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Original research

Antioxidant capacity and chemical composition of different parts of saffron flowers

Akram Azghandi Fardaghi[a], Ali Es-haghi[a,*], Javad Feizy[b], Rajasekhar Lakshmipathy[c]

[a] *Department of Biology, Mashhad Branch, Islamic Azad University, Mashhad, Iran*
[b] *Department of Food safety and quality control, Research Institute of Food Science and Technology, Mashhad, Iran*
[c] *Department of Chemistry, KCG College of Technology, Chennai, Tamil Nadu, India – 600097*

ABSTRACT

Saffron is a spice derived from the flower of *Crocus sativus* L. The present study reports the antioxidant capacity and chemical compositions of various parts of saffron. The IC50 for stigma, style, stamen, petal and corm was found to be 4.94 ± 0.25, 123.68 ± 8.52, 58.97 ± 3.63, 46.02 ± 2.89 and 720.49 ± 14.32 µg/mL, respectively. The ferric reducing antioxidant power assay for these parts of saffron flower was determined and the stigma of saffron has the highest (2.36 ± 0.15 g Fe^{+2}/100 g sample) and the corm of saffron has the lowest value (0.03 ± 0.001 g Fe^{+2}/100 g sample). The Folin-Ciocalteu method was used to estimate the total phenolic content and the highest amount was found in stigma (183.25 ± 16.42 g quercetin/100 g sample) and the lowest amount in style of saffron (3.72 ± 0.89 g quercetin/100 g sample) respectively. The stigma and stamen of saffron were found to have 597.67 ± 11.12 and 159.14 ± 5.03 µg/g sample of carotenoid content which is highest among the selected parts of saffron. The aim of this study was to provide information about all of the saffron flower parts.

Keywords: DPPH; GC-MS; Petal; Saffron; Stamen

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1. Introduction

Herbal medicines have been an extremely important source for the discovery of many drugs (Abou-Arab & Abou Donia, 2001). The presence of biologically active molecules and substances such as antioxidant and antimicrobials have made the medicinal plants and spices a desirable choice for cosmetics, food supplements, drugs and religious rituals (Srinivasan, 2014). *Crocus sativus* L. belonging to the *Iridaceae* family and commonly known as saffron is one of the popular spice among the others (Peter, 2012). *Crocus sativus* plants are grown globally and Iran and Spain top the list. (Haghighi et al., 2007). According to the Iranian Ministry of Agriculture, Iran are the leading producer and exporter of saffron with 230 t (93.7% of world production) in 2005 with an export value of $100 million (Ghorbani, 2008). High quality saffron is produced in Khorasan Razavi province of Iran which is also the largest among other provinces. Saffron is cultivated almost exclusively for its stigma, which is the world's most expensive spice by weight (Serrano-Díaz et al., 2013). The various components of saffron is shown in Fig. 1, each flower is composed of six violet-colored petals, three yellow stamens and a white filiform style culminating in a red stigma divided into three threads (Ulbricht et al., 2011).

The existence of three main secondary metabolites such as crocin, picrocrocin and safranal determines the value of saffron (dried stigmas of *Crocus sativus* L.). The main secondary metabolites are responsible for color, taste and odor of the dried saffron. Indeed, saffron possesses ample volatiles and aroma yielding compounds such as carotenoids, including zeaxanthin, lycopene, β-carotenes and polysaccharides. The presence of α-crocin is primarily responsible for golden yellow orange color of saffron (Abe & Saito, 2000; Khoshbakht Fabim et al., 2012). Saffron plant requires ambient light soil fields in order to retain their properties and exposure to poor weather conditions for a significant time might result in loss of their valued properties. Traditionally, the lower parts of its corolla are collected manually and post-harvest, a delicate process is adopted to produce saffron spice. In Iran, each kilogram of saffron flower contains 2200-2400 numbers. Each kilogram of saffron flower contains 50-55 g fresh

*Corresponding author.
E-mail address: ashaghi@gmail.com (A. Eshaghi).
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and 10-12 g dried stigma and 28-30 g fresh and 3-4 g dried style (Kafi et al., 2018).

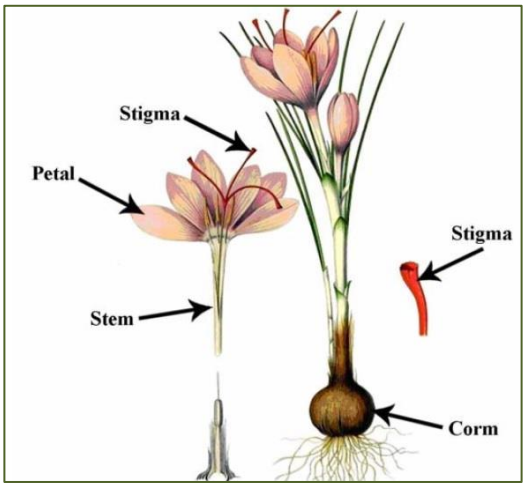

Fig. 1. Different parts of saffron *crocus sativus* L. plant.

The aim of this study is to understand the chemical compositions of stigmas, stamens, styles, petals and corms from *C. sativus* cultivated in Iran (Roshtkhar, Khorasan province). It is worth noting that these samples are related to a same farmland. The samples were evaluated for their DPPH radical, total phenolic content, FRAP assay, flavonoid and carotenoid contents and chemical compositions. There are enough papers citing the antioxidant activity of saffron metabolites, however, the other plant parts particularly the vegetative tissues are to be explored for its benefits.

2. Material and Methods

2.1. Plant material

Chemical free saffron plants cultivated in the region of Roshtkhar (Khorasan Razavi, Iran) were donated by a local farmer. Saffron flowers were picked manually in the month of October 2018 and different parts of the flower were separated. Collection of the corm of saffron was performed in the month of May 2019. Segregated parts of the flower were dried at room temperature in the dark and later dried samples were ground to a fine powder and stored in a refrigerator at 4 ℃ until ready for extraction.

2.2. Chemicals

Sodium carbonate, gallic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH), sodium acetate, quercetin, aluminum chloride, 2,4,6-tris (1-pyridyl)-5-triazine (TPTZ), ferrous sulfate, iron (III) chloride, hydrochloride acid and Folin–Ciocalteau phenol reagent were purchased from Sigma–Aldrich GmbH (Sternheim, Germany). Reagent-grade hexane, ethyl acetate, methanol, ethanol and acetone were from Panreac (Barcelona, Spain). Potassium acetate was purchased from Merck (Darmstadt, Germany). All other chemicals and solvents were of the analytical grade and obtained from Merck.

2.3. Sample preparation

For DPPH, 50 mg of powdered of stigma, style, stamen, petal and corm of saffron was weighted and was added 10 mL of methanol and stirred for 30 min in dark. The solution was filtered with filter paper. For FRAP assay, the total flavonoid content and the total phenolic content of 500 mg of ground powdered of stigma, styles, stamens, petals and corms of saffron were extracted by stirring in 25 mL of ethanol/water 70/30 (v/v) for 48 h in the dark. Later the supernatants obtained after centrifugation at 2800 ×g for 10 min were filtered (0.45 µm) and the extracts were freeze dried at -20 °C in sealed tubes.

2.4. DPPH radical-scavenging activity

A Shimadzu UV-1700 Pharma spec. (Tokyo, Japan) was used for the determination of polyphenolic compounds, phosphorus and antioxidant activity. Spectrophotometer equipped with a standard 10 mm path length spectrophotometer cell. The antioxidant capacity of the extracts were performed by 1,1-diphenyl-2- picryl hydrazyl (DPPH) free radical method described by Sánchez-Vioque et al. (Sánchez-Vioque et al., 2012). Two mL samples extract of style, stamen, petal and corm of saffron with concentrations of 25, 50, 100, 150, 200 and 250 mg/mL were mixed with 2 mL of 0.004% DPPH in methanol, incubated 1 h at room temperature, and the absorbance measured at 517 nm. The concentrations for stigma were 0.1, 0.2, 0.4, 0.6, 0.8 and 1 mg/mL. The mixture of methanol and DDPH were used as control and scavenging activity was calculated with the below formula:

$$\text{DPPH scavenging activity (\%)} = \frac{A_0 - A_1}{A_0} \times 100 \quad (1)$$

where A_0 was the absorbance of the negative control and A_1 was the absorbance of the extract of sample. The concentration of sample required to reduce 50% of DPPH radicals (IC_{50}) was calculated from linear regression analysis.

2.5. FRAP assay

The reducing power of the ethanolic extracts of saffron was determined by mixing freshly prepared 1.8 mL of FRAP reagent and 0.2 mL of sample solutions and standards. For the reagent blank, 1.8 mL of FRAP reagent was mixed with 0.2 mL of distilled water. The incubation of test samples and reagent blank was carried out at 37 ℃ for 30 min in a water bath. The FRAP reagent contained 20 mL of 10 mmoL/L TPTZ solution in 40 mmoL/L HCl plus 20 mL of 20 mmoL/L $FeCl_3 \cdot 6H_2O$, 24 mL of distilled water and 200 mL of 300 mmoL/L acetate buffer, pH 3.6. Post incubation period, the readings were recorded swiftly with a spectrophotometer at 593 nm. The calibration curve was plotted with five standard solutions of known Fe(II) concentration ranging between 0.1 and 0.8 mmoL/L ($FeSO_4 \cdot 7H_2O$) (Benzie & Strain, 1999).

2.6. Total phenolic content

Folin-Ciocalteau assay was employed for the total polyphenol determination (Singleton & Rossi, 1965). Folin-Ciocalteau method actually measures a sample's reducing capacity and can be considered as another antioxidant (electron transfer) capacity assay (Nithiyanantham et al., 2013). For the assay, aliquots (200 µL) of each extracts of samples and Gallic acid standards were taken in test tubes. Then 1.5 mL of Folin-Ciocalteau phenol reagent (1:100

with water) was added sequentially in each tube. The solution was thoroughly mixed by vortexing and incubated for 5 min at ambient temperature. Then the mixture was alkalinized with 2.5 mL of Na_2CO_3 (20%). After that, the test tubes were placed in dark for 60 min and the absorbance was recorded at 780 nm against the reagent blank. Total polyphenol concentration was calculated with respect to a gallic acid calibration curve (100 - 500 mg/L) and results were expressed as g of gallic acid equivalent per 100 g of dry weight of the sample. The experiment was done in triplicates.

2.7. Total flavonoid content

Total flavonoid content of the different extracts of Crocus stigma, style, stamen, petal, and corm was determined using colorimetric method as described by Baba et al. (2015). This methods did not react uniformly, which indicates that it is inadequate for evaluation of total flavonoid content in unknown samples (Pękal & Pyrzynska, 2014). Ten milligrams of quercetin were dissolved in methanol and then diluted to 12.5, 25, 50, 75 and 100 µg/mL to draw the calibration curve. The sample extracts and diluted standard solutions (0.5 mL) were separately mixed with 1.5 mL of methanol, 0.1 mL of 10% aluminum chloride, 0.1 mL of 1M potassium acetate and 2.8 mL of distilled water. The absorbance of the reaction mixture post incubation at room temperature for 30 min was measured at 415 nm with a spectrophotometer. The blank was made by substituting distilled water instead of 10% aluminum chloride.

2.8. Total carotenoid content

The estimation of total carotenoids were extracted with some modifications to de Carvalho et al. (2012) method. The extract mixture was prepared by crushing 0.5 g of saffron flower parts and adding 10 mL of acetone and the filtrate was transferred to a separating funnel which contained 20 mL of petroleum ether. The removal of acetone was carried by gradual addition of ultrapure water and the procedure was repeated until no residual solvent remained. The extract was then transferred to a flask and 5 g of anhydrous sodium sulfate was added. Using petroleum ether, the volume was made up and the absorbance was recorded at 450 nm. The total carotenoid content was calculated as follows:

$$\text{Carotenoid Content } \left(\frac{\mu g}{g}\right) = \frac{A \times V \times 10000}{EC \times m} \quad (2)$$

where A was the absorbance of the sample, V was the total volume of extraction, m was the sample weight and EC was 2592 (extinction coefficient of β-carotene in petroleum ether).

2.9. GC-MS analysis

Gas chromatography analysis was performed using an Agilent-7890 B GC (Agilent Technologies, USA) with a 5975 MS detector operated by ChemStation software. The identification of the extracted compounds was performed with a HP-5MS capillary fused silica column with the specifications: length of 30 m, internal diameter of 0.25 mm, and film thickness of 0.25 µm. The oven temperature program initiated at 60 ◦C, held for 1 min then rises at 5 ℃/min to 200 ℃, held for 1 min, and finally rises at 20 ℃/min to 280 ℃ and held for 21 min. Other operating conditions were as follows: carrier gas, He (99.999%), with a flow rate of 1mL/min; injector temperature, 200 ℃; interface temperature, 290 ℃; solvent delay 6 min and split ratio, 1:5. Mass spectra were taken at 70 eV. Mass range was from m/z 35–450 amu.

For GC-MS analysis, 1 g ground powdered of stamens, petals and styles, 0.1 g of ground powdered stigma and 3 g of ground powdered of corms were extracted by stirring in 25 mL of ethanol/water 70/30 (v/v) for 48 h in the dark. Then, for another half an hour, it was placed under ultrasound and was filtered with filter paper. The solution was concentrated twice with rotary evaporator and was extracted with 30 mL of diethyl ether (in 3 step; each one 10 mL). The extract was then transferred to a flask and 2 g of anhydrous sodium sulfate was added and was filtered. The extract was dried with rotary evaporator and desorbed with 3 mL of HPLC grade of hexane (Jalali-Heravi et al., 2009).

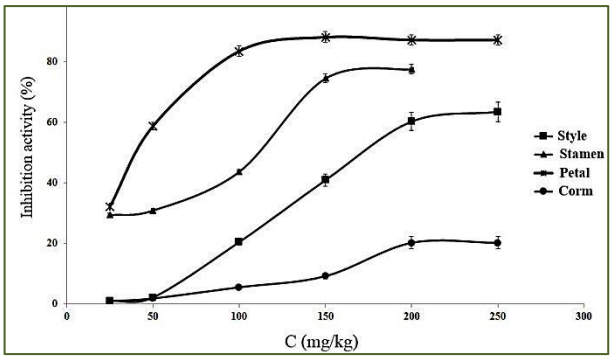

Fig. 2. Reducing power determination of corm, petal, stamen and style extracts at different concentration. Data are mean ± SD of three independent extracts.

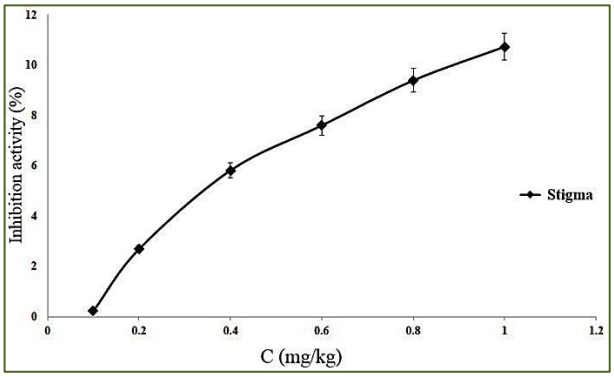

Fig. 3. Reducing power determination of stigma extracts at different concentrations. Data are mean ± SD of three independent extracts.

3. Results and Discussion

3.1. DPPH radical-scavenging activity

In this section, the antioxidant capacity of different parts of the saffron plant was evaluated with DPPH. The method is based on the reduction of the stable free radical DPPH in the presence of a hydrogen-donating antioxidant, and the formation of the non-radical form DPPH-H as result of the reaction. This reduction can be monitored at 517 nm by measuring the bleaching of DPPH (violet) to DPPH-H (yellow).

Table 1. Total phenolic, total carotenoid, total flavonoid content and antioxidant activity of extract of different part of saffron flowers.

Tissue sample	DPPH (IC50, mg/kg)	FRAP (g Fe (II)/100 g sample)	Total Phenolic Content (g/100 g sample)	Total Flavonoid Content (g quercetin/100 g sample)	Total Carotenoid Content (µg/g sample)
Stigma	4.94±0.25	2.36±0.15	2.72±0.18	183.25±16.42	579.67±11.12
Stamen	58.97±3.63	0.83±0.19	1.06±0.07	7.80±1.23	159.14±5.03
Style	123.68±8.52	0.31±0.08	0.76±0.08	3.72±0.89	4.50±0.80
Petal	46.02±2.89	1.62±0.26	1.96±0.12	20.18±2.18	2.38±0.49
Corm	720.49±14.32	0.03±0.001	0.56±0.09	ND	0.41±0.033

The obtained results for the antioxidant activity of the stamen, style, petal and corm of saffron plant were shown in Fig. 2. The obtained results for the stigma of saffron were shown in Fig. 3. Comparing the results, stigma showed the highest antioxidant activity followed by petals, stamens, styles and corms. The activity of all the extracts was found to be concentration dependent and increased with increase in the concentration of the extract. The IC_{50} values for extracts of stigma, petals, stamens, styles and corms were 4.94 ± 0.25, 46.02 ± 2.89, 58.97 ± 3.63, 123.68 ± 8.52 and 720.49 ± 14.32 mg/kg, respectively (Table 1). The obtained results show that the stigma and corms have the highest and lowest antioxidant activity with DPPH method, respectively.

The results are consistent with the results obtained by Vioque et al. (Sánchez-Vioque et al., 2012). In this research, the antioxidant properties of saffron leaves, petals and corms were investigated. The obtained results in this research showed that the antioxidant property of saffron leaves is more than petals and more than corms, and among these three parts of the plant, saffron corm showed the lowest antioxidant activity.

3.2. *Ferric reducing/antioxidant power (FRAP) assay*

Ferric reducing antioxidant power (FRAP) assay was employed for the measurement of total antioxidant activity (Benzie & Strain, 1999). The reduction of ferric tripyridyl triazine (Fe III TPTZ) complex to ferrous form (which has an intense blue color) at low pH was monitored by measuring the change in absorption at 593 nm. This reaction needs to prepare FRAP reagent. This colorless or yellow reagent is a mixture of Fe (III) chloride and TPTZ in adequate buffer. When extracted from different parts of saffron plant was added to this solution, the complex was reduced and the created complex has a blue color with a maximum absorption of 593 nm. A calibration curve was prepared with aqueous solution of $FeSO_4.7H_2O$ (5.6, 11.2, 22.4, 33.6, 44.8 and 56 mg/L). FRAP values were expressed as g of ferrous per 100 g dry weight of sample (g Fe (II))/100g sample). Similar to the DPPH results, the stigma and corms have the highest and lowest antioxidant activity with FRAP method, respectively (Table 1). In literature it was reported that saffron extract showed a total antioxidant activity calculated as sum of hydrophilic and lipophilic antioxidant activity of about 73.94 mmoles of Fe^{+2}/100 g dry weight (Ferrara et al., 2014).

3.3. *Total polyphenol content*

Total phenolic content was expressed in gallic acid by reference to a standard curve. The results on total phenolic compound of saffron plants extracts are presented in Table 1. The amounts of the total phenolic compounds of stigma, styles, stamens, petals and corms extract of saffron were 2.72 ± 0.18, 0.76 ± 0.08, 1.06 ± 0.07, 1.96 ± 0.12 and 0.56 ± 0.09 g/100 g sample, respectively. Similar to the DPPH and FRAP results, the stigma and corms have the highest and lowest polyphenolic compounds with Folin-Ciocalteau method, respectively. The polyphenol content of corm, stigma and leaf of saffron was investigated by Ahmad Baba and co-workers (Baba et al., 2015). In this research, the content of total polyphenol in aqueous and ethanolic extract was found to be 0.828 and 0.654 g/100 g for stigma and 0.597 and 0.707 g/100 g for corms, respectively. GCMS studies of saffron corms also proved existence of gallic acid, 4-hydroxybenzoic acid, p-coumaric acid, salicylic acid, cinnamic acid, vanillic acid, trans-ferulic acid, syringic acid and caffeic acid.

3.4. *Total flavonoids content*

Flavonoids are found throughout the vegetative kingdom. These colored compounds are obtained from secondary metabolites of vegetables. Flavonoids are present in leaf and skin of fruits and play an important and diverse role, including protection against ultraviolet light and resistance to disease. There are quantitative and qualitative methods for extraction and determination of these compounds in plants. Phenols and flavonoids are commonly used as free radical scavenging (Pieroni et al., 2002).

The results of the flavonoid content of the extracts of different parts of the saffron plant are shown in the Table 1. According the obtained results, the highest flavonoid content is related to the stigma whereas the saffron corm has the least amount. The results suggest that saffron corms are flavonoid free and the flavonoid content of petal was more than the stamen. Torabi & Zarrinkamar, (2015) investigated flavonoid content of petal and leaf of saffron. The mean flavonoid content of petal was 6.42, 4.02 and 3.96 mg rutin/g on October 26, 27 and 28, 2015 respectively. This investigation for leaf was 1.63, 3.42 and 2.53 mg rutin/g on September 27, October 27 and November 27, 2015, respectively. According to the obtained results by Karimi et al. (2010), flavonoid content of saffron was 5.88 mg rutin/g.

3.5. *Total carotenoid content*

Carotenoids are a member of the triterpenoid, which are yellow, orange and red in color. In Table 1, the total carotenoid content of different saffron plant can be observed. According to the obtained results, red stigma showed total carotenoid content levels of 579.67 ± 11.12 µg/g and yellow stamen contains total carotenoid content levels of 159.14 ± 5.03 µg/g. Despite of purple color of saffron petals, carotenoids content was found to be low (2.38 ± 0.49 µg/g). In the study by Ahmad Baba et al. (2015), total carotenoid content was determined in different *Crocus* tissue types for by spectrophotometric method and the results showed that the plant is rich in carotenoids with stigma containing maximum amount (546.55 µg/g) followed by leaf (171.12 µg/g) while corm has minimum content (45.64 µg/g).

Table 2. Compositions of the volatile component in saffron flower parts.

Row	Stigma		Style		Stamen		Petal		Corm	
	Name	Formula	Name	Formula	Name	Formula	Name	Formula	Name	Formula
1	Ethane-1,1-dioldibutanoate	$C_{10}H_{18}O_4$	Formic acid, decyl ester	$C_{11}H_{22}O_2$	benzeneacetaldehyde	C_8H_8O	2(3H)-Furanone, dihydro-4-hydroxy-	$C_4H_6O_3$	4-Nonenal,(E)	$C_9H_{16}O$
2	Undecane,4,4-dimethyl	$C_{13}H_{28}$	Decane	$C_{10}H_{22}$	Pheneylethyl alchol	$C_8H_{10}O$	Benzoic acid	$C_7H_6O_2$	Nonanal	$C_9H_{18}O$
3	Nonanal	$C_9H_{18}O$	Undecane	$C_{11}H_{24}$	Octane,4,5-diethyl-	$C_{12}H_{26}$	Anethol	$C_{10}H_{12}O$	2(3H)-Furanone,dihydro-4- hydroxy-	$C_4H_6O_3$
4	Octane,3,4-dimethyl	$C_{10}H_{22}$	Decane,4-ethyl-	$C_{12}H_{26}$	Benzoic acid	$C_7H_6O_2$	Undecan, 4,6-dimethyl-	$C_{13}H_{28}$	Decanal	$C_{10}H_{20}O$
5	Pentane,3-methoxy-	$C_6H_{14}O$	Hexacosane	$C_{26}H_{54}$	3-Ethyl-3-Methyl-2-Pentanol	$C_8H_{18}O$	Cholesterol	$C_{27}H_{46}O$	Anethole	$C_{10}H_{12}O$
6	Decane,5,6-dimethyl-	$C_{12}H_{26}$	Cyclopentane,1-hexyl-3-methyl-	$C_{12}H_{24}$	Benzeneacetic acid	$C_8H_8O_2$	Dodecanoic acid	$C_{12}H_{24}O_2$	2(3H)-Furanone,5-hexyldihydro	$C_{10}H_{18}O_2$
7	Undecane,5-methyl-	$C_{12}H_{26}$	Dodecane	$C_{12}H_{26}$	Anethole	$C_{10}H_{12}O$	Hentraiacontane	$C_{31}H_{64}$	(E)-9-Octadecanoic acid ethyl ester	$C_{20}H_{38}O_2$
8	3-Ethyl-3-methyl-2-pentanol	$C_8H_{18}O$	Naphthalene,decahydro-2,3-dimethyl	$C_{12}H_{22}$	Tetradecane	$C_{14}H_{30}$	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	Linoleic acid ethyl ester	$C_{20}H_{36}O_2$
9	Anethole	$C_{10}H_{12}O$	Naphthalene,decahydro-2,6-dimethyl	$C_{12}H_{22}$	Dodecanoic acid	$C_{12}H_{24}O_2$	Linoelaidic acid	$C_{18}H_{32}O_2$	9-Oxononanoic scid	$C_9H_{16}O_3$
10	Ethanone,1-(2,3,4-trimethylpt,enyl)	$C_{11}H_{14}O$	Naphthalene,decahydro-1,5-dimethyl	$C_{12}H_{22}$	Chalcone	$C_{15}H_{12}O$	Octadecanoic acid	$C_{18}H_{36}O_2$	Hexadecane	$C_{16}H_{34}$
11	4-Hydroxy-2,6,6-trimethyl-3-oxocyclohexa-1,4-diencarbaldehyde	$C_{10}H_{12}O_3$	Tridecane	$C_{13}H_{28}$	1-Nonadecene	$C_{19}H_{38}$	4,8,12,16-Tetramethylheptadecan-4-	$C_{21}H_{40}O_2$	Benzophenone	$C_{13}H_{10}O$
12	Tetradecane	$C_{14}H_{30}$	Tridecane,5-mmethyl-	$C_{14}H_{30}$	2-Phenylethyl docosanoate	$C_{30}H_{52}O_2$	Tetracosane	$C_{24}H_{50}$	Cis-Vaccenic acid	$C_{18}H_{34}O$
13	4-Hydroxy-2,6,6-trimethylcyclohex-1	$C_{10}H_{16}O$	Tridecane,3-methyl-	$C_{14}H_{30}$	Tetradecanoic acid	$C_{14}H_{28}O_2$	Hexacosane	$C_{26}H_{54}$	1-Hexadecanol	$C_{16}H_{34}O$
14	Hexadecane	$C_{16}H_{34}$	Cyclotetradecane	$C_{14}H_{28}$	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	Hexadecanoic acid,2-hydroxy 1-(hydroxymethyl)ethyl ester	$C_{19}H_{38}O_4$	n-Hexadecanoic acid	$C_{16}H_{32}O_2$
15	Hexacosane	$C_{26}H_{54}$	Tetradecane	$C_{14}H_{30}$	Chalcone	$C_{15}H_{12}O$	3-Methylpentacosane	$C_{26}H_{54}$	Hexadecanoic acid,ethyl ester	$C_{18}H_{36}O_2$
16	Octadecane	$C_{18}H_{38}$	Tetradecane,2-methyl	$C_{15}H_{32}$	Octadecanoic acid	$C_{18}H_{36}O_2$	Octacosane	$C_{28}H_{58}$		
17	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	Pentadecane,3-methyl	$C_{16}H_{34}$	Hexadecanoic acid,2-hydroxy1-(hydroxymetyl) ester	$C_{19}H_{38}O_4$				
18	Eicosane	$C_{20}H_{42}$	Hexadecane	$C_{16}H_{34}$	Tetracosane	$C_{24}H_{50}$				
19	1-Tetradecanol	$C_{14}H_{30}O$	Octadecane	$C_{18}H_{38}$	Linoelaidic acid	$C_{18}H_{32}O_2$				
20	Octadecanoic acid	$C_{18}H_{36}O_2$	Tetradecan	$C_{24}H_{50}$	9,12,15-Octadecatrienoic acid,(Z,Z,Z)-	$C_{18}H_{30}O_2$				
21	9-Octadecanomide,(Z)	$C_{18}H_{35}NO$	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	Octadecanoic acid	$C_{18}H_{36}O_2$				
22			Eicosane	$C_{20}H_{42}$	Squalene	$C_{30}H_{50}$				
23			Linoelaidic acid	$C_{18}H_{32}O_2$	9-Octadecanamide, (Z)-	$C_{18}H_{35}NO$				
24			9-Octadecanoic acid	$C_{18}H_{34}O_2$						
25			Ocatdecanoic acid	$C_{18}H_{36}O_2$						

3.6. GC-MS analysis

Active substances are chemical compounds that naturally occur in plants and usually refer to compounds that have more medical effects than nutrition. The active compound from the different parts of saffron were extracted and identified with GCMS. The results of this analysis are shown in Table 2. The compounds that identified in different parts of saffron have characteristics that can be used in the pharmaceutical, cosmetics and food industries. Many of these compounds can be extracted and purified for using in many industries, although more research is needed on the amount of these compounds and possibility of extract them economically.

4. Conclusion

The extracts prepared from various parts of saffron collected from Roshtkhar (Khorasan Razavi province, Iran) were assessed for its total polyphenol, flavonoids, carotenoids contents and bioactive molecules. It was found that the stigma extracts contain high antioxidant, carotenoid and polyphenol contents compared to the other part selected in this study. The GC-MS results highlight the presence of various bioactive molecules which support the findings. The presence of gallic acid, 4-hydroxybenzoic acid, p-coumaric acid, salicylic acid, cinnamic acid, vanillic acid, trans-ferulic acid, syringic acid and caffeic acid in the extracts are responsible for the superior antioxidant, total polyphenol, and flavonoid and carotenoid contents.

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Conflict of interest

The authors declare that they have no conflict of interest.

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