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# Evaluation of the Effects of Organic and Conventional Cultivation Practices on Phytochemical and Anti-Cancer Activities of Saffron (*Crocus sativus* L.)

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## ABSTRACT

**Saffron (*Crocus Sativus* L.) is a medicinal plant with high nutritional, medicinal value, and anticancer properties that have great cytotoxic effects on cancer cells. To evaluate the anticancer effects of stamen and tepal extracts of saffron on human breast cancer cells (MCF-7), a factorial experiment based on a completely randomized block design with three replications was conducted at the Agricultural Research Field of the University of Birjand, Iran. The treatments included field age (one-, two-, and three-year-old farm), as well as organic and conventional cultivation with different levels of manure and chemical fertilizer (low, medium, and high levels of chemical fertilizer and manure), respectively. The studied traits including Total Phenolic Content (TPC), antioxidant activity, and cytotoxicity were evaluated by using, respectively, 2,2-Diphenyl-1-Picrylhydrazyl (DPPH), Ferric Reducing Antioxidant Power (FRAP), and A 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenylte- tetrazolium bromide (MTT) tests. The results showed a significant difference among the phytochemical, antioxidant, and anti-cancer properties of the extracts obtained from organic and conventional conditions, the highest of which was obtained from organic cultivation. In addition, the content of antioxidants and therapeutic compounds in the extracts increased by increasing the level of manure. The result of the MTT test showed that both tepal and stamen extracts of saffron had an anti-proliferative effect on cancer cells, with stronger anti-cancer properties for stamen extract. Therefore, the use of stamen extract as an effective and inexpensive source for the pharmaceutical industry would open up new dimensions to prevent the therapeutic challenges of breast cancer.**

**Keywords:** Breast cancer, Cytotoxicity, DPPH, FRAP, MTT Test, Pharmaceutical industry.

## INTRODUCTION

Saffron (*Crocus sativus* L.) is one of the oldest medicinal plants and a member of the large family of Iridaceae that have been popular in medical science because of anti-inflammatory and anti-cancer properties. Pharmacological experiments have established numerous beneficial attributes including radical scavenging, antimutagenic and immunomodulating effects (Cardone *et al*., 2020). Chemical analysis has shown the presence of more than 150 active metabolites in saffron stigmas. The more powerful components of saffron are flavonoids and phenols, along with the main secondary metabolites, which have a strong potential for cleansing free radicals. They prevent free radical reaction through different methods because of possessing

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antioxidant properties, which increase cancer cell death and decrease cancer cell viability (Farahi *et al*., 2018). Most of these phenolic compounds are identified in saffron petals, which possess considerable antioxidant activity and are considered responsible for their medicinal properties in treating a lot of important human diseases (Pandita, 2021; Kianimanesh *et al*., 2021).

Breast cancer is the most frequent cancer in women all over the world, and is still the second leading cause of cancer-related death (Riis, 2020). Currently, a combination of various techniques such as radiation therapy, chemotherapy, immunotherapy, and surgery are used. Despite recent progress, most of these strategies and combinations adversely affect the patient's quality of life through severe side effects (Raju *et al*., 2020). Unfortunately, conventional chemical drugs also cause adverse side effects on normal cells/tissue (Nguyen, 2020). Therefore, one of the main challenges in breast cancer research is to look for new, effective therapeutic options with fewer side effects to control morbidity and mortality rates (Preveen *et al*., 2021). Utilizing medicinal plant extract for treating cancers, especially breast one, can be regarded as an accessible and cheap source for patients, along with decreasing the side effects of synthetic drugs (Agrawal *et al*., 2020; Alabi *et al*., 2020).

Considering the importance of saffron as a medicinal plant, its proper and optimum cultivation through different farming managements should replace its current cultivation. Therefore, proper nutritional management through utilizing organic and chemical fertilizers, along with appropriate soil and climatic condition, is considered essential in growing saffron for exploiting the potential of the environment and achieving maximum yield (Behdani *et al*., 2008). Organic inputs can guarantee both agricultural production and nature conservation. The current approach is to find an appropriate substitute of chemical fertilizers by organic compounds that are cost-effective and eco-friendly (Bakhtiari *et al*., 2020). Besides, it is proved that various cultivation ways of medicinal plants can improve their profitability and make an important contribution to human health (Sing *et al*., 2017). Nowadays, the tendency for medicinal plant production and demand for natural crops are increasing in the world. In addition, many herbal drug manufacturing companies prefer the plant compounds produced through organic cultivation because of increasing their medicinal quality by utilizing organic and bio-fertilizers (Hazell, 2019).

Recently, many researches have focused on assessing the essential metabolites of saffron stigma as an antioxidant-rich source. To the best of our knowledge, no study has been conducted on the antioxidant properties of the saffron stamen, therefore, the present study was aimed to evaluate the antioxidant and anti-cancer properties of aqueous extracts of tepal and stamen extracted from saffron cultivated in organic and conventional conditions in relation to human breast cancer cells.

## MATERIALS AND METHODS

In order to evaluate the antioxidant and anticancer effects of stamen and tepal extracts of saffron plants on human breast cancer cells (MCF-7) an experiment was conducted in a factorial form based on Randomized Complete Block Design (RCBD) with three replications at the Agricultural Research Field of University of Birjand, (32° and 53' North and longitude of 59°and 13' East), 1,480 m asl. Experimental factors included organic and conventional cultivation each of them at three levels (low, medium, and high levels of chemical and organic fertilizer) and field age. For this reason, three farms with different ages were selected: three-years-old farm (cultivated plants in 2016), two-years-old farm (cultivated plants in 2017), and one-year-old farm (cultivated plants in 2018). In every year, the treatments (organic and conventional cultivation with different managements) and their sub treatments (low,





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medium, and high levels of chemical and organic fertilizer) were done in the same places (with the same soil analysis). The harvesting time of flowers at these farms with different ages was only in the 2019 year.

After preparing the seedbed and before planting, the needed manure and chemical fertilizers were added to the soil. For this reason, plots of the organic and conventional treatments were separated by two meters to prevent their interaction. The results of the chemical analysis of animal manure are shown in Table 1. Studied traits included the Total Phenol Content (TPC) as well as anthocyanin and the destructive effect of the extract on oxygen free radicals (DPPH), antioxidant property (FRAP), IC50 [Different concentrations of Tepal and Stamen (0– 13.5 mg mL$^{-1}$ were added to the cells], along conducting cellular experiments to assess the effect of cytotoxicity on breast cancer cells (MTT test).

## Experimental Treatments Management:

- Organic Farming System (Low levels of manure, OFS-L): Use of 15 t ha$^{-1}$ of cow manure.
- Organic Farming System (Medium levels of manure, OFS-M): Use of 30 t ha$^{-1}$ of cow manure.
- Organic Farming System (High levels of mManure, OFS-H): Use of 45 t ha$^{-1}$ of cow manure.

In this cultivation way, weed control was done manually.

- Conventional Farming System, (Low levels of chemical fertilizer, CFS-L)

Disinfection of saffron corm with benomyl before cultivation at the rate of 1.5%. The use of 100 kg ha$^{-1}$ of triple superphosphate, annual application of 100 kg NPK (in three times), annual application of supergiant herbicide (1.5 L ha$^{-1}$), spraying liquid fertilizer containing micronutrients in late March each year (2 L ha$^{-1}$).

- Conventional Farming System, (Medium levels of chemical fertilizer, CFS-M)

Disinfection of saffron corm with benomyl before cultivation at the rate of 1.5%. The use of 150 kg ha$^{-1}$ of triple superphosphate, annual application of 150 kg NPK (in three times), annual application of supergiant herbicide (1.5 L ha$^{-1}$), spraying liquid fertilizer containing nutrients in late March each year (4 L ha$^{-1}$).

- Conventional Farming System, (High levels of chemical fertilizer, CFS-H)

Disinfection of saffron corm with benomyl before cultivation at the rate of 1.5%. The use of 200 kg ha$^{-1}$ of triple superphosphate, annual application of 200 kg NPK (in three times), annual application of supergiant herbicide (1.5 L ha$^{-1}$), Spraying liquid fertilizer containing nutrients in late March each year (6 L ha$^{-1}$).

## Plant Materials

In this regard, the flowers were harvested for one week in early morning (6-8 am). Then, the tepal and stamen of different treatments were separately dried within specific dishes in a laboratory environment (shade condition and ambient temperature), powdered by using a specific mill and placed into various bags. Finally, they were kept under standard laboratory conditions (temperature: 20-25$^{o}$C; relative humidity: 50-55%; at shade condition) to extract and assess the intended traits.

**Table 1.** Chemical analysis of the manure used in the study.

| Organic Matter | Mg | Ca | Na | K | P | N | pH | EC (dS m$^{-1}$) |
|---|---|---|---|---|---|---|---|---|
| 80 | 0.32 | 1.9 | 0.15 | 2 | 0.5 | 2.5 | 8.5 | 14 |



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### Extracting and Preparing the Required Solutions

Regarding aqueous extraction, 5 g of tepal and 1.5 g of stamen powder from each treatment were separately weighed by using a digital balance with 0.00001 g precision and poured into dark glassware. In addition, 100 and 50 mL of distilled water were respectively added to the different treatments of tepal and stamen and the prepared samples were placed in Ben-Marie for 30 minutes. After taking out from Bain-Marie and reaching ambient temperature, the samples were transferred from beakers to falcon tubes and twice centrifuged for 20 minutes at 4,000 rpm. Further, the pure supernatant solution was poured into test tubes, stored in a -20°C freezer for 2 days, and converted to dry powder by placing in a lyophilizer (freeze-dryer, Backman) for 35 hours (Hoshyar *et al*., 2013). Finally, the powders were weighed and primary stock (10 mL or 10 mg $mL^{-1}$) was prepared to start the experiment.

### Measurement of Total Phenolic Content (TPC)

The Total Phenolic Contents (TPC) of saffron tepal and stamen extracts were measured using the Folin–Ciocalteu method (FCR) according to a modified procedure of Yoo *et al.* (2012). For this reason, the 96-well plates were selected and in each of their microplates, there was a mixed combination of 10 µL of the extracted sample, distilled water and 100 µL of Folin–Ciocalteu. Then, after 10 minutes, 10 µL of sodium carbonate (Na2CO3) were added. Microplates were incubated in the dark, at room temperature, for 60 minutes. The absorbance of all samples was measured at 760 nm using a spectrophotometer (Epotech, USA Biotech). The total phenolic content was calculated as µM Ggallic Acid Equivalent (µM GAE) by using Gallic Acid (GA) calibration curve.

### Measurement of Anthocyanin

Of the tepal and stamen extract, 0.25 mL was mixed with 10 mL of distilled water, then, the solution was acidified by hydrochloric acid. The red color at pH 3-4 and the color change with pH changes indicates the presence of anthocyanin. Solution absorbance was measured at 550 nm using a spectrophotometer (Epotech, USA Biotech). The concentration was calculated using a quenching coefficient of 33,000 $M^{-1}$ $cm^{-1}$ and the results were presented in terms of micromoles per gram (Chhabra *et al*., 1984).

### Measurement of FRAP (Ferric Reducing Antioxidant Power)

The FRAP ($Fe^{3+}$-Reduction, Ferric reducing antioxidant power) assay was conducted on each sample according to Parit *et al.* (2018). For this reason, 96-well plates were used and in each microplate there was 10 µL of different extracts of tepal and stamen that were mixed with 250 µL of FRAP (2,4,6-Tris (2-pyridyl)-1,3,5-triazine (TPTZ). and 15 µL of distilled water. Then, microplates were incubated at room temperature in the dark for 10 minutes, and the absorbance of solutions was estimated at 593 nm using a spectrophotometer (Epotech, USA Biotech). Also, 10 µM iron sulfate ($FeSO_4.7H_2O$) in different concentrations was used as the standard solution.

### Measurement of DPPH (2,2-Diphenyl-1-Picrylhydrazyl)

The DPPH (2,2-Diphenyl-1-Picrylhydrazyl) assay of each sample was conducted according to Hara *et al.* (2018). Ten µL of different extracts of tepal and stamen that were mixed with 250 µL of Methanolic solution of DPPH (65 $\mu$M, Sigma) and 50 $\mu$L of methanol used as blank, were kept in each microplate of 96-well plates. Finally, the microplates were





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incubated in the dark, at room temperature for 30 minutes. Determination of DPPH concentration was carried out spectrophotometrically. The absorbance of the resulting solution was measured at 517 nm using a spectrophotometer (Epotech, USA Biotech). To obtain a calibration curve, Trolox was applied; the relative inhibition of the antioxidants against DPPH was calculated according to the following equation:

Inhibition %= [(Ao-As /Ao]×100.

Where, Ao and As are the Methanol+DPPH and Extract+DPPH, respectively.

### Measurement of Anti-Cancer Property Traits

Among various organic and conventional treatments, the organic and conventional treatments with high antioxidant properties, which are expected to be effective in controlling cancer, were selected for assessing the anti-cancer property of the extracts obtained from saffron tepal and stamen on breast cancer cells (MCF-7).

### Cell Culture

The MCF-7 cells were kindly prepared from Birjand University of Medical Sciences. The cells were grown at 37°C in a humidified atmosphere containing 5% CO2 in DMEM containing 10% FBS, 100 units/mL penicillin, and 100 µg mL$^{-1}$ streptomycin. They were treated with different concentrations of tepal and stamen (0–13.5 mg mL$^{-1}$) at various time intervals (0–72 hours). All treatments were performed in triplicate.

### Evaluating Cell Viability through MTT Test

In this regard, $5\times10^3$ MCF-7 breast cancer cells were seeded in each well of 96-well culture plates. Plates were placed in a $CO_2$ incubator for 24 -hours to connect cells to the plate floor. Further, the different concentrations of the aqueous extracts of saffron tepal and stamen (0 to 13.5 mg mL$^{-1}$) were added for 24, 48, and 72 hours (Hoshyar *et al*., 2013). Then, each well was replaced with 10 µL of 5 mg mL$^{-1}$ MTT solution and plates were incubated at 37°C for 4 hours. After the incubation time, 100 µL of Dimethyl Sulfoxide (DMSO) were used to solve formazan crystals. Finally, the absorption of each well was determined by using Elisa reader (Biotech epoch, USA) at 540 nm and cell viability percentage was calculated through the following formula (Bathaie *et al*., 2013). For analysis of the cytotoxic efficiency, the IC50 value (Initial concentration of drug that reduced the number of live cells to 50%) of tepal and stamen were calculated using the time-dependent curves by linear interpolation.

Cell viability= (Number of viable cells/Number of all cells)×100

### Statistical Analysis

The measured characteristics were statistically analyzed based on a factorial experiment with a Randomized Complete Block Design (RCBD); and all treatments were performed in triplicate. As the data were homogeneous, the software SAS (V9.1), Excel, and GraphPad Prism (V.7.0) were used to analyze the data and draw the figures. Means were compared using the LSD test at a 5% probability level. Motic software was also used to draw the images.

## RESULTS AND DISCUSSION

### Total Phenolic and Anthocyanin Content in the Extracts of Saffron Tepal and Stamen

Results of variance analysis related to tepals showed that total phenolic content was affected by the main treatments of





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fertilizer and field age (P≤ 0.01), as well as the interaction effect of fertilizer and field age (P≤ 0.05) (Table 2). Additionally, a significant difference for the phenolic content of the tepal was observed between the various levels of fertilizer and the age of the fields. Further, the highest and lowest phenolic content in saffron tepal were obtained from the High level of manure in the Organic Farming System in the three-years-old farm (OFS-H×2016) and Low level of chemical fertilizer in the Conventional Farming System in the one-year-old farm (CFS-L×2018), respectively (Figure 1-a).

The results related to stamen indicated that the main effects of fertilizer and field age had a significant effect on total phenolic content (P≤ 0.01), while the interaction of fertilizer and field age had no significant effect on stamen total phenolic (Table 2). As shown in Figure 1-b, the highest and lowest total phenolic content in fertilizer treatments belonged to a High level of manure in the Organic Farming System (OFS-H) and a Low level of chemical fertilizer in the Conventional Farming System (CFS-L), respectively. In addition, there was a significant difference among the age of fields such that the highest and lowest of which were respectively achieved in the three-years-old and one-year-old farms (Figure 1-c). Further, the total phenolic content of saffron tepal and stamen under organic cultivation (manure application) was more compared with that of conventional cultivation (chemical fertilizer application) (Figures 1-a and -b).

Considering the results of analysis of anthocyanin content in tepal and stamen extracts, their anthocyanin content was significantly influenced by only the main treatments of fertilizer and different ages of field (P≤ 0.01), while interaction of fertilizer and field age had no significant effect on this trait (Table 2). The highest and lowest anthocyanin content was obtained from the High level of manure in the Organic Farming System (OFS-H), which had no significant difference with a Moderate level of manure one (OFS-M) and Low level of chemical of fertilizer (CFS-L), respectively (Figure 2-a). As shown, the highest and lowest anthocyanin content in tepals were achieved in the three-years-old and one-year-old farms, respectively (Figure 2-b).

The results of anthocyanin content of stamen indicated statistically significant difference between the different levels of

**Table 2.** Values of mean squares in the analysis of variance of TPC (Total Phenol Content), Anthocyanin, FRAP (Ferric Reducing Antioxidant Power) and DPPH (2, 2-Diphenyl-1-Picrylhydrazyl) in tepal and stamen of saffron.

| | | | Results of saffron tepal | | |
|---|---|---|---|---|---|
| Source | df | TPC | Anthocyanin | FRAP | DPPH |
| Replication | 2 | 52.47 ns | 0.48 ns | 4.12 ns | 1789.01 ns |
| Fertilizer | 5 | 3638.26** | 6.77** | 2261.77** | 10518.2** |
| Year | 2 | 1093.5** | 7.46** | 1698.27** | 10514.69** |
| F×Y | 10 | 353.83* | 0.35 ns | 247.24** | 1224.75** |
| Error | 34 | 143.04 | 0.65 | 76.21 | 374.15 |
| CV % | | 2.7 | 8.83 | 2.4 | 4.2 |
| | | | Results of Saffron Stamen | | |
| Source | df | TPC | Anthocyanin | FRAP | DPPH |
| Replication | 2 | 1052.72 ns | 0.0041ns | 357.39ns | 1933.12ns |
| Fertilizer | 5 | 5926.07** | 0.077** | 19008.8** | 35296.42** |
| Year | 2 | 2605.5** | 0.037** | 22231.5** | 26605.4** |
| F×Y | 10 | 130.41ns | 0.0012ns | 200.3ns | 529.34ns |
| Error | 34 | 237.76 | 0.001 | 262.56 | 1160.16 |
| CV % | | 2.7 | 5.9 | 3.27 | 6.32 |

* and **: Significant at 5 and 1% probability levels, respectively. ns: Non-significant.





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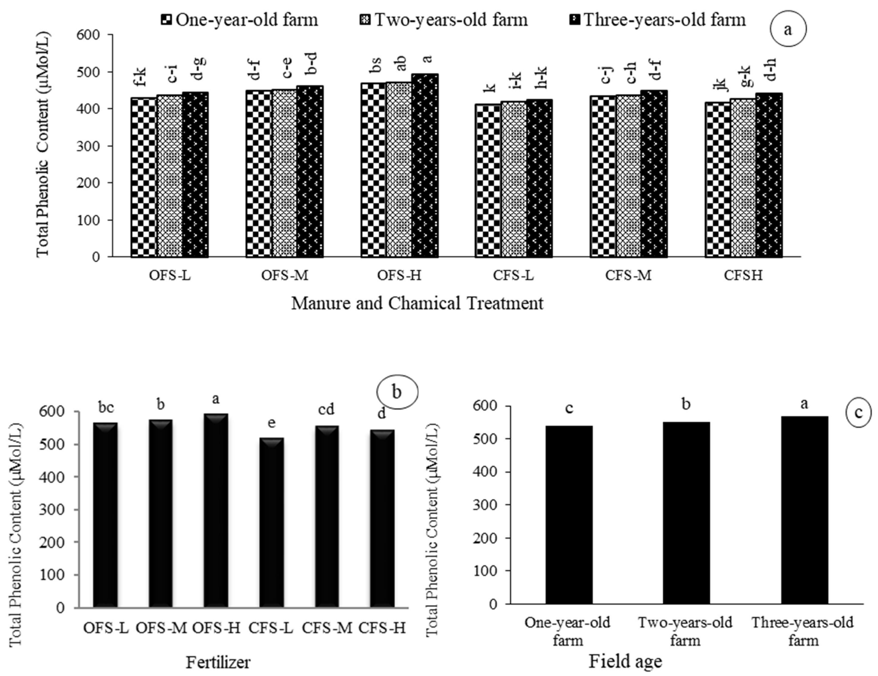


**Figure 1**. The interaction of different levels of fertilizer (manure and chemical treatment) in field age on the Total Phenol Content (TPC) (µmol $L^{-1}$) in saffron tepal (a), the effect of fertilizer on saffron stamen (b), and the effect of field age on saffron stamen (c). Differences of the columns that have the same letters are not statistically significant at 5% (LSD) level of significance.

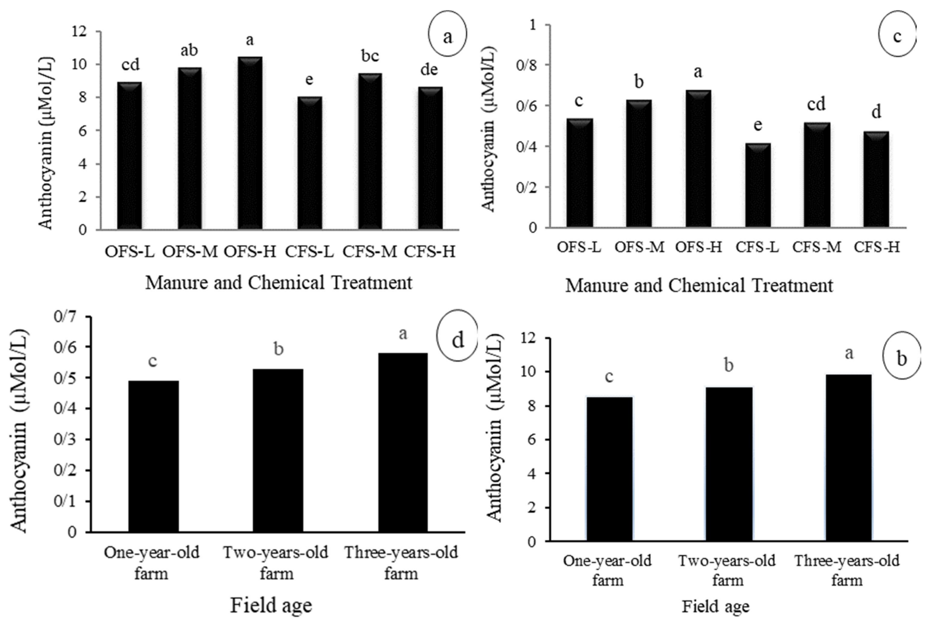


**Figure 2.** The effect of different levels of fertilizers (manure and chemical treatment) (a), and the effect of field age (b), on the anthocyanin (µmol $L^{-1}$) in saffron tepals and the effect of different levels of fertilizer (manure and chemical treatment) (c), and the field age (d) on the anthocyanin (µmol $L^{-1}$) in saffron stamen. Differences of the columns that have the same letters are not statistically significant at 5% (LSD) level of significance.

manure and chemical fertilizer. In addition, the highest and lowest anthocyanin content was obtained from a High level of manure in the Organic Farming System (OFS-H) and a Low level of chemical fertilizer in the Conventional Farming System (CFS-L), respectively. Regarding the levels of chemical fertilizer, the highest anthocyanin content was obtained by using a Moderate level of fertilizer in a Conventional Farming System (CFS-M), which had no significant difference with a High level of chemical fertilizer in this treatment (CFS-H) (Figure 2-c). The result of field ages also showed a significant difference among them such that the highest and lowest anthocyanin content of stamen were obtained from three-years-old farm and one-year-old farm, respectively (Figure 2-d).

Phenolic compounds have antioxidant properties that prevent oxidative stress or mitigate its effects on plant cells (Saki *et al*., 2019). Application of various fertilizers, especially organic fertilizers and plant growth promoters, increases production of phenolic compounds due to the increased access of plants to nutrients. An increase of total phenolic compounds of saffron stigma under biological and organic fertilizers was reported by Ghanbari *et al.* (2019), being consistent with the results of the present study.

The result of the present study showed that in conventional farming cultivation, with an increase in chemical fertilizer level, the total phenolic content of tepal and stamen decreased such that the highest content was achieved by using a moderate level of chemical fertilizer. Present results about phenolic content are in line with those of Kianimanesh *et al.* (2021) on *Crocus sativus* that reported the increasee of phenolic content at organic conditions. Based on the results of Behdani and Hoshyar (2016), possessing higher phenolic content is considered as the main reason for more antioxidant activity in the extracts of *Crocus sativus*. Recently, researchers have focused on assessing saffron stigma and tepals as an antioxidant-rich source (Bathaie *et al*., 2013; Hoshyar *et al*., 2013; Lahmass *et al*., 2017), however, to the best of our knowledge, no study has been reported on the antioxidant properties of the saffron stamen. Comparing the results related to saffron tepal and stamen in the present study showed that the total phenolic content of stamen was higher than that of tepal in both treatments of fertilizer and studied field age (Figure 1). Result showed that total phenolic and anthocyanin content decreased at the Highest level of chemical fertilizer treatment (CFS-H) compared with the Moderate level in Conventional one (CFS-M). In general, the excessive and unbalanced use of chemical fertilizers results in abundant negative consequences such as severe environmental destructive effects and considerable decrease in the productivity of the inputs (Cavagnaro, 2015; Reich, 2017).

## Evaluating the Antioxidant Potential of Tepal and Stamen by DPPH and FRAP Tests

DPPH: Table 2 shows that different levels of manure, chemical fertilizer, and different ages of farm, as well as their interaction, had significant effects on the antioxidant activity assessed by DPPH. Under organic conditions, radical scavenging activity (DPPH) occurred with higher speed. Indeed, the results related to the interaction of fertilizer and the field age on tepal extract indicated that the highest and lowest amounts of DPPH assay were obtained from a High level of manure in Organic cultivation in the three-years-old farm (OFS-H×2016) and the Low level of chemical fertilizer in the Conventional Farming System at the one-year- old farm (CFS-L×2018), respectively (Figure 3-a).

The results of variance analysis in the data related to stamen showed that the main effects of fertilizer and field age had significant effects on DPPH ($P \leq 0.01$), while their interaction effect had no significant effect (Table 2). An increase in the level of manure resulted in enhancement of the





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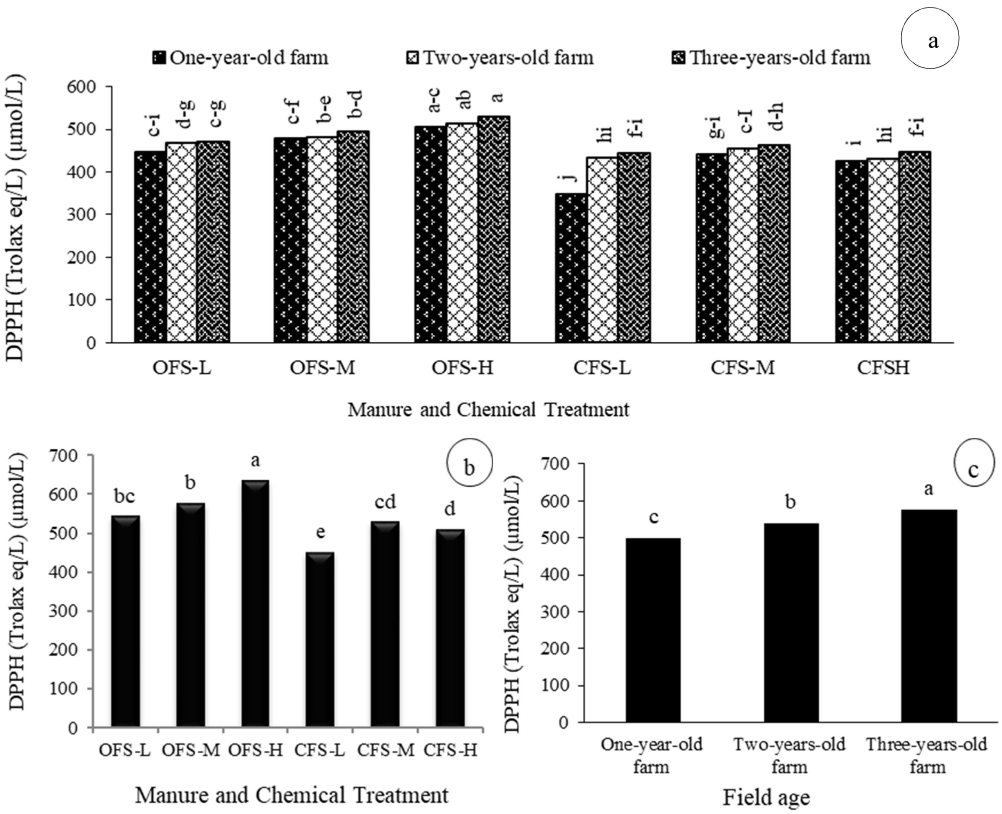


**Figure 3.** The interaction of different levels of fertilizer (manure and chemical treatment) in field age on the DPPH (2, 2-Diphenyl-1-Picrylhydrazyl) (Trolax eq $L^{-1}$, µmol $L^{-1}$) (a) in saffron tepal, and also the effect of different levels of fertilizer (manure and chemical treatment) (b), and field age (c) on DPPH in the saffron stamen.

amount of DPPH related to stamen, the highest of which was achieved by using a High level of manure in the Organic Farming System (OFS-H). Similar to the reaction of tepal extract, the amount of DPPH decreased by enhancing the level of chemical fertilizer in a High level of fertilizer in the Conventional Farming System (CFS-H) compared with that in a Moderate level of fertilizer one (CFS-M) (Figure 3-b). And the result from different ages of farm showed that the highest and lowest of DPPH of stamen were obtained from three-years-old farm and one-year-old farm respectively (Figure 3-c). As shown in Figure 4, the antioxidant capacity of free radicals scavenging in stamen extract is greater than tepal extract at all levels of manure and chemical fertilizer, which can represent its higher antioxidant property.

FRAP: The content of FRAP of tepal extract was affected by the treatments of fertilizer and field age, as well as their interaction effect ($P \leq 0.01$) (Table 2). The highest FRAP was attained from the Highest level of manure treatment in the three-years-old farm (OFS-H×2016). Regarding conventional cultivation, the antioxidant power of FRAP decreased by increasing the level of chemical fertilizer (CFS-H) such that the maximum and minimum of which were obtained at the Moderate level of fertilizer in the three-years-old farm (CFS-M ×2016) and the Low level in the one-year-old (CFS-L×2018) (Figure 5-a).

Additionally, the results showed significant difference between different farming years. Over time, the antioxidant capacity of plants increased in the two-years-old and three-years-old farm, which

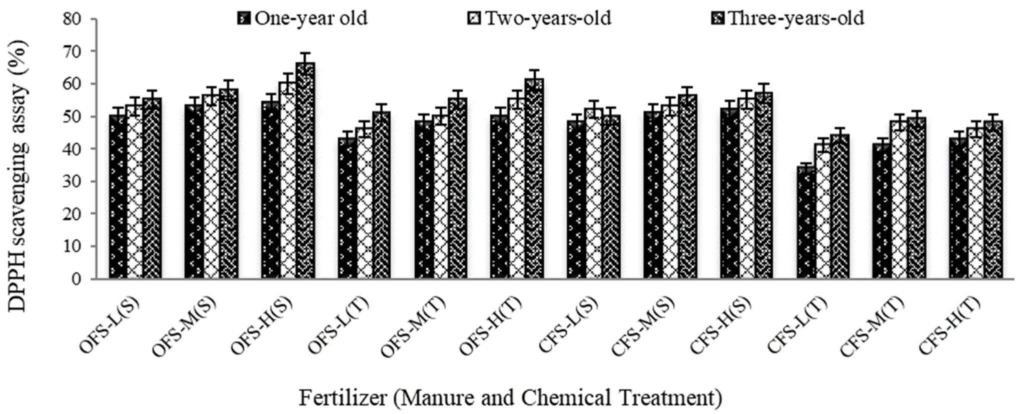


**Figure 4.** Comparison between Stamen (S) and Tepal (P) effects on reducing oxygen radicals in different levels of manure (OFS-L-OFS-H) and chemical (CFS-L-CFS-H) fertilizer in different ages of farm (field age).

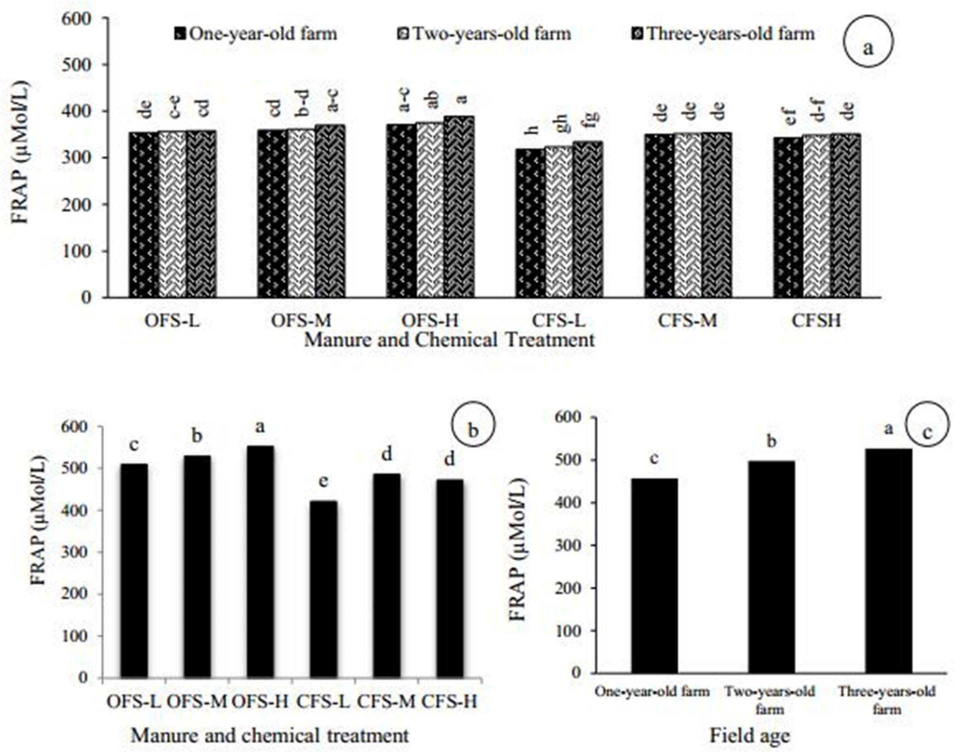


**Figure 5.** The interaction of different levels of fertilizer (manure and chemical treatment) in field age on the FRAP (µmol $L^{-1}$) in the saffron Tepals (a), and also the effect of different levels of fertilizer (manure and chemical treatment) (b) and different ages of farms (field age) (c) of FRAP in saffron Stamen. Differences of the columns that have the same alphabets one not statistically significant at 5% (LSD) level of significance.

can be related to the higher effectiveness of manure and even chemical fertilizers. In manure, as nutrient elements are gradually released, more nutrient sources are provided to plant, and their positive effects increase in the two-years-old and three-years-old farm. As illustrated in Table 2, the results related to FRAP in stamen extract are only influenced by the main effects of fertilizer and field age,





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while their interaction had no significant effect. Considering the treatments of fertilizer, the maximum and minimum of FRAP were achieved at the Highest level of manure (OFS-H) and the Lowest level of chemical fertilizer (CFS-L), respectively. Over time, FRAP increased in the two-years-old and three-years-old farms similar to DPPH results (Figures 5-b and -c).

In general, the phenolic and antioxidant compounds (DPPH, FRAP) related to the extract of saffron tepal and stamen increased in the study by raising the level of manure, indicating that the proper provision of nutrient elements to plant through supplying organic materials in soil affects the improvement of these traits. Enhancing the antioxidant activity of the plant exposed to manure can be related to the provision of the macro and micro-elements responsible for its antioxidant activity (Rostaei *et al*., 2019). The tepal and stamen extracts treated by manure had higher phenolic content and acceptable antioxidant efficiency because of absorbing the materials that play an important role in producing phenolic compounds, which are consistent with the results of Ibrahim *et al.* (2013).

Since high-antioxidant treatments can deal with cancer cells and possess greater therapeutic properties, the treatments with high antioxidant contents under organic and conventional conditions were selected for MTT to assess the effects of organic and conventional cultivation on the therapeutic property of tepal and stamen, along with comparing their therapeutic properties. These treatments included a High level of manure in the Organic Farming System in three-years-old farm (OFS-H×2016) and a Moderate level of fertilizer in Conventional one in the two-years-old farm (CFS-M×2017), as well as a High level of fertilizer in Conventional one in the three-years-old farm (CFS-H×2016) regarding tepal and stamen extracts, respectively.

## MTT Test and the Therapeutic Properties of Tepal and Stamen Extracts

The results of the MTT test represented the interaction effects of the dose and time of treatment by using tepal and stamen extracts with the viability of cancer cells. Thus, an increase in the dose of the extracts resulted in decreasing cell viability significantly, the results of which were more effective over time (Figures 6 and 7). The dose and time-dependent cytotoxic effects of the extracts on breast cancer cells were observed in the study. In general, adding treatment time resulted in increasing the toxicity of the extract and decreasing the percentage viability of breast cancer cell significantly. Based on the results of the MTT test on tepal extract under Organic (OFS-H×2016) and Conventional conditions (CFS-M×2017), a significant difference was observed concerning the effect on the therapeutic property of the extract (Figures 6-a and -b). In addition, the percentage of cell viability decreased by enhancing the concentration of the extract in Organic condition (OFS-H×Three-years-old farm) from 1.5 to 13.5 mg $mL^{-1}$, as well as for the time from 24 to 72 hours. Further, IC50, which is defined as a concentration of extract with at least 50% cell death, was determined as 12.4, 11, and 9.8 mg $mL^{-1}$ at 24, 48, and 72 hours, respectively (Figure 6-a and Table 3).

The results related to tepal extract in Conventional conditions (CFS-M×two-years-old farm) were completely different from those in the organic one. In conventional conditions, treatment by the different concentrations of the extract showed a dose-dependent cytotoxic effect only after 72 hours (Figure 6-b). Generally, the lower the IC50 concentration, the greater the impact on inhibiting breast cancer cells. In this study, IC50 was more compared with that in organic cultivation conditions and was 13.67, 11.94, and 10.79 mg $mL^{-1}$ at 24, 48, and 7 2 hours, respectively. Since fewer IC50 lead to much decrease in cancer cell viability and vice versa, the tepal extract cultivated under organic conditions was more effective in inhibiting breast cancer cells (Figure 6-a and Table 3).

Considering the results of the MTT test related to stamen extract in Organic (OFS-H×three-years-old farm) and Conventional (CFS-H×three-years-old farm) cultivation,





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their therapeutic properties were significantly different. Regarding organic condition, there was a significant difference between different concentrations of the extract, as well as treatment times. Thus, the maximum therapeutic properties (decreasing the viability of breast cancer cells) were achieved at 72 hours in all studied concentrations of the extract. Further, the percentage of cancer cell viability decreased overtime at 48 and 72 hours, by indicating the effectiveness of the extract in treating disease (Figure 7-a). Furthermore, IC50s at 24, 48, and 72 hours in stamen extract were obtained as 11.02, 10.63, and 8.59 mg $mL^{-1}$, respectively (Table 3). In conventional conditions, a significant difference was observed between the concentrations and times under study. These results were similar to those related to tepal extract in organic conditions and represented a non-uniform trend. Additionally, IC50s attained 12.47, 11.20, and 9.92 mg $mL^{-1}$ at 24, 48, and 72 hours, respectively (Figure 7-b and Table 3).

Although most of the previous studies focused on the therapeutic properties related to the stigma and tepals of saffron, the present study demonstrated lower IC50 and, consequently, stronger therapeutic properties in the extract of the saffron stamen. Accordingly, paying more attention to the issue is recommended for the optimal use of its therapeutic properties. In addition, the viability of cancer cells exposed to the stamen extract under organic conditions declined completely uniform and the percentage of cell viability decreased by increasing the concentration of the extract over time (24, 48, and 72 hours). Further, the decreasing trend of viability in the cells treated by the stamen extract under conventional conditions was more uniform than that of tepal extract under conventional one. Furthermore, the percentage of decreasing cancer cell viability depended on both concentration and time.

The extracts of saffron tepal and stamen can be an appropriate selection for manufacturing anticancer drugs. In addition,

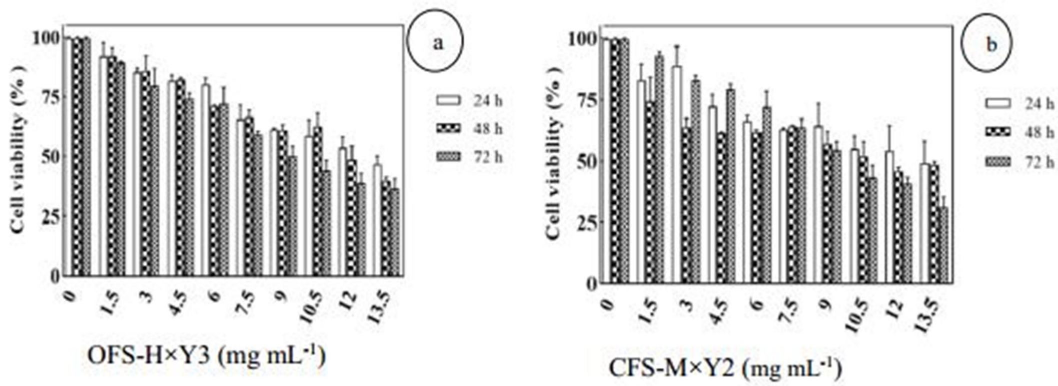


**Figure 6.** The effect of different concentrations of Tepal extract (0–13.5 mg $mL^{-1}$) cultivated in organic condition in three-year-old farm (mg $mL^{-1}$) (a) and the effect of different concentrations of Tepal extract (0–13.5 mg $mL^{-1}$) cultivated in conventional condition in two-year-old farm (mg $mL^{-1}$) (b) on MDAMB468 (Breast cancer) cell viability after 0–72 hours.

**Table 3.** IC50 values (mg $mL^{-1}$) of saffron tepal and stamen on MDA-MB cell line.

| Treatment | 24 h | 48 h | 72 h |
|---|---|---|---|
| CFS-M×Two-year-old farm (Tepal) | 13.67 | 11.94 | 10.97 |
| CFS-H×Three-year-old farm (Stamen) | 12.47 | 11.20 | 9.92 |
| OFS-H×Three-year-old farm (Tepal) | 12.4 | 11 | 9.8 |
| OFS-H×Three-year-old farm (Stamen) | 11.92 | 10.63 | 8.59 |





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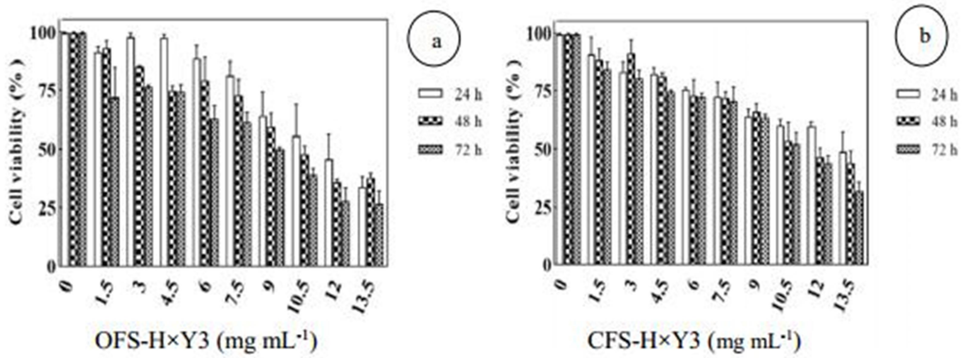


**Figure 7.** The effect of different concentrations of Stamen extract (0–13.5 mg mL$^{-1}$) cultivated in organic condition in three-year-old farm (mg mL$^{-1}$) (a) and the effect of different concentration of Stamen extract (0–13.5 mg mL$^{-1}$) cultivated in conventional condition in the three-year-old farm (mg mL$^{-1}$) (b) on MDAMB468 (Breast cancer) cell viability after 0–72 hours.

they can be used along with chemotherapy drugs and decrease the side effects of consuming high doses of conventional chemical drugs by improving the effectiveness of therapy. In addition, Bathaie *et al.* (2013), reported the role of saffron stigma in treating colorectal (SW480, NIH3T3) and Gastric cancer cells (AGS) because of possessing crocin and crocetin, as well as lack of adverse effects on healthy cells. Behdani and Hoshyar (2016) studied the antioxidant and therapeutic properties of the saffron stamen cultivated under organic conditions and mentioned its anticancer properties, which are in line with the results of the present study.

## CONCLUSIONS

The present study investigated the anticancer properties of tepal and stamen extracts derived from saffron, which is used traditionally in the treatment and management of cancer in the world. Based on the facts and information provided in the current research, it is concluded that the tepal and stamen extracts of *Crocus sativus* are effective against breast cancer cell lines. According to the results, antioxidant compound content, phytochemical, and therapeutic properties related to saffron tepal and stamen in organic conditions were higher compared with the conventional one. Further, the antioxidant and therapeutic properties of tepal and stamen extracts under organic cultivation increased by enhancing the level of manure from OFS-L to OFS-H. However, different results were obtained for conventional conditions such that the highest and lowest values of the mentioned properties were achieved at moderate and high levels of fertilizer, respectively. Based on the results of the MTT test, saffron stamen had greater and more uniform therapeutic properties than those of saffron tepals, which should be further evaluated in the therapy field. Additionally, higher DPPH radical scavenging activity was obtained from stamen extract compared with tepal, which is considered as important in inhibiting cancer. By considering the results of the present study, it can be concluded that organic cultivation (different levels of manure) is superior to conventional one (different levels of of chemical fertilizers) because of the enhancement in the antioxidant activity in treating breast cancer. This suggests that the cultivation and growth conditions of saffron are better to be developed in organic farms to use as an alternative cancer treatment. Also, among the different ages of the farm, the most antioxidant and anti-cancer properties were obtained from the three-year-old farm (cultivated plants in 2016). These results can





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be due to the greater effectiveness of the organic system in three-year-old farms compared to two- and one-year-old farms.

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# بررسی تأثیر دو روش کشت ارگانیک و متعارف بر فعالیت‌های فیتوشیمیایی و ضد سرطانی زعفران (*Crocus sativus* L.)

م. عسکری، م.ع. بهدانی، ه. ملایی، و ح. ر. فلاحی

## چکیده

زعفران (*Crocus Sativus* L.) یک گیاه دارویی با ارزش غذایی، دارویی و خواص ضد سرطانی بالا است که اثرات سیتوتوکسیک زیادی بر سلول های سرطانی دارد. در این پژوهش، آزمایشی به‌صورت فاکتوریل در





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قالب طرح بلوک‌های کاملاً تصادفی با سه تکرار در مزرعه تحقیقاتی دانشکده کشاورزی دانشگاه بیرجند به منظور بررسی اثرات ضد سرطانی عصاره‌های پرچم و گلبرگ زعفران بر سلول‌های سرطان سینه انسان (MCF-7) انجام شد. تیمارهای مورد بررسی شامل: سن مزرعه (مزرعه یک ساله، دو ساله و سه ساله) و همچنین کاشت ارگانیک و رایج با سطوح مختلف کودی (کم، متوسط و زیاد) بودند. همچنین صفات مورد مطالعه به ترتیب شامل محتوی فنل کل، فعالیت آنتی اکسیدانی و سمیت سلولی ( DPPH)، قدرت آنتی اکسیدانی (FRAP) و سنجش تکثیر یا مرگ سلولی(MTT) و در نهایت IC50 بودند. نتایج نشان داد که بین خواص فیتوشیمیایی، آنتی‌اکسیدانی و ضد سرطانی عصاره‌های به‌دست آمده از شرایط ارگانیک و رایج تفاوت معنی‌داری وجود دارد که بیشترین آن از کشت ارگانیک به دست آمد. همچنین محتوی آنتی‌اکسیدان‌ها و ترکیبات درمانی عصاره‌ها با افزایش سطوح کود دامی افزایش یافت. نتایج آزمایش MTT نشان داد که عصاره‌های گلبرگ و پرچم زعفران دارای اثر ضد تکثیری بر سلول‌های سرطانی هستند اما خواص ضد سرطانی عصاره پرچم بیشتر از گلبرگ بود. بنابراین، استفاده از عصاره پرچم به‌عنوان یک منبع موثر و ارزان برای صنعت داروسازی، می‌تواند ابعاد جدیدی را برای جلوگیری از چالش‌های درمانی سرطان سینه، بوجود بیاورد.



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