INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print), 2222-5234 (Online)
http://www.innspub.net
Vol. 14, No. 3, p. 438-442, 2019

RESEARCH PAPER OPEN ACCESS

Total phenolic contents of two varieties of *Crocus sativus* and their antioxidant activity

Zafar Iqbal[1*], Mohsan Javed[2], Samina Gull[3], Mian Habib-Ur-Rahman Mahmood[3], Zhang Hai[4]

[1]*Applied Chemistry Research Centre, PCSIR Laboratories Complex, 54000-Lahore, Pakistan*

[2]*Department of Chemistry University of Management and Technology, Lahore, Pakistan*

[3]*Chemistry Discipline, Division of Science & Technology, University of Education, Township Lahore, Pakistan*

[4]*School of Chemistry and Chemical Engineering, Qianbei Featured Resources Applied Research Key Laboratory, Zunyi Normal College, Guizhou Zunyi 563006, China*

Key words: *Crocus sativus*; Extraction; UV absorption; Total phenolic contents; Antioxidant activity.

http://dx.doi.org/10.12692/ijb/14.3.438-442 Article published on March 27, 2019

Abstract

Methanolic extract of two varieties of *crocus sativus* dried stigmas was taken for total phenolic contents and antioxidant activity by using 1,1-diphenyl-2-picryl hydroxide scavenging. UV absorption of both samples separately showed three maximum wavelengths 236 nm and 234 nm (maximum absorbance of picrococine), 320 nm and 308 nm (maximum absorbance of safranal) and 436 nm and 434 nm (maximum absorbance of crocine) respectively. The electrochemical studies showed the oxidation reduction peaks. Total Phenolic content of red and brown saffron by using FC reagent was 5.05 mg and 4 mg of gallic acid equivalent/g of saffron extract respectively. At 50 µL concentration, both red and brown *crocus sativus* showed 71.68% and 73.70% inhibition respectively.

* **Corresponding Author:** Dr ZafarIqbal ✉ zafarmayo2000@yahoo.com

Introduction

A kind of spice, saffron is obtained from *crocus sativus* flower that is mostly known as saffron crocus. It is an utmost advantageous plant amongst the Iridaceae family in the world. About for the last thirty five hundred years saffron crop is being grown as a spice. Saffron's dried stigmas are the much expensive which have been fascinating since beginning, owing to its medicinal benefits (Plessner *et al.*, 1989). Both spice and the plant are commonly known with the name of saffron. The domestic use of saffron dates back two thousand to fifteen hundred years BC as per study of Archeology and history (Grilli, 2004).Its colour is due to crocin, smell is due to safranal and the special bitter taste is due to the presence of glycoside picrocrocine (Basker *et al.,* 1999). Its taste and idoform or hay like scent is due to the chemicals picrocrocine ($C_{16}H_{26}O_7$) that is considered chief sour constituent of *crocus sativus*. Picrocrocine function as a monoterpene glycosides predecessor for safranal ($C_{10}H_{14}O$) that is volatile oil causing aroma (Lozano *et al.,* 2000).

Reactive oxygen species (ROS) are produced by living organisms as a result of normal cellular metabolism and environmental factors, such as air pollutants or cigarette smoke. ROS are highly reactive molecules and can damage cell structures such as carbohydrates, nucleic acids, lipids, and proteins and alter their functions. The shift in the balance between oxidants and antioxidants in favor of oxidants is termed "oxidative stress." Regulation of reducing and oxidizing (redox) state is critical for cell viability, activation, proliferation, and organ function. Aerobic organisms have integrated antioxidant systems, which include enzymatic and non-enzymatic antioxidants that are usually effective in blocking harmful effects of ROS (Kasparova *et al.*, 2005). However, in pathological conditions; the antioxidant systems can be overwhelmed. Oxidative stress contributes too many pathological conditions and diseases, including cancer, neurological disorders, atherosclerosis, hypertension, ischemia/perfusion, diabetes, acute respiratory distress syndrome, idiopathic pulmonary fibrosis, chronic obstructive pulmonary disease, and asthma (Nurul and Asmah, 2012).

Thus the current study was designed and colorants were extracted from the two varieties of crocus stivus and their dyeing properties on the leather were studied.

Materials and methods

The experiment was designed for the analysis of methanolic extract of two varieties of *Crocus sativus* for total phenolic contents and their antioxidant activity.

Collection of sample

The samples of two varieties (yellow and red) of saffron were collected from local market of Lahore (Pakistan). Dried stigmas were used as such for extraction.

Methanolic extract preparation of samples

Methanolic extract of two varieties of saffron were prepared through maceration with methanol. Solid to liquid ratio was 1:5 and it as macerated for 24 hours, followed by washing with *n*-hexane for the removal of any fatty material.

Determination of total phenolic contents (TPC)

Reagents and procedure

All types of solutions which were used to determine the total phenolic content were prepared using distilled water and analytical grade reagents. 1 M (1g/L) stock solution of Gallic acid was prepared in methanol solvent.

From the stock solution five dilutions of 10 parts per million, 20 parts per million, 30 parts per million, 40 parts per million and 50 parts per million were arranged for calibration curves. 0.2 M Folin Ciocalteu reagent and 1 M Sodium carbonate was also prepared using distilled water.

Then 1ml of each extract of saffron red and brown were mixed with 5ml 0.2N Folin-Ciocalteu reagent, after keeping 3 minutes 4ml of 1M Na_2CO_3 solution

was added and kept for 30 minutes. Then both the mixtures were run to the UV-Vis spectrophotometer (Model No Cecile CE 2041, 2000) at 765 nm (Leamsomrong *et al.,* 2009). Absorbance of Gallic acid dilutions were also taken to use as standard for the calibration curve.

Antioxidant propertis of Crocus sativus colorant using 2, 2-diphenyle-1-picrylhydrazyl

The antioxidant studies of natural colorants are commonly determined by the DPPH assay depending on the fact that an antioxidant is hydrogen donor. DPPH is preferably used to determine the scavenging of radical of the composites acting by means of hydrogen acceptor. DPPH is preferred as it is available in stable and commercially available organic nitrogen radicals. Maximum absorbance of DPPH was reported at 517 nm, higher the rate of disappearance of DPPH greater the antioxidant activity of the sample compound. By the absorption of hydrogen from the oxidant 2, 2-diphenyl-1-picrylhydrazyl formed resulting color change from purple to yellow (Sanja, 2009).

To prepare 0.04 % solution DPPH (0.04g) was dissolved in 150 ml of methanol (Espin *et al*., 2000). Five samples having concentration dilutions of 10 microliter, 20 microliter, 30 microliter, 40 microliter and 50 microliter using methanol as solvent of both red and brown saffron were prepared and each was kept in open air for 30 minutes.

First of all a blank reading using only DPPH was taken at ƛ max 517 nm using UV-Visible spectrophotometer (Model No Cecil CE 2041, 2000 series).

Then the readings of different samples with different dilutions named as 1,2,3,4 and 5 were also recorded at ƛ max 517 nm. The property to scavenge the radicle was found the equation given below:

Percent Inhibition = $[(A_B\text{-}A_S)/A_B] \times 100$

A_B = absorbance of control at 30 minute.

A_S = absorbance of sample at 30 minute.

Results and discussion

Two varieties of *Crocus sativus* (red and brown) were collected and the dyes were extracted through maceration using methanol as solvent and washing with *n*- hexane. These dyes were stored in dark at room temperature.

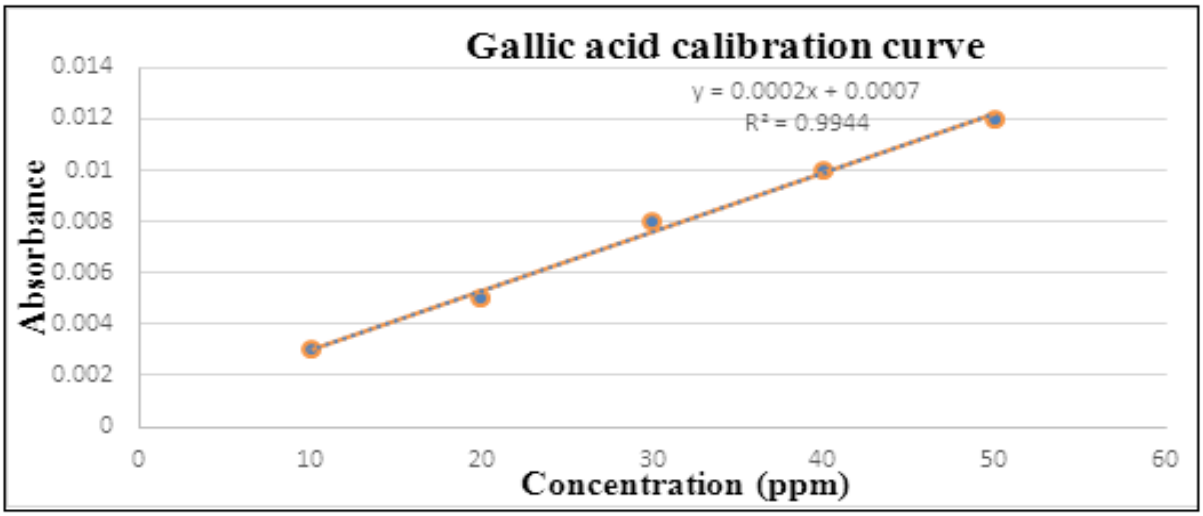

Fig. 1. Gallic acid calibration curve.

Total phenolic contents (tpc)

Total phenolic content measurement was done by using Folin-ciocalteu reagent. Total phenolic content was calculated with respect of gallic acid standardization graph and outcomes were communicated in milligram of gallic acid corresponding per gram of extract of threads of *Crocus sativus*.

Absorbance of Red *Crocus sativus* = 0.00171
Absorbance of Brown *Crocus sativus* = 0.0015

Total Phenolic Content of red and brown saffron sample was calculated 5.05 mg and 4mg gallic acid equivalent/g of extract of respective *crocus sativus* stigmas. In a study total phenolic component was reported 6.55 mg gallic acid equivalent that was higher than the values obtained from the ethanolic and boiling water.

The methanol appeared to be the best solvent to extract the active components (Karimi *et al.*, 2010).

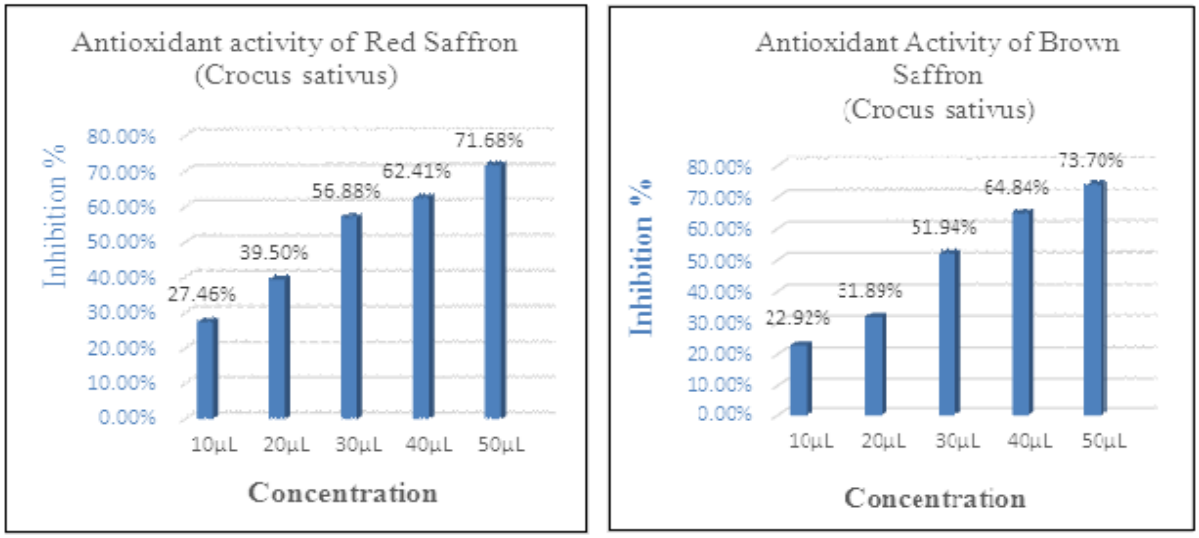

Fig. 2. Antioxidant activity of Red and Brown saffron (*Crocus sativus*) Blank reading = 0.9925.

Antioxidant activity of Crocus sativus colorant

Antioxidant activity of methanolic extracts of both varieties of saffron was measured by using DPPH free radical scavenging activity.

At 10µL concentration both red and brown *crocus sativus* showed 27.455% and 22.921% inhibition at 20µL, 39.50% and 31.89% at 30µL,56.88% and 51.94% and at 40µL, 62.41% and 64.84% respectively. Up to 50µL concentration both showed 71.68% and 73.702% inhibition respectively. High antioxidant activity represented the least activity of vitamin C or ascorbic acid. SA Ordoudi *et al.*, 2009 reported antiradical capacity and bioactive compounds of saffron using FC reagent, different free radical species produced in cell model system. The free radical scavenging and ferric reducing power activities in methanolic extract was found higher at concentration of 300µg/mL with the value of 68.2% and 78.9%.

Conclusion

Two natural colorants of red and brown saffron were selected and dyes were extracted through maceration using methanol as solvent. The dyes of red and brown saffron were applied on wet blue goat leather by using mordant potassium dichromate, sodium dichromate and formic acid. The colorant were applied to the leather surface and its color fastness to mild washing evaluation was done according to standard procedure. Color fastness to mild washing evaluation showed almost no color change in red *crocus sativus* and same with sodium dichromate (4) and slight color change (3-4) in all other samples which showed the retaining capacity of colorant at leather surface. The best results were represented that of red saffron with and without mordant as compare to brown saffron.

References

Basker D, Negbi M. 1999. The uses of saffron.Economic Botany **3,** 228-236.

Espin JC, Soler-rivas C, Wichers HJ. 2000. Characterization o the total free radical scavenging capacity of vegetable oils and oil fractions using 2,2-

diphenyl-1-picrylhydrazyl radical. Journal of Agriculture and Food Chemistry **48,** 648-656.

Grilli CM. 2004. Saffron reproductive biology.Acta Horticulture **650,** 25-37.

Inayat A, Khan SR, Waheed A, Deeba F. 2010. Applications of eco-friendly natural dyes on leather using different modrants. Proceeding Pakistan Academy Science **47,** 131-135.

Karimi E, Oskoueian E, Hendra R, Jaafar HZE. 2010. Evaluation of *Crocus sativus* L. stigma phenolic and flavonoid compounds and its antioxidant activity. Molecules **15,** 6244–6256.

Kasparova S, Brezova V, Valko M, Horecky J, Mlynarik V, Liptaj T, Dobrota D. 2005. Study of the oxidative stress in a rat model of chronic brain hypoperfusion. Neutochemistry International **46,** 601-61.

Learsomrong K, Suttajit M, Chantiratikul P. 2009. Flow injection analysis system for the determination of total phenolic compounds by using folin-ciocalteu assay. Asian Journal of Applied Sciences **2,** 184-190.

Lozano P, Castellar M, Simancas M, Iborra L. 1999. Quantitative high-performance liquid chromatographic method to analyse commercial saffron (Crocus sativus L.) products. Journal of Chromatography **830,** 477-483.

Musa AE, Madhan B, Madhulatha W, Raghava RJ, Gasmelseed GA, Sadulla S. 2009. Coloring of leather using henna-natural alternative material for dyeing. The Journal of the American Leather Chemists Association **104,** 183-190.

Onem E, Gulumser GS, Ocak B. 2011. Evaluation of natural dyeing of leather with rubiatinctorum Extract. Ekoloji Dergisi **20,** 81-87.

Ordoudi SA, Befani CD, Nenadis N, Koliakos GG, Tsimidou MZ. 2009. Further examination of antiradical properties of *crocus sativus* stigmas extract rich in crocins. Journal of Agriculture Food Chemistry **57,** 3080–3086.

Paschal FD, Danladi AA, Myek B, Sunday DJ, Joshua I, Ferdinand N. 2015. Extraction of dyestuff from justiciacarnea hooker and its application in the dyeing of wool, leather and cotton, International Journal of Mordern Chemistry **7,** 81-90.

Plessner O, Negbi M, Ziv M, Basker D. 1989. Effects of temperature on the flowering of Crocus (*Crocus sativus L.):* Induction of hysteranthy. Israel Journal of Botany **38,**1-7.

Sanja SD, Sheth N, Patel D, Patel B. 2009. Characterization and evaluation of antioxidant activity of Portulacaoleracea. International Journal of Pharmacy and Pharmaceutical **1,** 31-36.

Sivakumar V, Anna JL, Vijayeeswarri J, Swaminathan G. 2009. Ultrasound assisted enhancement in natural dye extraction from beetroot for industrial applications and natural dyeing of leather. UltrasonicsSonochemistry**16,** 782-789.

Nurul SR, Asmah R. 2012. Evaluation of antioxidant properties in fresh and pickled papaya. International Food Research Journal **19,** 1117-1124.