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Essential Oils from Flower Petals of Saffron *(Crocus sativus)*

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Abstract

Saffron petal is the main by-product of saffron processing which is produced at a high level but is not applied and thrown out. Saffron petal is containing several compounds such as mineral agents, anthocyanins, flavonoids, glycosides, alkaloids and kaempferol. As saffron petal is cheaper and produces in large amounts compared to saffron stigma, so it can be considered as an appropriate source for different purposes. The field experiment was carried out under the dry land farming system in 2015 and 2016. The vegetative parts of the plants were treated with spraying foliar fertilizer as Delfard (D) (7 kg ha-1) and Floral (P) (2.5 kg ha-1) with three replications. In this study essential oils from flower petals of saffron extracted by water distillation method (Calavenger apparatus), and volatile also phytochemical compounds were evaluated by gas chromatography (GC), and gas chromatography-mass spectrometry (GC/MS). A total of 27 volatile components were identified. Main components from Saffron petal essential oil foliar fertilizer treatment D1 were Methyl pentanoate (67.2), Isophorone (17.2%) and Hexanal (3.0%) and Saffron petal essential oil foliar fertilizer treatment P1were Methyl pentanoate (57.6%), 3,3,5-trimethyl-cyclohexene (57.6%), Hexanal (4.3%). Differences in compound proportion and composition were observed among the treatments. This is the first research conducted on Iranian saffron volatiles according to their geographical origin. Various methods were selected for extracting of essential oil from *Crocus sativus* L. Finally, the results of this experiment showed the compatibility and success of growth and production in saffron quantitatively and qualitatively in dry land conditions with a relatively high area.

1. Introduction

Crocus sativus L. (saffron) is belonging to Iridaceae family. It is used in foods as color and flavor agent and also used in cosmetic preparations (Fernández, 2004). Saffron is one of the strategic medicinal and spice plants of Iran. The cultivated species of saffron is *C. sativus* L. It is mainly cultivated in Khorasan and in some other region of Iran. Saffron, the dried red stigmata of *C. sativus* L. flower, is the most expensive of spices. Its price in retail market is five euros per gram. Saffron adds its faint, delicate aroma, pleasing flavor and magnificent yellow color to foods. In 2011, Iran was the largest producer of saffron in the world in terms of quantity and quality, with a surface area of about 66,497 hectares and an annual production of 211,565 kg (Kafi *et al*., 2006). As mentioned, saffron corm remains in the field for 7 to 10 years and do not require replanting. So, after 7 years (in some places up to 10 years) of saffron plantation, corm is transferred to new land. Obviously, when moving and replanting corms, it should be noted that the land should be selected for the new farm where the saffron has not been cultivated for at least 7 to 10 years. The usual and current care in saffron fields is the same from the second year to the end of the seventh year as follows. Harvesting of saffron products involves picking flowers and removing stigma. The edible part of the plant known as saffron, from the main part of the flower. Saffron harvest time varies from late October to early December depending on the climatic conditions of saffron points. Saffron flowers usually appear in late October and gradually increase in number. In comparison to other parts of plant, the stigma has more applications in food, cosmetic and treatment of diseases.

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Phytochemical studies have shown saffron stigma is containing crocetin, crocin, picrocrocin and safranal. The color of saffron is related to presence of crocin, while pharmacological properties are linked to crocetin. Also other components are found in saffron as flavonoids, anthocyanins, vitamins such as riboflavin and thiamine, proteins, starch, amino acids, mineral matter and gums (Rios *et al*., 1996).
In traditional medicine, it is used as an aphrodisiac, antispasmodic, expectorant, stomachache, relieving tension, depression and insomnia. Also the powdered stigma of saffron was used in treatment of cataract. Other traditional applications are antibacterial, antiseptic and antifungal effects (Schmidt *et al*., 2007; Hosseinzadeh and Nassiri- Asl, 2013; Mollazadeh *et al*., 2015). In modern medicine other pharmacological properties of stigma including neuroprotective (Khazdair *et al*., 2015), antitussive, hypolipidemic (Hosseinzadeh and Ghenaati, 2006; Asdaq and Inamdar, 2010), anticonvulsant (Hosseinzadeh and Khosravan, 2002), antinociceptive (Hosseinzadeh and Younesi, 2002), antidepressant (Hosseinzadeh *et al*, 2004), anxiolytic activity (Hosseinzadeh and Noraei, 2009), cardiovascular protective (Imenshahidi *et al*., 2010), anticancer (Abdullaev and Espinosa-Aguirre, 2004) and antioxidant (Hosseinzadeh *et al*., 2005; Hosseinzadeh *et al*., 2009) have been reported. Saffron petal as a by-product is produced at high level but it is not used and thrown away after harvesting. However, it is worth to pay attention to the petal as it is cheaper than stigma. Based on evidences, most studies are about stigma of saffron and there is low information about saffron petal. In this review, we collected all studies about saffron petal properties and its pharmacological effects.

2. Material and Methods

In order to study the effect of nutrition on phonological, morphological, phytochemical and saffron traits in dryland conditions an experiment was carried out at Rangelands and medicinal plants Research Station in Damavand, affiliated to the Research Institute of Forests and Rangelands (Ministry of Agriculture) an investigation was carried out in 2015-2016.
This station is located 65 km east of Tehran with geographical location 90° 40' 35" north latitude and 35° 5' 52" east longitude and it is located 1960 meters above sea level. Researh station Hmand Absard topographic, uncomplicated plain, with 4% slope, alluvial soil, brown with calcareous layers at the bottom and the supernatant is a loamy texture with an alkaline pH 7.7. The climate in the cold semi steppe region is so that the rain started from mid-October until December and it runs until June. Average of 42 years of annual rainfall, is 333 mm which of these, 49.8, 38.7 and 13.5 mm belong to April, May and June, respectively. The field experiment was carried out under drayland farming system. The vegetative parts of the plants were treated with spraying of foliar fertilizer using Delfard (D) (7 kg ha-1) and Phloral (P) (2.5 kg ha-1) with three replications. This research also was carried out using saffron (*C. sativus* L.) corms originating from Qaein city.

2.1. Plant Material Collection
Two flower petals of saffron samples were collected for analysis during 2018. Sample 1 Saffron petal essential oil foliar fertilizer treatment D, 102 grams gives 0.06gram essential oil. Sample 2 Saffron petal essential oil foliar fertilizer treatment P, 102 grams gives 0.03gram essential oil. Fertilizer treatment D and P are given in table 1, and keep in two liters balone with 500 ml distilled water the volatiles in saffron were extracted by water distillation (Clevenger apparatus). NaCl (2.5 g) and water (0.5 ml) were placed in the collecting vial. The samples were kept at 4 °C in absence of light until their analysis.

2.2. Gas Chromatography
GC analyses were performed using a gas chromatography, Ultera Fast Module –GC, made in Italia. Profile column machine brand Ph-5 capillary column, manufactured by Shimadzu with Length of 30 mm and an inner diameter of 1/0 mm thick 25/0 mm, the inner surface of the stationary phase material is covered Phenyl Dimethyl Siloxane 5%. Column temperature program: initial temperature 60 °C to start the final temperature of 280 °C. The initial 3 °C per minute to be added and then injected into the chamber to a temperature of 280 °C. Run time: 8.63 min., Detector: FID, 280 °C, Injectore: 280 °C; Carrier gas: He, 0.5 ml/min. The carrier gas inlet pressure to the column: helium with a purity of 99/99% of the inlet pressure to the column equal to 5/1 kilogram per square centimeter is set.

2.3. Gas Chromatography-Mass Spectrometry
The GC/MS unit consisted of a Varian Model 3400 gas chromatograph coupled to a Saturn II ion trap detector was used. The column was same as GC, and the GC conditions were as above. Mass spectrometer conditions were: ionization potential 70 eV; electron multiplier energy 2000 V. The identity of the oil components was established from their GC retention indices, relative to C7- C25 n-alkanes standards mixture, and by comparison of their mass spectra and retention indices with those reported in the literature (Shibamoto, 1987; Davies, 1990; Adams, 2017), and by computer matching with the Wiley 5 and NIST mass spectra library, whenever possible, by co-injection with standards available in the laboratories.

Table 1 Ingredient of fertilizer treatment D1 and P1

Chemical name	Symbol name	Formula	ppm	Delfard 15	Floral phosphor
Ammoniacal	N	-	-	12%	11%
Phosphoric anhydridd	P	P_2O_5	-	8%	20%
Potassium oxide	K	K_2O	-	4%	11%
Magnesium	Mn	EDTA	1000	0.10%	0.10%
Zinc	Zn	EDTA	1000	0.10%	0.10%
Copper	Cu	EDTA	500	0.05%	0.05%
Iron	Fe	EDTA	2000	-	-
Sulfur	S	So2	-	-	22%
Boron	B	-	-	-	0.01%

EDTA= Ethyl Diamine Tetra Acetic acid

3. Result and Discussion

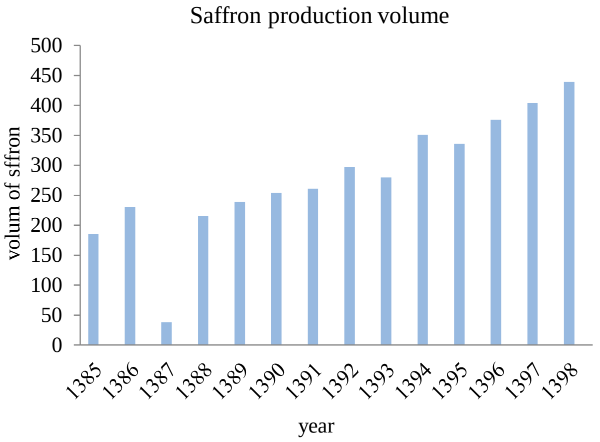

Fig. 1 Growth of saffron in the Iran in recent years

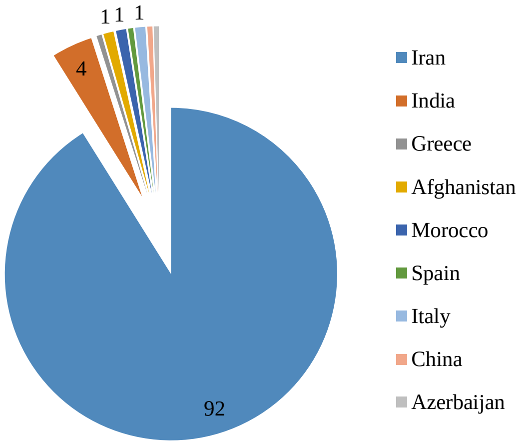

Fig. 2 Amount of saffron production in different countries

The amount of flowers required to produce one kilogram of saffron varies between 150,000 to 200,000 flowers. Each kilogram of flower is about 2000 to 2700 flowers and each kilogram gives between 12.55 to 37.37 grams and on average between 37.65 to 112.11 grams of saffron (Table 2). At present, Iran has a 90% share in world production and is the most important and largest producer of saffron in the world (Sabzevari, 1996).

3.1. Saffron Industry in Iran

The saffron industry, from the point of view of various companies, has a smaller and less economical economy than other medicinal plants. Companies are proud of the long history of saffron in the country and believe that with the expansion of saffron products in the future, they should accelerate the economic growth of this industry.

Today in the country, about 400 tons of saffron is produced annually and information about the volume of saffron production in recent years (Table 3) is given in the following table.

Ph-5 capillary column

As can be seen, in recent years, the growth of saffron in the country has increased steadily (Fig. 1). In addition, the amount of saffron production in different countries is as follows.

Iran alone supplies about 92% of the world's saffron production (Fig. 2), and as can be seen, this number is much higher than the share of other countries in production. Iran's high share in saffron production indicates a stable competitive advantage for Iranian producers over other producers.

On average, it can be said that the total saffron industry has a volume of about 880 million dollars, of which about 500 million dollars is the share of the food sector, of which Iran has a share of about 40 to 60 percent of this market (Table 4). Iran's low income from industry compared to its production volume indicates a major problem in the industry, which from the perspective of manufacturing companies, this problem is due to intermediation and whole sale in the industry, crude sales and low processing of saffron.

Table 2 The largest exporters of saffron in the world in 2018 and the value of their exports

No.	Countery	Exports (million dollars)	Share of global exports	Percentage of annual export growth
1	Iran	101.32	36.7%	- 10.8%
2	Espania	57.08	20.7%	-15.3 %
3	Afghanestan	44.58	16.2%	142.3%
4	India	9.48	3.4%	420.3%
5	Greece	9.48	3.4%	26.6%

Global Saffron Market., behinexir company, 2019-12-16 upto 2020-10-27 (https://behinexir.com › global-saffron-market).

Table 3 Production status and cultivation area of Iranian saffron (Source: Statistics of the Ministry of Jihad Agriculture)

Iranian Solar year	year	Area under cultivation (ha)	Percentage of surface growth compared to the previous year	Production rate (tons)	Percentage of production growth compared to the previous year	Yield (kg/ha)
1385	2006	58358	-	186	-	3.2
1386	2007	58906	0.9	230	23.7	4
1387	2008	61936	5.1	38	-83.5	0.6
1388	2009	66497	7.4	215	465.8	3.2
1389	2010	70044	5.3	239	11.2	3.5
1390	2011	73119	4.4	254	6.3	3.5
1391	2012	79394	8.6	261	2.8	3.3
1392	2013	81882	3.1	297	13.8	3.7
1393	2014	87925	7.4	280	-5.7	3.2
1394	2015	95125	8.2	351	25.4	3.8
1395	2016	105270	10.7	336	-4.3	3.3
1396	2017	108083	2.7	376	11.9	3.5
1397	2018	113947	5.4	404	7.4	3.5
1398	2019	120223	5.5	439	8.6	3.7

Table 4 Percentage of dollar value growth in saffron

Iranian solar year	Year	Export rate (tons)	Dollar value (million dollars)	Percentage of dollar value growth
1385	2006	142	76	-
1386	2007	76	51	-32.9
1387	2008	70	92	80.4
1388	2009	65	180	95.7
1389	2010	107	333	84.4
1390	2011	122	368	10.8
1391	2012	139	419	13.9
1392	2013	137	200	-52.3
1393	2014	159	228	14
1394	2015	125	183	-19.7
1395	2016	203	286	56.3
1396	2017	237	325	13.6
1397	2018	280	351	8
1398	2019	270	296	-15.7

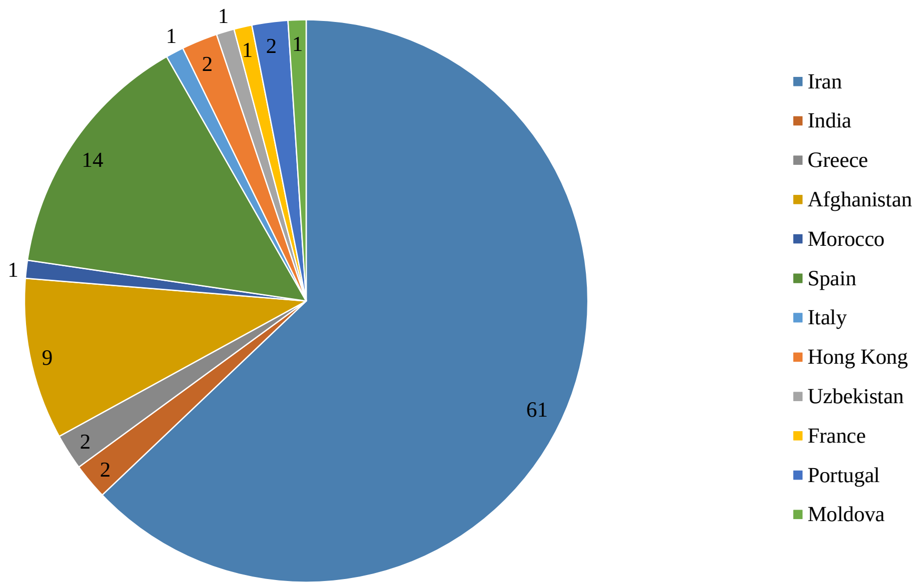

Fig. 3 Share of saffron market in different countries, Production status and cultivation area of Iranian saffron (Source: Statistics of the Ministry of Jihad Agriculture)

Table 5 Chemical composition eessential oils from flower petals of Saffron *(C. sativus)*

Compounds	R.I.	Saffron petal essential oil foliar fertilizer treatment D	Saffron petal essential oil foliar fertilizer treatment P
Hexanal	805	3.0	4.3
Methyl pentanoate	821	67.2	57.6
3,3,5-trimethyl-cyclohexene	824	-	34.9
3,5,5-trimethyl cyclo hex-3-en-1-one	832	0.4	0.5
Heptanal	903	-	0.2
1-heptanol	971	-	0.5
β- pinene	980	-	0.6
2,6,6-trimethyl-1,4-cyclohexadiene-1 carboxaldehyde	1049	0.4	-
(Phenyl ethyl alcohol	1052	0.3	-
Isophorone	1061	17.2	0.8
6-camphenone	1091	0.2	-
2,2,6-trimethyl-1,4-cyclo hexanedione	1102	0.6	-
4-hydroxy-3,5,5-trimethyl-2-cyclohexen-1-one (isomer 1)	1171	0.4	-
E-4-(2,6,6-trimethyl-cyclohexyl) but-3-en-2-one	1316	0.4	-
10- undecenol	1361	1.0	-
Ethyl- (4E)- decenoate	1380	0.3	-
(E)- β- ionone	1488	0.7	-
Germacrene B	1557	0.5	-
Hexyl phenyl acetate	1623	0.3	-
α- cadinol	1652	1.8	-
Safranal (= 2,6,6-trimethyl-1,3-cyclohexadien-1-carboxaldehyde)	1664	0.4	-
(2Z, 6Z)- farnesal	1683	0.6	-
Methyl eudesmate	1719	1.2	-
Dihydro-4-oxoisophorone (= 2,6,6-trimethtl-1,4-cyclohexandione)	1784	0.7	-
Nonadecane	1900	1.1	-
Dihydro- β- ionol	1967	-	0.4
2 α-acetoxy-amorpha-4,7(11)-diene-8-one	1982	0.4	-

They believe that Iranian saffron is processed with very low processing and practically raw, and the processing and packaging of saffron, which creates the most added value for this product, is done by intermediary countries such as Spain, and therefore, the section A large part of the income of the saffron industry goes to these countries (Fig. 3).

Table 5 shows the essential oil from flower petals of saffron extracted by water distillation method (Calavenger apparatus), and volatile also phytochemical compounds were evaluated by gas chromatography (GC), and gas chromatography–mass spectrometry (GC/MS). A total of 27 volatile components were identified. Main components from Saffron petal essential oil foliar fertilizer treatment D1 were Methyl pentanoate (67.2), Isophorone (17.2%) and Hexanal (3.0%) and Saffron petal essential oil foliar fertilizer treatment P1were Methyl pentanoate (57.6%), 3,3,5-trimethyl-cyclohexene (57.6%), Hexanal (4.3%),

Methyl pentanoate in Saffron petal essential oil foliar fertilizer treatment D with 67.2% and in Saffron petal essential oil foliar fertilizer treatment P with 57.6%, obtained._Methyl pentanoate is commonly used in fragranes, beauty care, soap, laundry detergenta at levels of 0.1 to 1 percentage. In a very pure from (greater than 99.5 %), it is used as a plasticizer in the manufacture of plastics. It is also used as an insecticide. Methyl valerate is other names of methyl pentanoate. With chemical formula $C_6H_{12}O_2$, molar mass 116.160 g·mol^{-1}, density 0.89 g/cm^3, melting point <25 °C, boiling point 126 °C (259°F; 399K) (MSDS). Isophorone in Saffron petal essential oil foliar fertilizer treatment D with 17.2%, obtained. Isophorone is an α, β-unsaturatred cyclic ketone. It is a colorless liquid with a characteristic peppermint-like odor, although commercial samples can appear yellowish. Used as a solvent and as a precursor to polymers, it is produced on a large scale industryally (Hardo and Manfred, 2005). Isoacetophorone is other name of isophorone. With chemical formula $C_9H_{14}O$, molar mass 138.210 g·mol^{-1}, appearance colorless to white liquid, odor peppermint-like (Sabzevari, 1996), density 0.9255 g/cm^3, melting point -8.1 °C (17.4°F; 265.0K), boiling point 215.32 °C (419.58°F; 488.47K), solubility in water 1.2 g/100 ml, solubility with ether, acetone, hexane, dichloromethane, benzene, toluene, alohol. Isophorone undergoes reactions characteristic of an α, β-unsaturatred ketone. Hydrogenation gives the cyclohexanone derivative. Epoxidation with basic hydrogen peroxide affords the oxide (Richard, 1957). Isophorone is degraded by attack of hydroxyl radicals (Retrieved, 2016). The partly hydrogenated derivative trimethylcylohexanone is used in production of polycarbonates. It condenses with phenol to give an analogue of bisphenol A. Polycarbonates produced by phosgenation of these two diols produces a polymer with improved thermal satability (Serini, 2000). Trimethyladipic acid and 2,2,4-trimethyl hexamethylene diamine are produced from trimethylcyclohexanone and trimethylyclohexanol. 3,3,5-trimethyl cyclohexene in Saffron petal essential oil foliar fertilizer treatment P with 34.9%, obtained. They are used to make specialty polyamides. Hydrocyanation gives the nitrile followed by reductive amiation gives isophorone diamine. This diamine is used to produced isophorone diisoyanate which has certain nihe applications (Hardo and Manfred, 2005).

4. Conclusion

Recently, according to research on the properties of various parts of saffron have been done. They concluded that the properties of the purple petals of saffron are nothing less than its precious stigmas. For this reason, recently the purple petals of saffron have attracted a lot of attention and are used as a tail. The antioxidants in saffron petals provide the body with the necessary immunity against free radicals. When it became clear that the properties of saffron flowers were as important as the amount of red saffron filaments, there was no news of discarding these petals. These petals have health and medicinal benefits. The role of this petal is also very important in the paint industry.

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