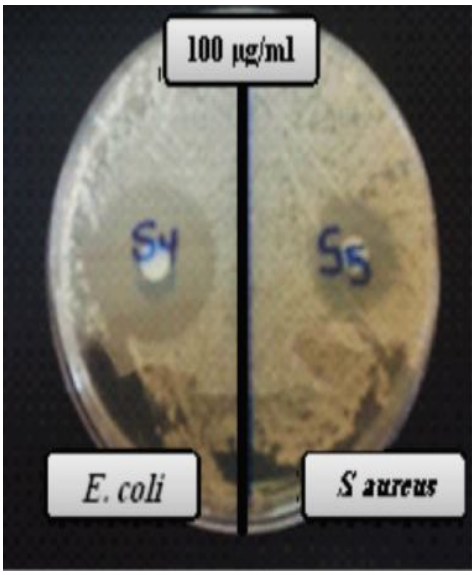

Fig. 5 : The antibacterial activity of carotenoid pigment against *Staphylococcus aureus* and *Escherichia coli* bacteria.

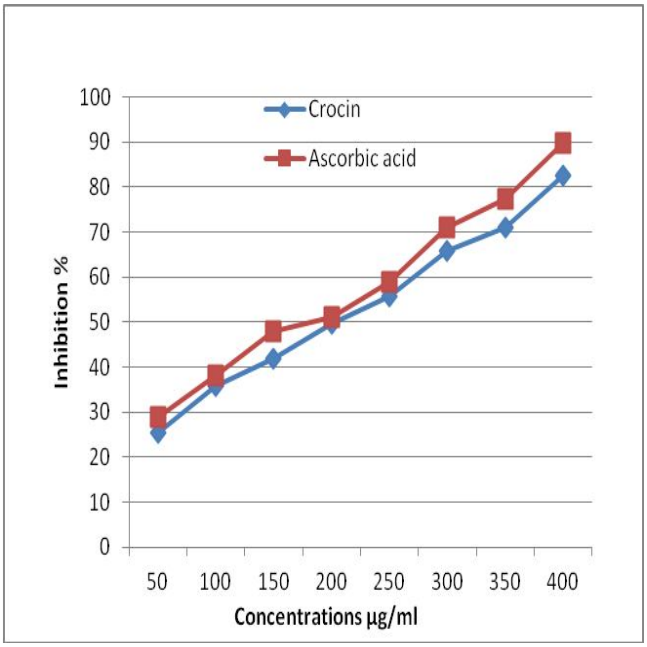

Fig. 6 : DPPH radical scavenging activity of crocin pigment of *Crocus sativus* plant.

The antioxidant activity is presented in fig. 6, which revealed that the percentage inhibition of 300 µg/ml of crocin extract was 65.7% with IC50 of 202 µg/ml, which is comparable with the standard antioxidant activity of ascorbic acid (71%, IC 50 178µg/ml). The results show that the antioxidant activity of crocin was nearly similar

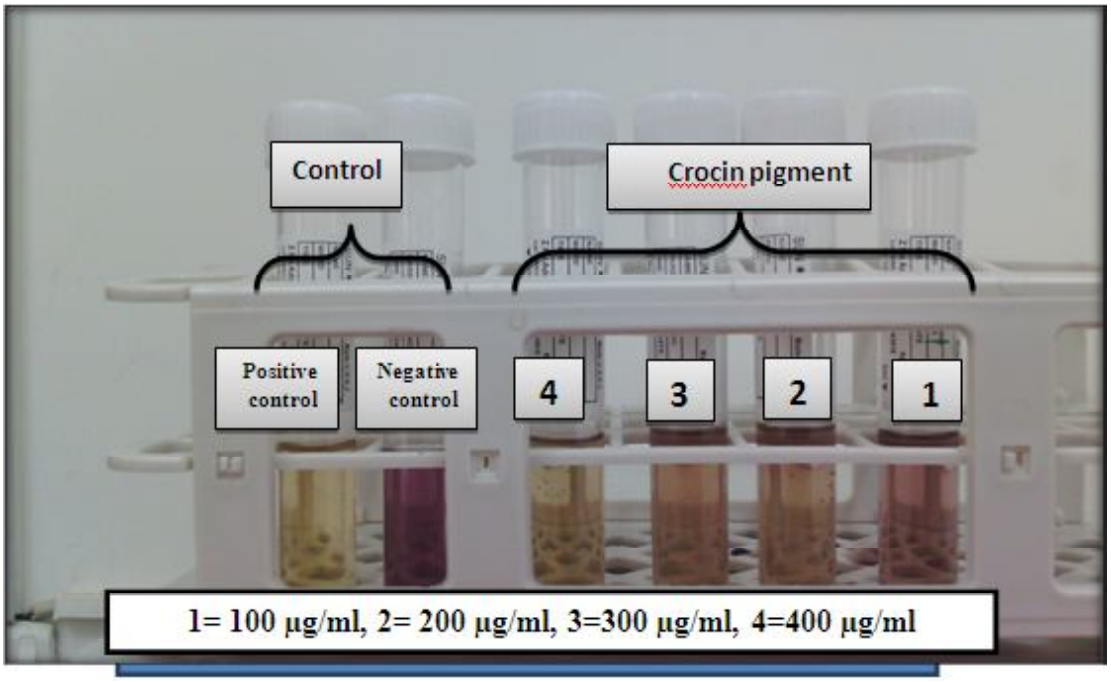

Fig. 7 : Reducing power of crocin saffron extract of *Crocus sativus* plant.

to that of ascorbic acid.

The antioxidant activity was augmented by increasing the concentration of the sample extract. DPPH antioxidant assay is established based on the ability of 1, 1-diphenyl-2-picryl-hydrazyl (DPPH), a stable free radical, to decolorize in the occurrence of antioxidants. The DPPH radical contains an odd electron, which is responsible for a visible deep purple color of DPPH in alcoholic solution and the color strength can be measured at absorbance 515 nm (Caroucho, 2013; Mai *et al*., 2012). When DPPH accepts an electron donated by an antioxidant composite, the DPPH is decolorized, which can be quantitatively measured from the changes in absorbance. Moreover, the DPPH radical scavenging activity has been shown to be directly related with the total phenolic content existing in the extracts as suggested by many previous reports (Andre *et al*., 2010).

The antioxidant activities of spices are chiefly attributed to their phenolic and flavonoid compounds. Saffron is one of the spices believed to possess antioxidant properties. The free radical scavenging and ferric reducing power activities were greater for the methanolic extract of saffron stigma at a concentration of 300 µg/mL, with values of 68.2%, as compared to the corresponding boiling water and ethanolic extracts (Hadizadeh *et al*., 2010). This results were near to the results obtained in the present study.

IC50 (concentration essential to inhibit 50% of DPPH radicals) of extracted crocin was 202 µg/mL and 178 µg/mL for standard ascorbic acid, these results were found to be similar to the results of Hadizadeh *et al*. (2010) they found that the IC50 for methanol, boiling water and ethanol extract were found to be 210.79, 255.44, and 299.44 µg/mL, respectively (Hadizadeh *et al*., 2010). Thus, crocin carotenoid pigment had high potential DPPH radical scavenging activity and was similar to those of synthetic antioxidants.

Conclusion

The significant antimicrobial and antioxidant activities of crocin compound isolated from saffron plant suggest that it could serve as a source of compounds with potent biological importance.

References

Abdullaev, F. (2009). Biological Properties and Medicinal Use of Saffron (*Crocus sativus* L.). *Altern. Med*., **4** : 44-47.

Heydari, S. and G. S. Haghayegh (2014). Extraction and microextraction techniques for the determination of compounds from saffron. *Canad. Chem. Trans*., **2(2)** : 221-247.

Rios, J. L., M. C. Recio, R. M. Giner and S. Manez (1996). An update review of saffron and its active constituents. *Phytother Res*., **10** : 189–193.

Fernández, J.-A. and S. Pandalai (2004). Biology, biotechnology and biomedicine of saffron. *Recent Res. Dev. Plant Sci.*, **2** : 127–159.

Winterhalter, P. and M. Straubinger (2000). Saffron—Renewed interest in an ancient spice. *Food Rev. Int.*, **16** : 39–59.

Mayer, M. A., C. Hocht, A. Puyó and C. A. Taira (2009). Recent advances in obesity pharmacotherapy. *Curr. Clin. Pharmacol*., **4** : 53–61.

Nakayama, T., S. Suzuki, H. Kudo, S. Sassa, M. Nomura and S. Sakamoto (2007). Effects of three Chinese herbal medicines on plasma and liver lipids in mice fed a high-fat diet. *J. Ethnopharmacol*., **109** : 236–240.

Abdullaev, F. I. (2003). Antitumor Effect of Saffron (*Crocus sativus* L.): Overview and Perspectives. In : *Proceedings of I International Symposium on Saffron Biology and Biotechnology* 650, Albacete, Spain, **22–25** : 491–499.

Xi, L., Z. Qian, P. Du and J. Fu (2007). Pharmacokinetic properties of crocin (crocetin digentiobiose ester)following oral administration in rats. *Phytomedicine*, **14** : 633–636.

Singla, R. K. and V. G. Bhat (2000). Crocin: An overview. *Indo Glob. J. Pharm. Sci*., **1** : 281–286.

Akhondzadeh, B. A., S. A. Ghoreishi, A. A. Noorbala, S. H. Akhondzadeh and S. H. Rezazadeh (2008). Petal and stigma of *Crocus sativus* L. in the treatment of depression: A pilot double-blind randomized trial. *J. Med. Plants*, **7** : 29–36.

Assimopolou, A. N., Z. Sinakos and V. P. Papageorgiou (2005). Radical scavenging activity of *Crocus sativus* L. extract and its bioactive constituents. *Phytother. Res.*, **19**:997–1000.

Nam, K. N., Y. M. Park, H. J. Jung, J. Y. Lee, B. D. Min, S. U. Park and W. S. Jung (2010). Anti-inflammatory effects of crocin and crocetin in rats brain microglial cells. *Eur. J. Pharmacol*., **648** : 110–116.

Omoigui, S. (2007). The biochemical origin of pain: the origin of all pain is inflammation and the inflammatory response. Part 2 of 3 – Inflammatory profile of pain syndrome. *Med Hypotheses*, **69** : 1169–1178.

Suleyman, H., B. Demican and Y. Karagoz (2007). Anti-inflammatory and side effects of cyclooxygenase inhibitors. *Pharmacol. Rep*., **59** : 247–258.

Tamaddonfard, E., A. A. Farshid, E. Ahmadian and A. Hamidhoseyni (2013). Crocin enhanced functional recovery after sciatic nerve crush injury in rats. *Iran J Basic Med Sci*., **16** : 83–90.

Tamaddonfard, E., A. A. Farshid, S. Asri-Rezaee, S. Javadi, V. Khosravi, B. Rahman and Z. Mirfakhraee (2013). Crocin improved learning and memory impairments in streptozotocin- induced diabetic rats. *Iran J Basic Med Sci*., **16** : 91–100.

Tamaddonfard, E., A. A. Farshid and L. Hosseini (2012). Crocin alleviates the local paw edema induced by histamine in rats. *Avicenna J Phytomedicine*, **2** : 97–104.

Tamaddonfard, E. and N. Hamzeh-Gooshchi (2010). Effect of crocin on the morphine-induced antinociception in the formalin test in rats. *Phytother Res*., **24** : 410–413.

Tamaddonfard, E. and N. Hamzeh-Gooshchi (2010). Effects of intraperitoneal and intracerebroventricular injection of crocin on acute corneal pain in rats. *Phytother Res*., **24** : 1463–1467.

Tamaddonfard, E., N. Hamzeh Gooshchi and S. Seiednejhad-Yamchi (2012). Central effect of crocin on penicillin-induced epileptiform activity in rats. *Pharmacol. Rep*., **64** : 94–101.

Schmidt, M., G. Betti and A. Hensel (2007). Saffron in phytotherapy: Pharmacology and clinical uses. *Wien. Med. Wochenschr*., **157** : 315–319.

Liu, N., Y. Yang, S. Mo, J. Liao and J. Jin (2005). Calcium antagonistic effects of chinese crude drugs: Preliminary investigation and evaluation by 45ca. *Appl. Radiat. Isot.*, **63** : 151–155.

Hosseinzadeh, H., H. R. Sadeghnia, F. A. Ghaeni, V. S. Motamedshariaty and S. A. Mohajeri (2012). Effects of saffron (*Crocus sativus* L.) and its active constituent, crocin, on recognition and spatial memory after chronic cerebral hypoperfusion in rats. *Phytother. Res*., **26**:381–386.

NCCLS (1990). *Performance for intimicrobial Disc susceptility test*, **4**th ed. Approved Standard. NCCLS Decument M2-A4. The National Committee for Clinical Laboratory Standards, Villanova PA.

Alam, M., A. Ghani, N. Subhan, M. Rahman, M. Haque and M. Majumder (2008). Antioxidant and membrane stabilizing properties of the flowering tops of *Anthocephalus cadamba*. *Nat. Prod. Commun*., **3** : 65-67.

Verma, S. and A. Bordia (1998). Antioxidant property of saffron in man. *Indian J. Med. Sci.*, **52** : 205–207.

Kamalipour, M. and S. Akhondzadeh (2011). Cardiovascular effects of saffron: An evidence-based review. *J. Tehran Univ. Heart Cent.*, **6** : 59–61.

Wang, Y., T. Han, Y. Zhu, C. J. Zheng, Q. L. Ming, K. Rahman and L. P. Qin (2009). Antidepressant properties of bioactive fractions from the extract of *Crocus sativus* L. *J. Nat. Med*., **64** : 24-30.

Hadizadeh, F., S. A. Mohajeri and M. Seifi (2010). Extraction and Purification of Crocin from Saffron Stigmas Employing a Simple and Efficient Crystallization Method. *Pakistan J. of Biol. Scie*., **13(14)** : 691-698.

Britton, G. (1983). *The biochemistry of natural pigments*. Cambridge Univ. Press: Cambridge.

Britton, G. (1995). UV-Visible spectroscopy. In carotenoids; Britton, G., S. Liaagen-Jensen, H. Pfander (eds.) Vol. **1** B : Spectroscopy, Chapter 2.

Dey, P. M. and J. B. Harborne (1997). *Plant biochemistry*. Academic press. London. P. 554.

Feeny, P. (1998). Inhibitory effect of oak leaf tannins and the hydrolysis of proteins by tyrosine. *J. Photochemistry*, **8** : 2116-2129.

Carocho, M. and I. C. Ferreira (2013). A review on antioxidants, prooxidants and related controversy: natural and synthetic compounds, screening and analysis methodologies and future perspectives. *Food Chem. Toxicol.*, **51** : 15-25.

Mai W., D. Chen and X. Li (2012). Antioxidant activity of Rhizoma cibotii *in vitro*. *Adv. Pharm. Bull*., **2(1)** : 107-114.

Andre, C., Y. Larondelle and D. Evers (2010). Dietary antioxidants and oxidative stress from a human and plant perspective: A review. *Curr. Nutr. Food Sci*., **6** : 2-12.