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Crocus Officinalis (L.) Extract on Human Colerectal Cancer Cell Line (HT-22): Investigation in Vitro

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Research Article

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ABSTRACT

Crocus sativus L. has been used as spice, food colouring and medicinal plant for thousands of years. In this study, antioxidant properties and cytotoxic effect of saffron extract were evaluated in HT-22 cell lines. Malignant and non-malignant cells (L929) were cultured in DMEM medium and incubated with different concentrations of saffron extract in different solvents (water, ethanol, ethyl-acetate, n-hexane). Cell viability was determined by MTT assay. Antioxidant parameters such as DPPH, FRAP, total polyphenol and flavonoid amounts were measured. The highest DPPH value was found as 15.90±0.12 µg/mL in ethyl acetate solvent. Total polyphenols were 89.06±0.02 mg GAE/g extract, flavonoids were 76.98±2.08 µg QE/g extract and FRAP was 67.93±0.98 mg/g extract in ethanol solvent. In colon cancer, IC50 value 94±1.37 µg/mL was found to be the highest value. This is thought to be due to the high antioxidant capacity in ethanol solvent. While antioxidant capacity was not found to be high, antiproliferation effect was found to be high. Saffron may also be considered as a promising chemotherapeutic agent in cancer treatment in the future.

Keywords: Antioxidant parameters, Crocus sativus L., HT-22 cell lines, Saffron.

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Introduction

Cancer is increasingly becoming the biggest health problem all over the world. Natural products have long been used in the prevention and treatment of many diseases, including cancer, due to their low side effects. For this reason, studies on natural plants are increasing today and it is being investigated whether they are good drug candidates [1]. Colon cancer, which is among the top five cancer types in the world, occurs in the gastrointestinal system. According to the 2018 estimated data of the World Health Organization, colon cancer was found in 1.80 million cases and ranks second in the world with 862 thousand among cancer cases resulting in death [2].

Today, many additives such as preservatives, colourants and oxidation inhibitors are widely used in food. However, in recent years, there has been a very rapid increase in use and 200.000 tonnes of additives are used in food annually. Despite the increasing use of additives today, the awareness of consumers increases the use of additives obtained from natural sources. In line with these developing demands, manufacturers pay more attention to the use of additives obtained from natural sources in their production [3]. Saffron is a spice of natural origin used to give colour and flavour in food and is a natural substance that has proven to have positive effects on health.

Saffron is a plant used as a medicine in Anatolia since the Hittite period. It is known that saffron has been used in herbal treatment since ancient times due to its antidepressant, sedative, anodyne, decongestant, antispasmodic, diaphoretic and expectorant properties [4]. Crocus sativus L. (saffron) is a 20-30 cm tall perennial cultivated plant of the crocus genus belonging to the Iridaceae (irises) family growing in Mediterranean and Asian countries [5]. Saffron (Crocus sativus L.) is widely cultivated in arid and semi-arid regions of Iran [6]. The stigmas and stigmas of the blue-purple coloured flower are called saffron [7]. Saffron is cultivated for flavouring food, for the production of cosmetics and perfumes, and for medicinal purposes. Carotenoids, phenolic groups and flavonoids in the structure of foods are the structures responsible for the antioxidant effect. Saffron (Crocus sativus L.) is an important plant that can be used in functional food production because it has high antioxidant capacity [8]. "Crocin" is a water-soluble natural dye among carotenoids with high colouring power and golden yellow colour. It also has a strong antioxidant and anticancer effect together with picroxin and safranal. In addition, picroxin and safranal are sought after special flavour and aroma substances. These compounds are also effective in determining the quality of saffron.

Saffron contains proteins, fats and carbohydrates, as well as some valuable compounds such as vitamin A, vitamin C and folic acid. Saffron is also a rich source of minerals such as iron, calcium, magnesium, copper, manganese, potassium, phosphorus and zinc. It also

contains some vital metabolites, monoterpenoids, flavonoids, carotenoids and anthocyanins. Saffron contains different chemical constituents that fluctuate greatly depending on growing conditions. Chemical composition analyses showed that saffron contains about 50%-63% sugar (dextrin, starch, pectin, gums and pentosans), 12%-13% protein, 10%-12% moisture, 5%-8% fat, 5%-7% minerals and 5%-10% crude fiber. According to the analysis, the essential oil of saffron tepals and tepals (one of the outer parts of the flower) differed in composition. The essential oil of tepals contains crocetin, crocin, picrocrocrocin and safranal as main components [9,10].

Studies conducted with saffron extract and its components have shown that they have anticarcinogenic [11], antigenotoxic [12], antioxidant [13], anti-inflammatory [14], antihypertensive [15], anti-obesity [16], liver protective [17] properties.

In this study, it is aimed to investigate the antioxidant and anticarcinogenic properties of saffron, which is also grown in Iran.

Materials an Methods

Preparation of Extracts

The collected Crocus Officinalis (L.) were collected Iranian. Iranian saffron was obtained from Bahraman company, which grows and supplies saffron to the market in Mashhad city, Khorasan Razavi province. Crocus Officinalis (L.) were washed with tap water, then distilled water and dried in the laboratory for 2 weeks. The dried samples were ground and 100 gram samples were extracted twice in 1000 mL of different solvents (water, ethanol, n-hexane and ethyl acetate solvents) at 45°C for 45 min in an ultrasonic bath. The extracts were filtered through Whatman No.1 filter paper and concentrated in vacuo to obtain the extracts. The extracts obtained were stored at -20 °C for use in experimental procedures.

Determination of Antioxidant Properties

Determination of radical scavenging activity (DPPH)

DPPH (2,2-diphenyl-1-picrylhydrazyl) assay was performed according to the procedure developed by Cuendet et al. Ascorbic acid was used as a reference standard. DPPH was dissolved in methanol solution (0.004 g/100mL). According to this method, the sample was mixed with DPPH radical, incubated in the dark for 30 minutes and the absorbance was measured at 517 nm in a spectrophotometer [18].

Determination of iron (Fe+3) reducing power in extracts

FRAP test developed by Benzie and Strain (1996) and modified by us was performed [19]. Reducing agents such as antioxidants cause the ferricyanide (Fe+3) complex to be reduced to Fe+2. In this method, this reduction property is utilised and the colour changes from yellow to green depending on the reducing power of the sample tested. The resulting green colour gives maximum absorbance at 700 nm. increasing absorbance indicates an increase in the reducing power. According to this method, trolox was used as standard antioxidant compound.

Total flavonoid content determination

Total flavonoid content (TFC) of the extracts was determined by aluminium chloride method. The determination of total flavonoids using $AlCl_3$ is based on the formation of stable complexes of flavones and flavonols between $AlCl_3$, keto and hydroxyl groups. In addition, $AlCl_3$ also involves the formation of complexes with ortho-dihydroxyl groups of A- or B- rings of flavonoids. According to this method, quercetin was used as standard [20].

Estimation of Total Phenolic Content (TPC) Determination

Folin–Ciocalteu's assay and colorimetric method were used to evaluate the total phenolic content [21]. The Crocus Officinalis (L.) extract (1 mg/mL) was mixed with 1 mL Folin-Ciocalteu reagent and then 5 mL distilled water was added. After 5 minutes, 1 mL of sodium carbonate (10%) was then added. The final mixture was then incubated in the dark at room temperature for 60 min and the absorbance was measured against the blank at 725 nm using a UV-VIS spectrophotometer. The total phenolic content of the extract was calculated from the standard gallic acid (10-250 mg/L) calibration curve and expressed as mg gallic acid equivalent (GAE) per gram dry extract weight.

Cell Culture

Cells

In this study, colon cancer (HT-22-HTB-38™) cell lines and fibroblast cells (L-929) were grown in DMEM medium containing 10% (v/v) fetalbovine serum (FBS), 1% penicillin-stretomycin, in an incubator at 5% CO2, 95% humidity and 37°C.

Cytotoxicity tests

The cytotoxic effects of Crocus Officinalis (L.) extract, which was found to be the most effective antioxidant, on colon and healthy cells were performed using MTT method. Firstly, cells were seeded in plates at 5×10^4 cells/well. All cells were treated with different concentrations (100, 50, 25, 12.5, 6.25, 3.125, 1.56 ug/mL) of the extracts and incubated for 24 hours. After incubation, MTT solution was added and incubated for 2-4 hours. After the plate was poured, 100 uL DMSO was added and formazone crystals were formed. Absorbance values were obtained by reading on an ELISA reader at 545 nm [22].

Results

Antioxidant Parameters

DPPH radical scavenging activity was determined for different concentrations of Crocus Officinalis (L.) extracts. For this purpose, the % inhibition values at each concentration were calculated from the absorption values obtained and plotted against the concentration. The following formula was used in the calculation.

$$I\,(\%) = \left[\frac{A_{blanc} - A_{sample}}{A_{blanc}}\right] \times 100$$

Table 1. DPPH values

	Water	Etanol	Ethyl acetate	n-hexane	Ascorbic Acid
µg/mL	23.04±0.02	31.67±0.30	15.90±0.12	22.03±0.06	4.94±0.06

** The values are given as mean ± standard deviation.

IC_{50} value of Crocus Officinalis (L.) extract was 23.04±0.02 µg/mL water, 31.67±0.30 µg/mL ethanol, 15.90±0.12 µg/mL ethyl acetate, 22.03±0.06µg/mL n-hexane. The IC_{50} value of ascorbic acid was found to be 4.94±0.06 µg/mL (Table 1).

Crocus Officinalis (L.) extract was analysed according to the method of Chang et al. The amount of total phenolic matter was calculated as gallic acid equivalent [19]. The concentrations of total flavanoid compounds in Crocus Officinalis (L.) extracts (200, 100, 50, 25, 12.5, 6.25, 3.125, 1.562 µg/mL) were calculated as quercetin equivalent from the equation obtained from the quercetin standard graph calibration curve given y = 0.0032x+0.0768 (R2=0.9997) [18].

Table 2. Total phenolic and flavonoid content of different solvents extracts

	Water	Etanol	Ethyl acetate	n-hexane
Total phenolic content (mg GAE/g extract)	44.16±0.04	89.06±0.02	53.26±0.04	48.43±0.01
Total flavonoids content (µg QE/g extract)	31.09±1.08	76.98±2.08	56.98±1.46	60.98±2.04

** The values are given as mean ± standard deviation.

In the analysis of extracts according to Benzie and Strain method, the amounts of FRAP antioxidant power were calculated as trolox equivalents. The concentrations of iron reducing compounds (200, 100, 50, 25, 12.5, 6.25, 3.125, 1.562 µg/mL) in extract were calculated as equivalent to trolox from the equation obtained from the calibration curve of the trolox standard graph.. The obtained graph equation was found as y = 0.0031x-0.0012 (R2=0.9999).

The results of FRAP antioxidant power of Crocus Officinalis (L.) extracts obtained from ethanol, water, ethyl acetate and n-hexane solvents are given in Table 3.

Table 3. FRAP values

	Water	Etanol	Ethyl acetate	n-hexane
(mg/g)	42.38±1.29	67.93±0.98	5.83±1.04	50.82±1.90

** The values are given as mean ± standard deviation.

Cell Culture

In the study, Crocus Officinalis (L.) extracts prepared in water, ethanol, ethyl acetate and n-hexane solvents were applied to colon cancer in cell culture with 24 hours incubation. IC_{50} values were calculated and given below (Table 4 and Table 5).

Table 4. IC_{50} values of Crocus Officinalis (L.) calculated in different solvents in colon cancer

	Water	Etanol	Ethyl acetate	n-hexane
(ug/mL)	178±1.73	94±1.37	157±2.09	143±1.03

** The values are given as mean ± standard deviation.

Table 5. IC_{50} values of Crocus Officinalis (L.) calculated in different solvents in healthy cell line

	Water	Etanol	Ethyl acetate	n-hexane
(ug/mL)	318±2.91	159±2.65	230±1.09	258±1.78

** The values are given as mean ± standard deviation.

Discussion

C. sativus L. is a plant widely cultivated in different parts of the world for nutritional and economic purposes [23]. Different solvent extracts of C. sativus L. show different pharmacological effects [24]. As a result, in the present study, four different extracts of C. sativus L. were obtained and their antioxidant activities and anticancer effects were investigated.

Flavonoids are important because of their capacity to act as various antioxidants [25]. They are commonly found

in phenolic compounds and are reported to be potent antioxidants [25]. Recent studies have shown that phenolic compounds have a possible protective role against oxidative disorders [26].

Karimi et al. (2010) analysed the total phenolic content of Greek methanol saffron extracts by Folin-Ciocalteu method and found the phenolic content to be 6.5 mg GAE/g. This study, they were found that Total flavonoid content was measured as 5.8 mg /g (26). Makhlouf et al. (2011) determined the total phenolic content of saffron flowers grown in Lebanon by Folin-Ciocalteu method and found 16 mg GAE/g. Havva et al. (2010) measured the total flavonoid content as 2.9 mg/g in a similar study on saffron extract. While Kariminin found the inhibition percentage of saffron extracts by DPPH method as 55% in his study, Hawa et al. (2010) found the inhibition percentage of ethanol and water extracts of saffron by DPPH method as 50% in pure ethanol and 59% in pure water [27,28,29].

IC50 value of Crocus Officinalis (L.) extract was found 23.04±0.02 µg/mL for water, 31.67±0.30 µg/mL for ethanol, 15.90±0.12 µg/mL for ethyl acetate, 22.03±0.06µg/mL for n-hexane. The IC50 value of ascorbic acid was found to be 4.94±0.06 µg/mL (Table 1).

Total phenolic different solvents extracts were found 44.16±0.04 mg GAE/g extract for water, 89.06±0.02 mg GAE/g extract for ethanol, 53.26±0.04 mg GAE/g extract for ethly acetat, 48.43±0.01 mg GAE/g extract for n-hexane. The flavonoid content were determined 31.09±1.08 µg QE/g extract for water, 76.98±2.08 µg QE/g extract for ethanol, 56.98±1.46 µg QE/g extract for ethly acetat, 60.98±2.04 µg QE/g extract for n-hexane. According to FRAP analyses were found 42.38±1.29 mg/g extract for water, 67.93±0.98 mg/g extract for ethanol, 45.83±1.04 mg/g extract for ethly acetat, 50.82±1.90 mg/g extract for n-hexane. The total phenolic, FRAP, and flavonoid content observed in our study showed that the antioxidant capacity of C. sativus was low. However, in our study, the highest antioxidant capacity was observed in the extract made with ethanol solvent.

In this study, the cytotoxic and apoptogenic effects of ethanolic saffron extract in HeLa and HepG2 cell lines were investigated. According to the data obtained, it was found that saffron extract had more cytotoxic activity against HeLa and HepG2 cell lines than non-malignant cells tested [30]. Studies from around the world have shown that polyphenols found in medicinal plants suppress cell proliferation and induce apoptosis [31]. A small number of cell line studies have proven the anti-cancer effect of C. sativus leaves. Saffron and crocetin have been reported to induce apoptosis in human breast cancer cells via p53-mediated apoptosis stimulation [32]. In our study, we applied saffron extracts to colon cancer cells. Our data showed that saffron extract had greater cytotoxic activity against HT-22 cell lines than against non-malignant cells tested, which is consistent with previous studies showing that saffron and its constituents have anti-tumour and anticarcinogenic activities.

Conflicts of interest

There are no conflicts of interest in this work.

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