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Micropropagation and potential of bioactive compounds of saffron (*Crocus sativus* L.) for nutrition and health

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Abstract

To inquire about the possibility of durable valorization of saffron, this review highlights the different techniques of *in vitro* culture and varietal creation of this specie. Then, a reveal main component of saffron with some pharmacological activities that make this product a serious therapeutic hope. Saffron (*Crocus sativus* L.) is a sterile triploid geophyte that propagated by corms. To respond to the increasing global demand for saffron, it is necessary to expand the area under cultivation. Thus, *in vitro* techniques can produce a large quantity of propagating material in reduced time. It is well-known that saffron is traditionally used as a coloring or flavoring agent, but recent research showed its potential for health promotion. The interest components include crocin, crocetin, picrocrocin, and safranal have all demonstrated a wide range of use in medical field. Previous studies have reported that biological activities of saffron alleviates or prevents health problems such as stomach upset, cardiovascular disease, and depression. In addition, saffron is also promising in cancer prevention due to its antioxidant properties.

Keywords: biological activities; chemical compounds; *Crocus sativus*; *in-vitro* culture

Introduction

Crocus sativus L. was cultivated in North Africa since the 9th century (Pierlot, 1925). It is now recognized as one of the most expensive and valuable plants (Sedighara, 2003). Saffron, made from the dried stigmas of *C. sativus* flowers, is the main terroir of Morocco products (Figure 1). It is a quality spices with high commercial value (Abdullaev, 2007; Gresat *et al*., 2008). From antiquity to the present day, most of saffron produced was, and still used in cooking. In Morocco, saffron used in tea instead of mint, but also as a spice in the preparation of various traditional dishes including 'koftas' (meatballs and tomatoes) or 'mrouzia' (a sweet-

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salty dish made from mutton or dill). Saffron is also a central ingredient in the blend of chermoula herbs that perfume many Moroccan dishes (Tsatsaroni *et al*., 1998; Modaghegh *et al*., 2008). It is used in traditional medicine as a remedy to treat various human health problems such as diabetes, cancer, and Alzheimer's disease (Abdullaev and Espinosa, 2004; Mzabri *et al*., 2019).

(A) (B)

Figure 1. *Crocus sativus* plant morphology: (A) saffron plant; (B) Saffron Flowers and Stigma

Saffron is a triploid perennial plant that multiplies vegetatively (Fernández, 2004; Kumar, 2009). Improving the yield and the quality of saffron by crossing was not possible because of the sterility problems (Dhar *et al*., 1988; Chahota *et al*., 2003).

Thus, *in vitro* culture is very advantageous for large-scale multiplication and for the creation of new resistant cultivars (Lagram *et al*., 2016; Sevindik and Yalcin, 2016).

Our plant is one of the most valuable spices. The color, taste (flavor) and aroma constitute the particular traits of saffron, which are associated with three different molecular configurations: crocin, picrocrocin, and safranal (D'Archivio *et al.,* 2019). Therefore, saffron is the subject of several pharmacological studies (Mirhadi *et al*., 2020; Akhondzadeh *et al*., 2020). The antidiabetic, antioxidant, immunological, anticancer, and others biological activities of saffron were highlighted in the study realized by Amanpour *et al*. (2019).

In our review, we present an update of research on tissue culture, induced varietal creation, main compounds and some biological activities of *C. sativus*.

In Vitro Tissue Culture

Somatic embryogenesis

Somatic embryogenesis is one of the most important aspects of tissue culture in saffron (Slackand and Tufford, 1995). This technique allows mass production of healthy corms (Bagheri and Vasel, 2006). Various tissues were used for the induction of somatic embryogenesis and regeneration in saffron (Table 1), in particular, vegetative apices (George *et al*., 1992), apical and axillary buds (Blázquez *et al*., 2007; Zeybek *et al*., 2012), meristematic zone (Sharma *et al*., 2005; Sharifi *et al*., 2010), segments leave (Devi *et al*., 2014; Zaffar *et al*., 2014) and ovaries (Bhagyalakshmi, 1999; Namin *et al*., 2010).

For the propagation by somatic embryogenesis and regeneration of healthy plants from saffron corms, MS supplemented with indole-3-acetic acid (IAA) and 6-benzylaminopurine (BAP) in combination with 2,4-dichlorophenoxyacetic acid (2,4-D) were used (Ding *et al*., 1981). Results showed that the most effective treatment was 2.0 mg/L 2,4-D + 1.0 mg/L BAP (Ding *et al*., 1981; Rajabpoor *et al*., 2007).

Several studies obtained saffron organogenic callus from meristems using kinetin (Kn) or thidiazuron (TDZ) combined with 1-naphthaleneacetic acid (NAA) under dark (Vatankhah *et al*., 2010). In this way,

auxin and cytokinin are the key factors in determination of the embryonic response due to their participation in the regulation cycle and cell division (Francis *et al*., 2001).

Table 1. Effect of hormones on *C. sativus* callogenesis and organogenesis

Explants used	Culture medium	Growth regulators	Results	References
Somatic embryogenesis				
Meristem	LS	2 µM BA + 2 NAA µM	Somatic embryo	Ebrahimzadeh *et al*. (2000)
Corm	B5	1 BA + 2 NAA	Callus	Chen *et al*. (2003)
Corm	LS	4 NAA + 4 BA 1 2,4-D + 4 kn	Somatic embryo	Karamian (2004)
Apical bud	-	-	Callus Shoots Regeneration	Sharma *et al*. (2005)
Bourgeon apical	LS	2 NAA + BA 1 2,4-D + BA	Non-embryogenic calluses Embryogenic calluses	Darvishi *et al*. (2007)
Corm	MS	0.5 TDZ	Somatic embryo	Sheibani *et al*. (2007)
Corm	MS	2 2,4 D + 1 BAP	Somatic embryo	Ebrahimzadeh *et al*. (2000)
Corm	MS	2 BAP + 0.05 NAA	Somatic embryo	Blazquez *et al*. (2009)
Buds	LS	3,5 BAP + 2 NAA	Callus	MalekZadeh *et al*. (2009)
Protoplasts	MS	1 2, 4-D + 0.2 Kn	Callus	Chaloushi *et al*. (2007)
Corm	MS B5	4.54 µM TDZ 2.22 µM NAA + 2.68 µM BA	Shoots Regeneration	Sharifi and Ebrahimzadeh (2010)
Callus	MS	4,0 µM Kn + 10,0 µM picloram 2,0 µM TDZ + 1,0 µM NAA	Embryogenic calluses	Vatankhah *et al*. (2010)
Corm	MS	1 2,4-D + 4 Kn	Callus	Sharifi *et al*. (2012)
Corm Root	MS	2.5 2,4-D + 1 BAP	Callus	Zeybek *et al*. (2012)
Corm	MS	0.25 2,4 D + 1 BAP	Callus	Gantait and Vahedi (2015)
Leaf	MS	2.5 µM TDZ +2.0 µM picloram 26.64 µM BAP + 1 µM NAA	Somatic embryo Shoots Regeneration	Devi et al. (2014)
Corm Leaf	MS	1 BA + 1 2,4-D 1 BA + 1 NAA	Callus Shoots Regeneration	Zaffar *et al*. (2014)
Microcorms formation				
Flower Corm	MS	2 BA + 0.5 NAA	Microcorms	Karaoğlu *et al*. (2007)
Somatic embryo	½ MS	Without hormones	Microcorms	Sheibani *et al*. (2007)
Buds	½ MS	3 BA	Microcorms	Sharma *et al*. (2008)

Corm	½ MS	20 µM TDZ+ 10 µM NAA	Microcorms	Parray *et al*. (2012)
Apical Buds	MS	2 BAP + 0.5 NAA	Microcorms	Mir *et al*. (2014)
Buds	½ MS	1 BAP + 1 2,4 D	Microcorms	Lagram *et al*. (2016)
Direct organogenesis				
Style	MS	5 Kn + 5 NAA	SLS	Rajabpoor *et al*. (2007)
Stamen	MS	NAA	SLS	Zhao *et al*. (2001)
Flower buds	MS	5 Kn + 4 NAA	SLS	Zeng *et al*. (2003)
Style	MS	26.8 µM NAA + 31.1 µM BAP	Flowers	Jun *et al*. (2007)
Buds	MS	BAP + 2iP +TDZ	Shoots Regeneration	Majourhat *et al*. (2007)
Corm	MS B5	4.54 µM TDZ 2.22 µM NAA + 2.68 µM BA	Shoots Regeneration Roots	Sharifi *et al*. (2010)
Flower buds	MS	10 NAA + 10 BAP	SLS	Namin *et al*. (2010)
Corm	MS	26.64 µM BAP + 5 µM NAA	Shoots Regeneration	Devi *et al*. (2014)
Corm	MS	5 BAP	Shoots Regeneration	Dıaz-Vivancos *et al*. (2011)
Corm	½ MS	20 µM BAP + 15 µM NAA	Shoots Regeneration	Parray *et al*. (2012)
Corm	½ MS	5 BA + 0.5 NAA	Shoots Regeneration	Renau-Morataa *et al*. (2013)
Corm	MS	4 BAP 4 BAP + 4 NAA	Shoots Regeneration Roots	Sarhan *et al*. (2013)
Corm	MS	1 2,4-D + 1 BAP	Shoots Regeneration	Simona *et al*. (2013)
Callus	MS	1 NAA + 1 TDZ 1 NAA + 2 Kn	Shoots Regeneration Roots	Vatankhah *et al*. (2014)

MS: Murashige and Skoog medium; LS: Linsmaier & Skoog medium; B5: Gamborg B5 medium; BA or BAP: Benzyl adenine; NAA: Naphthalene acetic acid; 2,4-D: 2,4-Dichlorophenoxyacetic acid; Kn: Kinetin; SLS: stigma-like structures

Direct somatic embryogenesis is a viable method for saffron propagation with little risk of variation. TDZ and picloram, at various concentrations, are effective for induction and proliferation of somatic embryos, while maturation was favorited on ½ MS supplemented with 2.5 µM of TDZ and 2.0 µM of picloram. The different stages of somatic embryo development are determined by histological studies with an electron microscope (Devi *et al*., 2014).

Various studies were indicated that the somatic embryo maturation was carried out in a medium supplemented with abscisic acid (ABA) and the germination could realize in a medium without hormones (George *et al*., 1992; Sheibani *et al*., 2007; Sevindik *et al*., 2016) or supplemented with GA3 (Ebrahimzadeh *et al*., 2000). Picloram combined with other hormones, regulate the embryonic stages and produce a maximum frequency of somatic embryos and germination. It should be noted that the maturation and the germination could be combined using a medium supplemented with ABA (1.75 µM), BAP (0.5 µM) and GA3 (20.0 µM) (Little *et al*., 2000; Ahmed *et al*., 2011).

In vitro microcorm formation

Saffron is a sterile plant, therefore, the percentage of regenerated corms under natural conditions is low (Chahota *et al*., 2003). Consequently, *in vitro* production of microcorms is promising in terms of multiplication rate and number of microcorms produced (Table 1).

During micropropagation, it was found that the shoots, developed *in vitro*, tended to swell at the base and form microcorms (Gui *et al*., 1998; Sharma *et al*., 2008). Those microcorms are considered ideal for saffron

micropropagation. Some studies had also produced micro-corms from flower, leaf segments and bulbs grown on MS medium supplemented with 2 mg/L BAP and 0.5 mg/L NAA after 6 months of cultivation (Karaoğlu *et al*., 2007).

A Maximum number of microcorms was observed in MS supplemented with 2 mg/L BAP, 0.5 mg/L NAA, and 1.5 mg/L paclobutrazol or in ½ MS supplemented with TDZ (20 µM), IAA (10 µM), and sucrose (40 g/L). Those results showed an additional improvement compared to the previously developed protocols (Parray *et al.,* 2012; Mir *et al.,* 2014). Thus, a complete protocol for *in vitro* production of saffron corms was offered. The author's showed that the highest number of microcorms could be regenerated on ½ MS supplemented with TDZ (20 µM), IAA (10 µM) and sucrose (40 g/L). Maximum germination (90%) of those microcorms could be obtained on MS containing 20 µM BAP, and 15 µM NAA (Parray *et al*., 2012).

In vitro regeneration and formation of microcorms may be provided by direct organogenesis from corms meristematic regions. A study showed that MS supplemented with 1 mg/L IAA had the best results in terms of percentage of corm formation (76.7%). The protocol described in this study could contribute to the growth and modernization of saffron agriculture and biotechnology (Çavusoglu *et al*., 2009).

For many cultivated species, sucrose/carbohydrate concentration is the most determining factor in cormogenesis. A higher sucrose concentration (6 to 9%) promotes the production of microcorms. No form of microcorms in the absence of sucrose and very little in mannitol (Staikidou *et al*., 2005; Madubanya *et al*., 2006).

Direct plant regeneration

Direct organogenesis is an alternative method of somatic embryogenesis, as this latter is ineffective and the corms regeneration from this method remains low (Sharma *et al*., 2010). Direct shoot regeneration can be carried out from apical and/or lateral buds, corms, and ovaries (Table 1).

Direct shoots regeneration is an infrequent event in monocotyledons and especially in lridaceae family. Shoots that come directly from explant tissue can provide a micropropagation method and develop protocols for genetic transformation (George and Eapen, 1993).

Dozens of mediums supplemented by various growth regulators were used. Among these growth regulators, 2,4-D, BAP, IAA, Indole-3-butyric acid (IBA), Kn, NAA, TDZ, and Zeatin (Parray *et al*., 2012; Gantait *et al*., 2015). The effects of 2,4-D and BAP are initially tested. The combination of 1 mg/L 2,4-D and 1 mg/L BAP was favorable for direct organogenesis (Simona *et al*., 2013).

However, BAP, Kn, or Zeatine are the most frequently used in combination with auxins. Also, TDZ alone can induce multiple shoots from corm explants (Sharma *et al*., 2008). Direct shoots regeneration from *C. sativus* ovaries was influenced by medium components, incubation conditions and explant age (Homes *et al*., 1987). The best caulogenesis response (28%) was observed when MS was supplemented with NAA and BAP (Bhagyalakshmi, 1999). The cultivation in dark and at 20 °C was beneficial for shoot induction (Bagheri and Vesal, 2006). It should be noted that BAP is effective in inducing direct organogenesis from buds and corms (Majourhat *et al*., 2007).

An effective protocol was provided for saffron regeneration using apical bud cells, grown on MS supplemented with different concentrations of 2,4-D, BAP and NAA. The maximum percentage of shoot regeneration (75%) was obtained on MS containing 0.5 mg/L BAP. Results of this study revealed that the mice layers of bud cells are suitable for direct saffron organogenesis (Azadi *et al*., 2017). For shoots regeneration from callus, the combination of NAA (5 mg/L) and TDZ (5 mg/L) proved to be the best one (Vahedi *et al*., 2015).

Mutagenesis and Abiotic Stress

Induced mutagenesis at Crocus sativus

For plants with vegetative growth, such as saffron, explants mutagenesis could be an ideal technique to strengthen the genetic variation in the population and the rapid multiplication of mutant clones (Donini and Sonnino, 1998). The economic impact of saffron is important because of its high price, as it presents a strong added value. Considering this economic importance and the factors responsible for its low yield and the lack of interest in cultivation by producers, a study is carried out to establish an *in vitro* mutagenesis protocol and identify mutagenic concentrations appropriate in order to induce maximum variability in saffron. Ethyl Methane Sulfonate (EMS)-induced mutagenesis has a significant impact on growth percentage and *in vitro* plant regeneration. EMS concentration (0.1% to 0.5%) showed varied survival of the explants, while a concentration of more than 0.6% was inhibitory (Perera *et al*., 2015; Kashtwari *et al*., 2018).

In vitro mutagenesis using EMS in saffron has not yet received attention. Previous scientific reports on the mutation induction were entirely *in vivo* using physical and chemical mutagens (Nehvi *et al*., 2010; Khan *et al*., 2011; Salwee and Nehvi, 2014). It is widely emphasized that EMS can induce nuclear as well as a cytoplasmic mutation in cultivated plants (Akhund *et al*., 1975).

New variants are created to increase the production of saffron corms by inducing, *in vivo*, mutations using physical (gamma rays) and chemical mutagens (EMS, colchicine, and bromide ethidium) at different stages of corms growth. The three treatments, 0.1% EMS; 0.05% colchicine, and 0.2 Kr gamma rays, allowed to have the highest number of corms (Zaffer *et al*., 2004).

EMS, ethidium bromide, colchicine and physical mutagens at different doses had a pronounced effect on saffron growth. Corms were subjected to different doses of gamma rays ranging from 0.5 to 3.0 Krad to induce mutagenesis *in vivo*. Variations in germination time, plant height, flowering induction, number, and form of petals were highlighted. An increase in plant height at lower doses (0.5 and 1.0 Krad) and rapid induction of flowering at 1; 1.5, and 2.0 Krad were noted (Zaffer *et al*., 2004). It could be noted that Physical and chemical techniques of induced mutagenesis can be used to increase the multiplication rate of corms and create new traits.

Salt and water stress applied to Crocus sativus

According to its vegetative reproduction, saffron has a narrow genetic base, and induced *in vitro* variations offer opportunities for expanding new cultivars. A study was carried out by Shahabzadeh *et al*. (2013) to evaluate the variations in saffron culture induced by Sodium Azide (NaN_3) in order to increase salt tolerance. Some Corms explants were subjected to various concentrations of NaN_3 and NaCl. Thus, the active components (crocin, picrocrocin, and safranal) were measured by High-Performance Liquid Chromatography (HPLC). Results showed that the variations in NaN_3-treated plants were wider for fresh callus weight, embryo weight, and regenerated corms compared to untreated plants. It can be noted that sodium azide is known to create point mutation in the plant's genome and consequently to expand variations in treated plants (Khan *et al*., 2009).

Some experiments were designed by using nano-silver particles for saffron subjected to stress conditions in order to study the effects of those treatments on corms. Results showed that nano-silver with 50-80 ppm concentrations could reduce the deteriorating effects of salt stress and increase antioxidant activity (Sorooshzadeh *et al*., 2012; Sharma *et al*., 2012; Namin and Azari, 2017).

Salt stress and stress-related hormones as ABA are the strongest inducers of proline accumulation (Razem *et al*., 2006; Azizian *et al*., 2014). Hossain *et al*. (2015) were reported that a remarkable reduction in total soluble protein was observed with an increase in amino acid concentration, and antioxidant enzyme activities, which are essential to modulate oxidative stress induced by nano-silver particles.

Salinity effects on saffron growth rate was carried out by many studies. Results showed that water salinity, fertilizer contents, and planting methods had a significant effect on leaves dry matter. A decrease in leaves dry matter was observed when salinity increased to 4.0 dS. However, corms yield increases considerably with slight salt stress (Azizian and Sepaskhah, 2014; Yarami and Sepaskhah, 2015). Also, the effect of NaCl resulted in a decrease of chlorophyll but an accumulation of proline, soluble sugars, and total phenols. These metabolites acted to help saffron against salinity (Mzabria *et al*., 2017).

Some studies revealed the possibility of using salt water, up to 5.5 dS/m, for saffron production in saline areas. In addition, foliar application of nitrogen could increase the production of salt-stressed saffron (Rajaei *et al*., 2009; Ghoreshiand and Moodi, 2015). The effects of irrigation levels and planting density on crocin, picrocrocin, and safranal contents were studied. It should be noted that relatively, high water stress increases secondary metabolites in saffron stigma and could decrease daughter corms growth (Koocheki and Sayyedi, 2016). In addition, several authors showed that total available water had an important role in determining the flowering time of saffron under salinity (Koocheki *et al*., 2014; Koocheki *et al*., 2016). Developed aerial organs stimulate root growth which improves the ability to absorb water and nutrients. A reduction in photo-assimilates can lead to sugar accumulation in leaves promoting their senescence. Renau-Morata and co-workers highlighted that water stress can decrease the photosynthesis rate and slightly reduce the osmotic potential of leaves in stressed plants. In contrast, proline was slightly elevated (Renau-Morata *et al*., 2012).

The problem of salinization of water and soil resources is greater in arid and semi-arid areas, where the main saffron areas are located. Good quality water resources in these areas are limited, therefore, saline water should be used for saffron production. Saffron is known as a salinity-sensitive plant (Yarami and Sepaskhah, 2015), however, it appears that by adopting the above approaches, it is possible to produce saffron using brackish water (Koocheki *et al*., 2016).

Main Compounds and Biological Activities

Significant progress made on various phyto-biochemical components, pharmacological properties and isolation of biological actives molecules of saffron (Gadd, 1971). Results of some studies revealed a chemical composition containing proteins, fats, wax, minerals, crude fiber, and sugars, including starch, reducing sugars, pentosans, gums, pectin, and dextrins (Table 2). Furthermore, traces of vitamins, and thiamine were identified (Rubio-Moraga *et al*., 2009; Harvanaghi and Arkun, 2018).

Phytochemical compounds of Crocus sativus

Saffron stamens, petals, styles and flowers were evaluated for polyphenols, flavonoids, tannins, alkaloids, proteins, fats, ashes, and fiber contents. Those results could contribute to better management and exploitation of saffron (Table 2). Chemical analysis of *C. sativus* stigmas showed the presence of approximately 150 volatiles and non-volatiles compounds (Bathaie and Mousavi, 2010). Volatiles contains more than 34 components as terpenes, terpene alcohols, and their esters, of which safranal is the main component. Most of non-volatile substances are carotenoids, including zeaxanthin, lycopene, and various α and β carotenes (Mir *et al.,* 2018).

Crocin and glucosyl esters of crocetin are the carotenoids responsible for characteristic saffron color. Picrocrocin is responsible for bitter spice taste, and Safranal is a monoterpene aldehyde responsible for its characteristic aroma (Figure 2) (Azarabadi and Özdemir, 2018).

Crocin ($C_{44}H_{64}O_{24}$) is one of few natural carotenoids easily soluble in water. Its high solubility is due to the carboxyl group at the end of polyene chain. This is one of the reasons why saffron is widely used as a color in food. Crocetin is mainly present as a trans-isomer and constitutes approximately 0.3% of total weight of stigmas (Melnyk *et al.,* 2010; Anjum *et al.,* 2015).

Picrocrocin ($C_{16}H_{26}O_7$) is the main element that gives saffron stability. It is a union of a safranal and a carbohydrate. During the process, picrocrocin releases aglycone ($C_{10}H_{16}O_2$) which is then, transformed into safranal (Figure 2). This latter, constitutes 70% of total volatiles (Tarantilis *et al*., 1997).

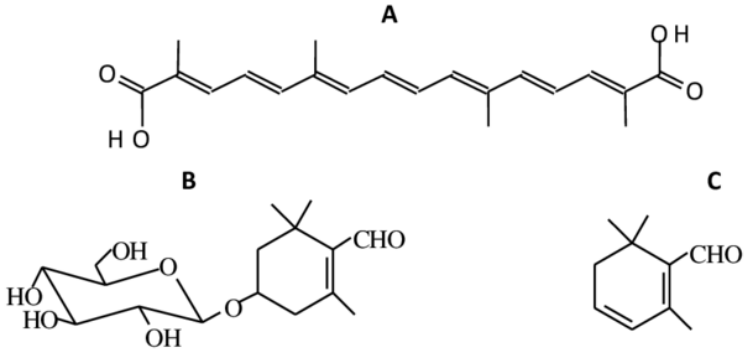

Figure 2. Chemical structures of main compounds of saffron plant. Crocetin (A); Picrocrocin (B); Safranal (C)

Table 2. Distribution and content of main phytochemicals components in *C. sativus*

Plants parts	Phytochemicals	Concentration (g/100g)	References
Stigma	Proteins	5.97-14	Srivastava *et al*. (2010); Muzaffar *et al*. (2015); Jadouali *et al*. (2018)
	Carbohydrates	12 - 65.18	Melnyk *et al*. (2010); Srivastava *et al*. (2010); Muzaffar *et al*. (2015); Jadouali *et al*. (2018)
	Riboflavin	5.02-13.86	Hashemi *et al*. (2019)
	Carotenoids	16.13	Lahmass *et al*. (2019)
	Cosine	37.80	Harvanaghi and Arkun (2018)
	Crocin	42.2	Mir *et al*. (2018)
	Flavonoids	5.88	Karimi *et al*. (2010)
	Total phenolics	0.139 – 16,63	Karimi *et al*. (2010) ; Lahmass *et al*. (2019)
	Safranal	0.65 – 64.1	Harvanaghi and Arkun (2018); Mir *et al*. (2018)
	Cis crocin 3	0.46-2.26	Alonso *et al*. (2001)
	Picrocrocin	3.69	Harvanaghi and Arkun (2018)
	Lipids	0.03 - 8	Dris and Jain (2004); Anjum *et al*. (2015)
	Fibers	4 – 13.45	Jadouali *et al*. (2018)
Petals	Protein	10.20	Fahim *et al*. (2012)
		6.35	Jadouali *et al*. (2018)
	Carbohydrates	71.16	Jadouali *et al*. (2018)
	Lipids	5.3	Fahim *et al*. (2012)
	Ashs	18.36	Kumar *et al*. (2011)
		7-7.30	Fahim *et al*. (2012) ; Jadouali *et al*. (2018)
	Fibers	11.25	Kumar *et al*. (2011)
	Linoleic acid	28.48	Kumar *et al*. (2011)
	Linolenic acid	21.06	
	Palmitic acid	16.21	
	Anthocyanins	8.52 - 16.6	Kumar *et al*. (2011); Serrano-Díaz *et al*. (2012); Feizy *et al*. (2016)
	Carotenoids	0.6	Khazaei *et al*. (2001)
	Crocetins	6.4	Khazaei *et al*. (2001)

	Flavonoids	60.64 – 129.16	Zeka *et al*. (2015); Jadouali *et al*. (2018)
	Kaemferol	12.6	Termentzi and Kokkalou (2008)
	Total phenolics	3.42 – 96.6	Sah *et al*. (2012)
Flowers	Proteins	4.3	Jadouali *et al*. (2018)
	Carbohydrates	67	
	Lipids	0.035	
	Ashs	10.95	
	Fibers	10.48	
	Flavonoids	30 - 34.23	Jadouali *et al*. (2018)
	Total phenolics	54.59 - 95	Serrano-Díaz *et al*. (2012); Jadouali *et al*. (2018)
Styles	Proteins	6.11	Jadouali *et al*. (2018)
	Carbohydrates	69.7	
	Lipids	0.015	
	Ashs	7.86	
	Fibers	7.93	
	Flavonoids	6.6 -12.17	Jadouali *et al*. (2018)
	Total phenolics	25.24 - 93.1	
Stamens	Flavonoids	14.10 - 20.2	Muzaffar *et al*. (2015); Jadouali *et al*. (2018)
	Total phenolics	35.69 - 73.2	
Corms	Total phenolics	4.23 - 6.64	Esmaeili *et al*. (2011)

Mineral composition of Crocus sativus

The mineral composition, as reflects the soil type and environmental growing conditions, is a powerful tool for saffron geographic origin identification. Mineral analysis of *C. sativus* showed that iron, sodium and zinc are major mineral elements with 727.95, 54.1, and 49.96 mg/kg respectively (Table 3).

Minerals are minor components found in saffron, which play a key role in quality control and food traceability, while also affecting human health. Moreover, the chemical elements in saffron samples from different countries have a wide range of concentrations. These differences can be affected by geographic conditions such as soil physicochemical properties, weather conditions, and other environmental conditions such as saffron cultivation and its genotype (Noori *et al*., 2022).

Table 3. Distribution and content of minerals in different parts of *C. sativus*. Mineral contents are expressed in mg/kg

Elements	Petals	Stamens	Styles	Flowers	Reference
Sodium (Na)	45.85 ± 5.44	53.35 ± 4.59	54.1 ± 2.40	45.85 ± 5.44	Jadouali *et al.* (2018)
Potassium (K)	23.75 ± 2.75	26.35 ± 1.34	23.65 ± 0.91	23.75 ± 2.75	
Calcium (Ca)	39.25 ± 1.06	14.8 ± 1.13	42.9 ± 2.26	39.3 ± 0.98	
Iron (Fe)	149.50 ± 6.2	94.76 ± 4.13	-	727.95 ± 6.4	
Zinc (Zn)	47.23 ± 5.04	49.96 ± 4.3	-	42.68 ± 4.4	
Nitrogen N (%)	1.01 ± 0.08	0.952 ± 0.012	0.38 ± 0.014	0.7 ± 0.02	

Biological activities of Crocus sativus

Since ancient times, plants have been used in all civilizations as a source of traditional medicine. For more than 3000 years, saffron has been considered a panacea. Some therapeutic properties attributed to saffron are listed below.

Antitumor and anticancer effects

Saffron is one of candidate spices whose effect on neoplastic cells has seen a renaissance in the last decade. Several scientific studies suggest the chemo-preventive effects of saffron and its extracts, both *in vivo* and *in vitro* (Salomi *et al*., 1991; Nair *et al*., 1995; Das *et al*., 2004).

The four major components of saffron were evaluated for inhibitory effects on the proliferation of human cervical carcinoma Hela cells. Results indicated that crocin produced the best inhibitory effects at concentrations >200 μM (Chryssanthi *et al*., 2007).

The significant anti-tumorigenic effects of crocetin on pancreatic cancer were highlighted. Most of these *in vivo* studies focused on bioactive compounds isolated from saffron. The additive and synergistic effects between different saffron compounds could, however, strengthen anti-cancer properties (Liu *et al.,* 2004). However, further evaluation of anti-proliferative role of saffron extract and its components in other cancer cell lines is needed. Such studies could constitute an important step in understanding of antiproliferative effect, hence the application of saffron to develop safe, and effective anticancer treatments.

The study of Dhar *et al*. (2009) showed that the potential utility of crocetin was similar both *in vitro* and *in vivo*. It has been also, reported that the LD_{50} of crocetin was very high at 2 g/kg.

Anti-cancer, chemo-preventive and tumoricidal properties of saffron molecules were reported by several studies (Magesh *et al*., 2006; Bakshi *et al*., 2020). It has been reported that crocin induced pancreatic cancer cell death via caspase 3 cleavage and mitochondrial release. Microarray data reveal that crocin upregulated genes involved in different checkpoints (cell cycle and DNA damage), and downregulated genes involved in arachidonic acid metabolism. A toxicity study showed that crocin is safe and reduces tumor growth *in vivo* (Bakshi *et al*., 2020).

Saffron extracts are already used against different types of tumors and cancers (liver, spleen, kidney, stomach, and uterus tumors) (Hartwell *et al*., 1982; Abdullaev and Espinoza, 2004; Bukhari *et al*., 2018).

Antioxidant effects

Ethanol extract from *C. sativus* had free radical scavenging activity, suggesting its use in cosmetics as a dietary supplement to treat age-related diseases (Zhang *et al*., 2019). Crocin was also found to have a higher antioxidant capacity than alpha-tocopherol in differentiated pheochromocytoma cells, the absence of which resulted in peroxidation of their membrane lipids and a decrease in the activity of intercellular superoxide dismutase (Cerd´a-Bernad *et al*., 2020).

Numerous studies showed that saffron had an important antioxidant activity for both stigmas and its by-products (Nassar *et al*., 2020; Wali *et al*., 2020). This was proved by the study conducted by Chichiricc`o *et al*. (2019) who showed that the anther water extract was found to be well tolerated by several cell lines and capable of modulating reactive oxygen species (ROS) levels, without exerting genotoxic or cytotoxic effects. The same extract was also able to blunt lipopolysaccharide (LPS)-induced nitrite and malondialdehyde (MDA) in isolated rat tissues.

Additionally, Nasser *et al*. (2020) used human myoblast cells to evaluate the protective effects of *C. sativus* L. extracts, (crocin and safranal) on hydrogen peroxide (H2O2)-induced oxidative stress using human myoblast cells. Results showed that the *in vitro*, pretreatment with safranal exhibited the lowest antioxidant effect whereas pretreatment with *C. sativus* L. extracts (0.3 μg/ml) and more notably with crocin (0.3 μM) attenuated the toxic impact of H_2O_2 (50 μM, 24 h) and also restored the capacity of adhesion among LHCN-M2 cells.

Several studies also showed that saffron extract inhibits lipid peroxidation in human platelet membranes induced by ascorbic iron-acid system (Jessie and Krishnakantha, 2005). A study showed that saffron extract was more potent than crocin, which can be attributed to the presence of extensive constituents (crocin, crocetin, dimethyl-crocetin, and flavonoids) (Magesh *et al*., 2006). Other studies found that aqueous saffron

extracts protect against genetic damage caused by antitumor agents and also inhibit genotoxin-induced oxidative stress in liver of mice and increases glutathione levels (Premkumar *et al*., 2003; Premkumar *et al*., 2006).

Antidepressant effects

Depression is a serious and widespread mental disorder. Natural herbal products and mainly saffron are widely used in traditional medicine as a tonic and antidepressant agent (Schmidt *et al.,* 2007; Lopresti and Drummond, 2007), and can be considered an alternative to synthetic antidepressants with fewer side effects (Moghadam *et al*., 2021; Razavia *et al*., 2021; Musazadeh *et al*., 2022). The antidepressant effect of saffron has been confirmed by several studies. Ahmadpanah *et al*. (2019) evaluated the effect of saffron treatment (60 mg/d) and (100 mg/d) for six weeks of 50 elderly patients using Hamilton Depression Rating Scale to assess participants' degree of depression. These results were all the more relevant, as saffron appears to be a potent antidepressant for the elderly, who may be more reluctant to use synthetic drugs (Ahmadpanah *et al*., 2019).

Anti-nociceptive and anti-inflammatory effects

Saffron extracts showed anti-nociceptive effects in induced pain tests, as well as acute and/or chronic anti-inflammatory activity (Byrami *et al*., 2013; Hashemzaei *et al*., 2020). The preventive effects of *C. sativus* extracts on tissue inflammation were indicated using different animal models. The treatment of sensitized animals with *C. sativus* extracts (0.1, 0.2, and 0.4 mg/mL) prevented the increase in total white blood cells (WBC), eosinophil, and lymphocyte numbers. In addition, it was observed that the effect of extracts on WBC was similar to dexamethasone (Bayrami *et al*., 2012).

The anti-inflammatory potential of tepal extracts was evaluated *in vitro* (human red blood cells) and *in vivo* (rats). Tepal extract (400 mg/mL) compared with Diclofenac (10 mg/ml) for *in vitro* and *in vivo* models showed similar anti-inflammatory activity. the percentages were 63.16% and 71.05%, respectively for tepal extract and Diclofenac (Bhat *et al*., 2012). Anti-nociceptive and anti-inflammatory effects of *C. sativus* extracts were attributed to their content in flavonoids, tannins, anthocyanins, alkaloids, and saponins (Hosseinzadeh *et al*., 2000).

Potential role of saffron during COVID-19

COVID-19 represents the most significant pandemic of the 21st century. This situation has led some researchers to exploit traditional medicine, in particular natural products that stimulate the immune system (Florindo *et al*., 2020; Mertes *et al*., 2021)). Saffron has been used for centuries to treat fever, bronchitis, colds, and other immune and respiratory disorders (Mokhtari-Zaer *et al.,* 2020). According to recent studies, there are several side effects of COVID-19 which include fatigue, depression, sleep abnormalities and deterioration in quality of life (Chopra *et al*., 2020; Huang *et al*., 2021).

Saffron may be a suitable candidate for managing anxiety, depression, neuropsychiatric disorders, and other side effects. The anti-inflammatory, antioxidant, and other medicinal properties attributed to bioactive compounds in saffron may help in pre- and post-COVID-19 infection management (Husaini *et al*., 2021).

The constituents of saffron are used in several drug formulations that can be used in the treatment of cardiovascular and central nervous system diseases as well as boosting immune function and treating depression (Mohajeri *et al*., 2020). The development of a saffron-based formulation and its commercialization may help provide a drug for effective management of adverse effects of COVID-19.

Diabetes disease

Diabetes is one of the diseases that can damage cells in various parts of the body. Studies have shown that the number of diabetic patients is increasing dramatically worldwide (Kooshki *et al*., 2020; Ceriello *et al*.,

2022). Thus, the use of complementary and alternative medicine in diabetic patients seems to be growing. Certain herbs such as saffron can control inflammation and play a major role in reducing the effects of diabetes (Azgomi *et al.,* 2022). Numerous studies showed that consuming 15 mg of saffron extract daily for eight weeks remarkably reduced fasting blood sugar (Milajerdi *et al*., 2018; Ebrahimi *et al*., 2019). Mobasseri *et al*. (2020) conducted a study of the saffron powder on 60 patients with type 2 diabetes divided into two groups. The first one was treated with 100 mg/d of saffron powder and the other group received one capsule of starch per day for a period of 8 weeks. Saffron has been reported to modulate glucose levels as well as the state of inflammation in patients with diabetes by decreasing the expression levels of certain inflammatory mediators (Mobasseri *et al*., 2020). Samarghandian *et al*. (2017) were able to show that saffron significantly reduced blood sugar, malondialdehyde, nitric oxide, total lipids, triglycerides, and cholesterol levels in diabetic groups treated with saffron compared to the control group. Thus, diabetic rats treated with saffron inhibited the expression of inflammatory cytokines compared to untreated diabetic rats. These results validate the use of saffron as a treatment for diabetes.

Conclusions

Saffron is the most valuable medicinal food product due to its importance in the sustainable development of the production areas of this spice. The dried stigmas of the *C. sativus* plant are used as a well-known spice which has other utilization in the pharmaceutical industries, textile dyes, and cosmetics.

The micropropagation of saffron still has many difficulties encountered mainly in conventional systems that only modern biotechnology-based breeding methods have the potential to solve. For example, certain fields must be exploited very intensively, such as somatic embryogenesis, synthetic seed production by encapsulation, *in vitro* conservation and especially the intervention of genetic engineering. The effect of carbon sources on regeneration via organogenesis or somatic embryogenesis could be studied using different carbon sources (glucose, fructose, maltose and sucrose) at different concentrations.

The phytochemical compounds of *C. sativus* are recognized as the most effective bioactive constituents in medicine and various studies have confirmed the anti-inflammatory, the immunomodulatory and the antioxidant effects of our plant. Therefore, *C. sativus* and its constituents could have therapeutic value. Besides the well described and widely used antioxidant properties, saffron has multiple interests for cosmetic applications such as anti-sun, anti-pigmentation and anti-aging activities, and could also be used as a pigment or in perfumes. Although the data from the experimental studies appear compelling, more clinical trials are needed to confirm the commercial use of *C. sativus* as a drug for the treatment of different diseases.

Authors' Contributions

Data curation: CS, CR; Supervision: YG, MB, LG, AL, Writing -original draft: CS; Writing-review and editing: CS.

All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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