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Inhibitory impact of aqueous, ethanolic and methanolic extracts of saffron (*Crocus sativus*) petals against some pathogenic bacteria

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Abstract

The aim of this study was to investigate the antimicrobial properties of aqueous and alcoholic extracts of saffron (*Crocus sativus* L.) petals against some of the most important food-borne bacterial pathogens such as *Staphylococcus aureus, Listeria monocytogenes, Bacillus cereus* and *Escherichia coli in vitro.* According to the results, all three aqueous, ethanolic and methanolic extracts of saffron petals (at concentration of 350 mg/ ml) showed significant inhibitory effect on the studied food-borne bacteria. However, the aqueous extract of saffron petal showed higher antibacterial activity against the studied bacteria compared to other two extracts and its inhibitory effect on the studied gram-positive bacteria, *S. aureus*, *L. monocytogenes*, *B. cereus* was significantly higher than gram-negative bacterium *E. coli*. The results of the MIC determinations of aqueous extract of saffron petals showed that concentrations lower than 43.75 mg/ml did not inhibit the growth of *B. cereus* and *S. aureus* bacteria, while MIC values were lower for *E. coli* and *L. monocytogenes* bacteria. According to the results, saffron petal, especially its aqueous extract, has the potential to be used as a natural preservative in the food industry.

Keywords: alcoholic extract; antibacterial activity; aqueous extract; food-borne bacterial; saffron petals

Introduction

Diseases caused by the consumption of food contaminated with pathogenic bacteria are of great importance for public health and cause great financial and human losses to communities annually. Every year, about 600 million people (one in every ten people) worldwide become ill after ingesting contaminated food, resulting in 420000 deaths and the loss of 33 million healthy life years (DALYs) in which many of them are young children (Lee and Yoon, 2021; WHO, 2022). More than 90 percent of food poisoning cases are caused by bacteria such as, *Staphylococcus aureus, Salmonella, Shigella, Clostridium botulinum, C. perfringens, Campylobacter jejuni, Listeria monocytogenes, Vibrio parahaemolyticus, Bacillus cereus* and Entero-pathogenic *Escherichia coli* (Bintsis, 2017). Using the antimicrobial agents or preservatives can be effective in the prevention of bacterial growth, but the extensive use of antibiotics in the treatment of bacterial infections has

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led to the emergence and spread of resistant strains (Seyfried *et al*., 2010; Terreni *et al*., 2021). Their residues in food might also cause many destructive effects on the environment and human health (Arsène *et al*., 2021; Shahid *et al*., 2021; Ghimpețeanu *et al*., 2022). Therefore, the research for finding suitable and natural alternatives for antibiotics and chemical preservatives is essential. In recent years, special attention has been paid to plant extracts and their active components as a good candidate for the production of food preservatives due to their various antimicrobial compounds and their multiple effects on the cellular structure of microorganisms (e.g., simultaneous physical and chemical degradation of bacterial cell membranes) (Thery and Arendt, 2018; Cejudo Bastante *et al*., 2019; Zhao *et al*., 2019; Adnan *et al*., 2020; Chen *et al*., 2020; Marquez-Rodriguez *et al*., 2020; Rubab *et al*., 2020; Bouhanna *et al*., 2021; Gong *et al*., 2021). Moreover, plant extracts are needed in very small amounts to inhibit the growth of bacteria, and they are unlikely to have significant adverse effects on food properties and consumer (Shahnia and Khaksar, 2013).

Saffron is a small and agriculturally perennial plant belonging to Iridaceae family (Srivastava *et al*., 2010). Its dried stigma is used in food industry as aromatic spice and for food coloring, and in pharmaceutical industry as sedative and analgesic for asthma, pertussis and inflammation (Mirheidar, 2005). Iran, especially the provinces of South Khorasan and Razavi Khorasan, is one of the most important hubs of saffron production in the world, producing more than 90% of the world production. In the process of saffron production, stigma is used as commercial saffron, and other parts of the flower are discarded, which has a very high volume (over 20 thousand tons annually) (Jafari-Sales and Pashazadeh, 2020). Saffron petal is a main source of bioactive compounds with different physiological and antioxidant activities including flavonoid compounds (quercetin, isoramentin, kaempferol), anthocyanins (dolphinidine, petonidine and malvidin), new monoterpenoids (crocusatin-J with antityrosinase activity and 4-dihydroxybutyric acid) (Serrano-Díaz *et al*., 2012; Jadouali *et al*., 2018; Hosseini *et al*., 2018). Nowadays, the only usage of saffron petal is dye extraction, which is not flourished yet (Goli *et al*., 2012). Therefore, finding a suitable solution for recycling this huge volume of waste is very important. Using the saffron petal as a natural preservative to prevent spoilage of processed foods in the food industry could be a one solution. Due to the fact that saffron is cultivated only in Iran and a few countries with old civilization such as Spain, India, Greece, and Morocco (Gohari *et al*., 2013), there is limited information about the pharmacological properties of this valuable plant and most studies are about stigmas (Muzaffar *et al*, 2016; Hashemi *et al*., 2018; Jomehpour *et al*., 2019; Valizadeh *et al*., 2022). Askari et al (2023) 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. However, according to the toxicological studies, saffron petals contain relatively low toxicity compounds compared to the stigma. Most of the effective compounds of petals are anthocyanins, which have low toxicity (Hosseini *et al*., 2018). Therefore, the aim of the present study was to investigate the antimicrobial potential of aqueous and alcoholic extracts of saffron petals on some of the most important food-borne bacterial pathogen such as *S. aureus, L. monocytogenes, B. cereus* and *E. coli*. If such an effect is proven, in addition to satisfying consumer demand for natural preservatives and increasing the shelf life and safety of food, it will also be prevented the loss of saffron by-product.

Materials and Methods

Preparation of plant extract

Saffron petals were collected from a saffron farm in Torbat Heydarieh, Khorasan-Razavi, Iran on November 2018. Extraction was performed according to the method of Alizadeh Behbahani *et al.* (2013) with slight modifications. Briefly, the petals were dried in the shade at room temperature and then pulverized using electric grinder. Powdered petals were separately extracted by maceration with three solvents distilled water, 80% ethanol and 80% methanol (1:10 ratio) (Nekkaa *et al*., 2022). The mixture was left on a shaker at room temperature for 72 hours to allow for proper extraction. The elucidated extracts were filtered with Whatman

No.1 filter paper then centrifuged at 1000 g for 5 min. The resultant supernatant was concentrated in an oven at 40 °C for 48 h to a concentration of 350 mg/ml. The extracts were poured into dark sterile glass bottles and kept at 4 °C for one week.

Antibacterial activity

Bacterial strains

Antibacterial activity of saffron petal extracts was evaluated against some of food-borne bacterial pathogens including both gram- negative bacteria (*E. coli*) and gram- positive bacteria (*S. aureus, L. monocytogenes, B. cereus*). All strains were obtained from the Iranian Research Organization for Science and Technology and grown on nutrient agar plates for 16 h at 37 °C

Agar disc diffusion method

Antibacterial activity of the extracts was evaluated by the standard disc diffusion method. Briefly, paper discs (6 mm in diameter) were impregnated with 30 µl of extract (at a concentration of 350 mg/ml) and placed on Mueller Hinton agar plates, which were evenly inoculated with 100 µl of bacterial cell suspension. The suspension is adjusted with sterile 0.9% saline to give a turbidity equivalent to the 0.5 McFarland standard at 600 nm (optical density = 0.08-0.1 corresponding to 1.5×10^8 CFU/ml). The plates were then incubated at 37 °C for 18 - 24 h. The bactericidal activity was determined by measuring the diameter (in mm) of clear inhibition zones around the discs using vernier caliper (Abolfathi *et al*., 2022). Standard disks of Doxycycline 30 ug (D30) and Gentamisin 10 ug (G10) were served respectively as positive controls for gram- positive and gram- negative bacteria. Solvent impregnated discs were used as negative control for both gram- positive and gram- negative bacteria.

Minimum Inhibitory Concentration (MIC)

The minimal inhibitory concentration (MIC) of extracts against sensitive bacteria was determined by the broth microdilution method using 96-well microtiter plates. Briefly, 50 µL of the extract (at a 350 mg/ml concentration) was serially diluted two-fold with 50 µL of Mueller Hinton broth in a 96-well polypropylene microtiter plate (except the control wells). Then, equal volume of overnight bacterial cell suspension (optical density = 0.08-0.1 corresponding to 1.5×10^8 CFU/ml) was added into all wells. After incubation at 37 °C for 16 - 18 h temperature, the last well with visible bacterial inhibition (lack of cloudiness in the well) was considered as the minimal inhibitory concentration. The well without extract was used as positive control (100% growth or 0% bactericidal activity). The negative control well (blank) contained solvent and Mueller-Hinton broth (Saquib *et al*., 2019).

Statistical analysis

Data were assessed for normality and homogeneity of variance using Kolmogorov–Smirnov test and Levene's test, respectively. Antibacterial activity of saffron petal extracts was analyzed using one-way ANOVA ($p < 0.05$). Means were compared with Duncan's multiple range test. Results are presented as mean ± S.D. All Statistical analyses were performed using SPSS software edition 21.

Results

As shown in Table 1, all three aqueous, ethanolic and methanolic extracts of saffron petals (at concentration of 350 mg/ ml) showed significant inhibitory effect on the studied food-borne bacteria (*S. aureus*, *L. monocytogenes*, *B. cereus* and *E. coli*). However, the aqueous extract of saffron petal showed higher antibacterial activity against the bacteria compared to other two extracts ($p < 0.05$) and its inhibitory effect on

the studied gram-positive bacteria, *S. aureus*, *L. monocytogenes*, and *B. cereus* (with inhibition zone diameters ranging from 16.5 to 18.39 mm) was significantly higher compared to gram- negative bacterium *E. coli* (inhibition zone diameter of 13.9 mm) ($p < 0.05$). However, no significant differences were observed among the sensitivity of the studied gram-positive bacteria to aqueous extract of saffron petals ($p > 0.05$) (Table 1) (Figure 1).

Table. 1. Mean diameter of non-growth zone of extracts of saffron petal against studied bacteria in millimeters

Bacteria/extract	Aqueous extract	Ethanolic extract	Methanolic extract	Doxycycline (D30)	Gentamisin (G10)
E. coli	13.9 ± 1.9 [b]	1.34 ± 0.4 [c]	9.3 ± 3.3 [c]	-	21.34 ± 2.3
S. aureus	16.5 ± 1.57 [ab]	2.48 ± 1.47 [c]	14.85 ± 0.75 [b]	31.74 ± 1.26	-
B. cereus	18.39 ± 0.32 [a]	7.7 ± 1.92 [cd]	5.46 ± 0.91 [d]	29.99 ± 1.17	-
L. monocytogenes	16.4 ± 0.55 [ab]	16.22 ± 1.81 [ab]	6. 02 ± 1.49 [d]	25.09 ± 1.34	-

Values are means ± S.D. (n =3). Different letters indicate significant differences ($p< 0.05$).

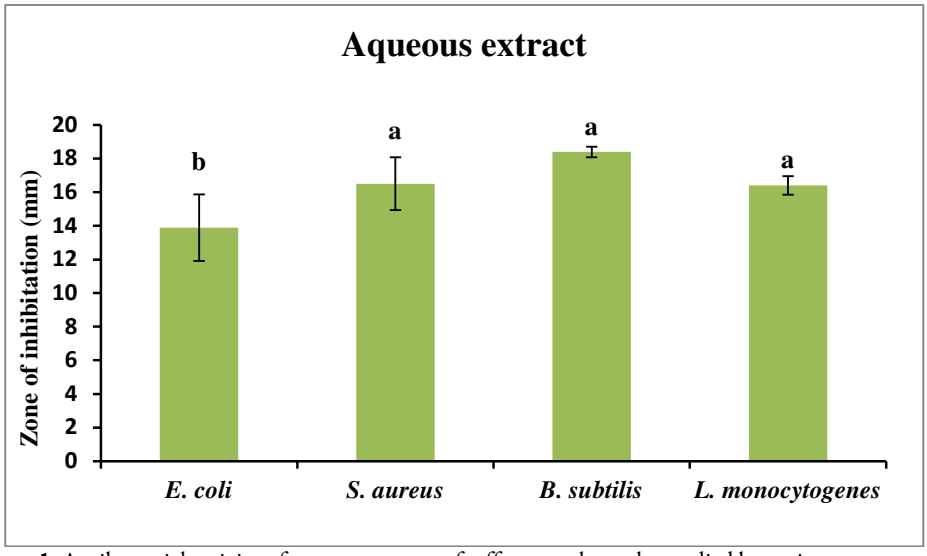

Figure 1. Antibacterial activity of aqueous extract of saffron petals on the studied bacteria
Values are means ± S.D. (n =3). Different letters indicate significant differences ($p< 0.05$)

Antibacterial activities of ethanolic and methanolic extracts were different depending on the bacterial strain (Figure 2 and Figure 3). The sensitivity of *E. coli* and *S. aureus* bacteria was higher to methanolic extract than to ethanolic extract of saffron petals ($p < 0.05$) (Figures 4 and 5), while the inhibitory effect of ethanolic extract of saffron petal on *L. monocytogenes* was higher than its methanolic extract ($p < 0.05$) (Figure 7). No significant difference was observed in the antibacterial activity of ethanolic and methanolic extracts of saffron petals against *B. cereus* ($p> 0.05$) (Figure 6). The most sensitive bacteria to the ethanolic and methanolic extracts of saffron petals were *L. monocytogenes* (16.22± 1.81 mm in diameter) (Figure 2) and *S. aureus (*14.85± 3.3 mm in diameter*),* respectively ($p < 0.05$) (Figure 3).

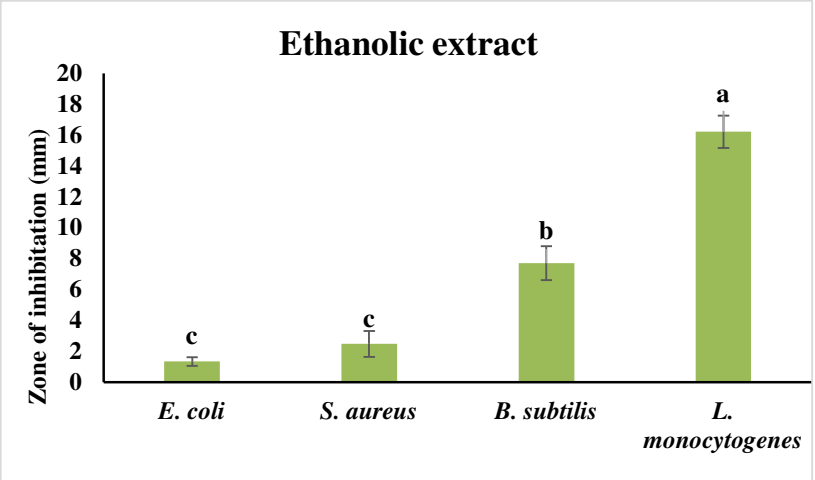

Figure 2. Antibacterial activity of ethanolic extract of saffron petals on the studied bacteria
Values are means ± S.D. (n =3). Different letters indicate significant differences (p< 0.05)

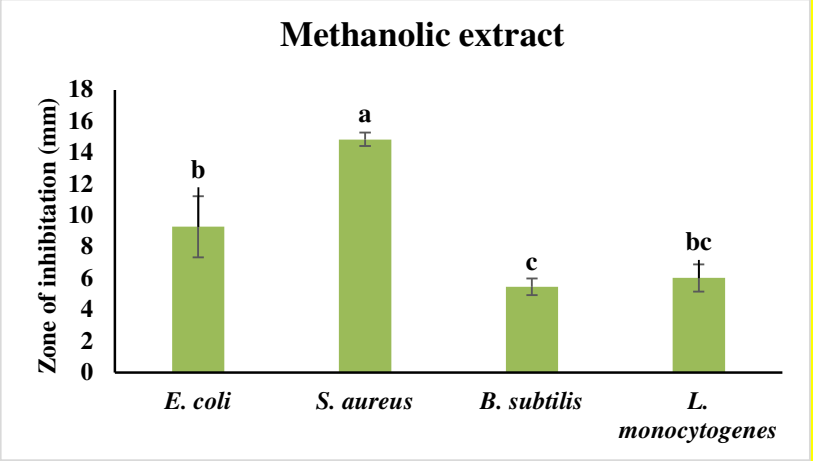

Figure 3. Antibacterial activity of methanolic extract of saffron petals on the studied bacteria
Values are means ± S.D. (n =3). Different letters indicate significant differences (p< 0.05)

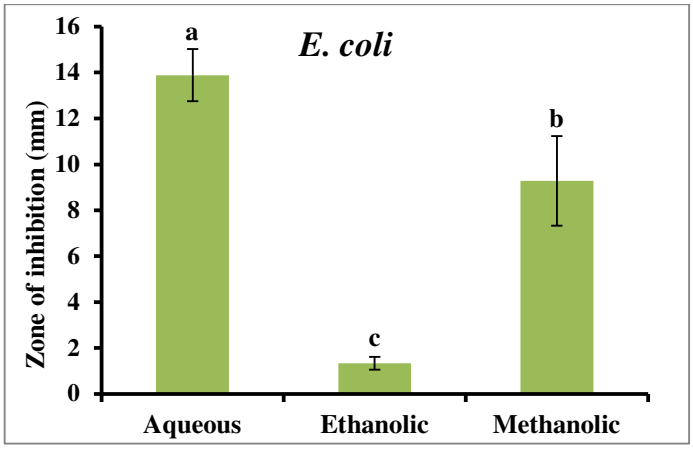

Figure 4. Antibacterial activity of saffron petal extracts against *E. coli*
Values are means ± S.D. (n =3). Different letters indicate significant differences (p< 0.05)

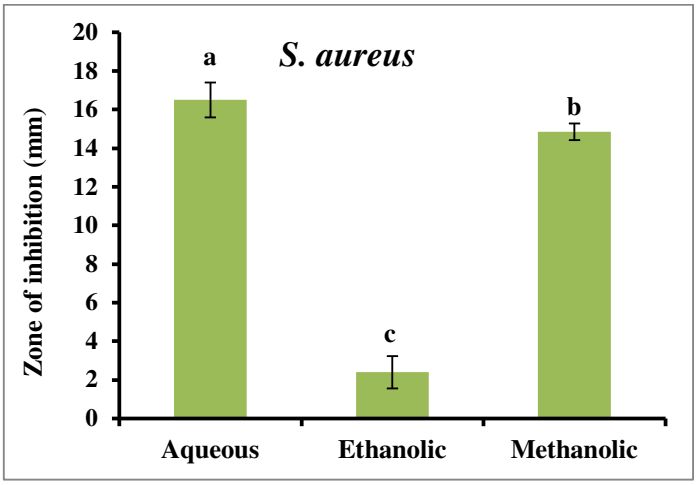

Figure 5. Antibacterial activity of saffron petal extracts against *S. aureus*
Values are means ± S.D. (n =3). Different letters indicate significant differences (p< 0.05)

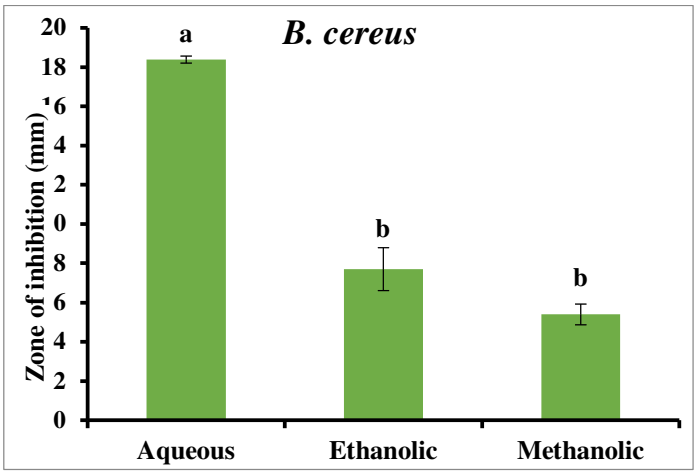

Figure 6. Antibacterial activity of saffron petal extracts against *B. cereus*
Values are means ± S.D. (n =3). Different letters indicate significant differences (p< 0.05)

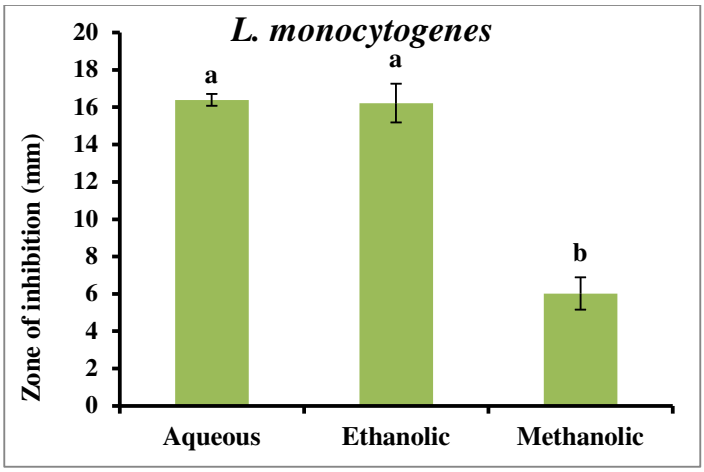

Figure 7. Antibacterial activity of saffron petal extracts against *L. monocytogenes*
Values are means ± S.D. (n =3). Different letters indicate significant differences (p< 0.05)

The results of the MIC determination of aqueous extracts of saffron petals showed no inhibitory effects of concentrations lower than 43.75 mg/ml the growth of *B. cereus* and *S. aureus* bacteria, while MIC values were lower for *E. coli* and *L. monocytogenes* bacteria (MIC= 21.78) (Table 2). However, according to the results of disc diffusion, it was expected that *E. coli* would show more resistance to the reduction of the extract concentration (Table 1).

Table 2. Minimum inhibitory concentration (MIC) of aqueous extract of saffron petals against the studied bacteria

Bacteria	MIC (mg/ml)
E. coli	21.87
L. monocytogenes	21.87
B. cereus	43.75
S. aureus	43.75

Discussion

Due to the public concerns about the side effects of chemical preservatives, the desire to consume products without preservatives or with natural preservatives has increased. Therefore, several studies have been conducted on the effect of essential oils and different plant extracts on pathogenic bacteria. Also, in recent years, the use of by-products of food industry such as grape peel and seeds, citrus peel and pulp, apple and pomegranate pomace, potato peel waste, petal waste and saffron corm has also been considered (Zambrano *et al*., 2019; Gebrechristos *et al*., 2020; Wali *et al*., 2020; Rahnemoon *et al*., 2021; Shehata *et al*., 2021;). Afshar Mohammadian *et al.* (2016) studied the antibacterial activity of methanolic extracts of petal of different saffron species, including *C. speciosus*, *C. sativus* and *C. caspius* on the bacteria causing infection and food poisoning. They found that the petal extract of *C. sativus* species had a high inhibitory effect on the investigated bacteria. The results of the present study also confirmed the antibacterial activity of aqueous and alcoholic extracts of *C. sativus* petals against some bacteria causing food spoilage including *E. coli*, *S. aureus*, *L. monocytogenes*, *B. cereus*. However, the inhibitory effect of aqueous extract of saffron petals on the studied bacteria was stronger than alcoholic extracts which may be due to the extraction of more antimicrobial compounds in saffron petals by water. These findings were consistent with the results of Okman *et al.* (2016), while Gandomi Nasrabadi *et al.* (2012) and Asgarpanaha *et al.* (2013) showed that methanol extract of saffron petal was effective against *B. cereus*, *L. monocytogenes*, *S. aureus, Salmonella enterica, S. typhimurium* and *Shigella dysenteria.* These differences can be due to the differences in biologically active compounds in saffron petals and their solubility. The composition of the extracts of a plant species can vary based on the region geography, harvest season, plant age, growth stage, the method of drying and extraction and the type of solvent used. In general, the plant extract has the highest antimicrobial activity during flowering or immediately after flowering. The extraction method and the type of solvent used also affected the antimicrobial effects of the extract (Farahmandfar *et al*., 2019; Adegbaju *et al*., 2020; AL-Hmadi *et al*., 2021; Makarova *et al*., 2021; Patnala and Kanfer, 2021). Afraze *et al*. (2014) investigated the effect of solvent on the extraction efficiency of the phenolic compounds of saffron petals and showed that the aqueous solvent had the highest extraction efficiency due to its polarity, and methanolic and ethanolic solvents were in the next ranks, respectively. However, the amount of phenolic compounds in the aqueous extract was lower than ethanolic and methanolic extracts. The antibacterial activity of saffron petals may be associated with the presence of a wide range of compounds with antimicrobial properties including phenolic compounds which may show additive or synergic activities due to their action on different cellular targets (Sidiq and Shrivastava, 2020). In general, the higher amount of phenolic substances in the extract, the greater their antibacterial properties against food pathogens (Shahnia and Khaksar, 2013).

Razaghi *et al*. (2003) and Pintado *et al.* (2011) showed that safranal and crocin in saffron inhibited the growth of *E. coli* and *S. aureus, S.* typhimurium *and S. enterica*. However, according to the results of the study by Afraze *et al*. (2014) other compounds besides phenolic compounds are also effective in the antibacterial properties of saffron petal extract, whose performance varies depending on the sensitivity and resistance of different bacterial strains.

Also, the results of this study showed that the aqueous extract of saffron petals had a higher antibacterial effect against the studied gram-positive bacteria, especially *B. cereus* compared to the gram-negative bacteria *E. coli*. Several studies have shown the higher susceptibility of gram-positive bacteria to various antimicrobial agents. Mohammadian (2016) showed that *B. subtilis* was the most sensitive bacterium and *E. coli* was the most resistant bacterium to petal and stigma extracts of different wild species of saffron which was in agreement with the results of present study. Tayel and El-Tras (2009) also showed that *S. aureus* and *B. cereus* were the most susceptible bacteria and *E. coli* and *Pseudomonas aeruginosa* were the most resistant bacteria to the saffron petal extract. However, Gandomi Nasrabadi *et al.* (2010) reported conflicting results. They showed that *S. Typhimurium* and *S. aureus* were the most sensitive and resistant bacteria against aqueous and alcoholic extracts of saffron petals, respectively. Azami *et al.* (2012) also showed that *S. typhimurium* is the most sensitive bacterium to the petal of saffron petals. The difference in the susceptibility of gram-negative and gram- positive bacteria to antimicrobial agents is probably due to the difference in their cell wall structure. The cell wall of gram-positive bacteria consists of thick layers of peptidoglycan and does not have an outer membrane on its cell wall, which can cause better penetration of active compounds into the cell, whereas the cell wall of gram-negative bacteria consists of thin layers of peptidoglycan and is more impermeable due to an outer membrane consisting of phospholipids molecules, lipopolysaccharides (LPS), lipoproteins and surface proteins (Silhavy *et al*., 2010). The extracts of saffron petal in the present study (especially alcoholic extracts) showed high antimicrobial activity against a gram-positive bacterium, but did not reveal high antibacterial activity against other gram-positive strains. For example, the ethanolic extract of saffron petals showed relatively high activity against *L. monocytogenes*, while other gram-positive bacterium, *S. aureus* was almost resistant to this extract. It is likely that the resistance of bacteria to antimicrobial agents is not necessarily due to differences in cell structure and other mechanisms are involved in this process. For example, resistance of gram-negative bacteria against antibiotics like penicillin originates from the secretion of the lactamase enzyme in the periplasmic space between the thin outer membrane and the cytoplasmic membrane (Elisha *et al*., 2017).

Conclusions

The results of the present study showed the saffron petal extracts (especially aquatic extract) can be used as a new and natural source of antibiotics compounds with potential active components against food-borne bacterial pathogen, especially gram-positive bacteria. On the other hand, due to the good taste and aroma of saffron petals, it is possible to use it in higher concentrations. Therefore, it is possible to use saffron petal extract as a natural preservative in the food industry. Taking into account that saffron petal is the main by-product of saffron production its usage in the food industry can avoid the waste of this by-product and if these petals are marketed or used, it will generate additional income for the producers. However, it should be noted that herbal medicines, like chemical medicines, may have unwanted side effects and cause irreparable damage to the user's body. Therefore, further in vivo and ex vivo confirmatory experiments are recommended for to verify efficacy of saffron petals as a natural preservative and its possible side effects on the consumer.

Authors' Contributions

H.F. was designing the experiment and reviewed and edited the manuscript. M.A. conducted the experiment and laboratory activities, analyzed data and wrote the draft of manuscript. N.A. assisted and conducted the extraction the materials in the laboratory.

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.

References

Abolfathi M, Akbarzadeh A, Hajimoradloo A, Joshaghani HR, Ross NW (2022). Seasonal variations in the skin epidermal structure and mucosal immune parameters of rainbow trout skin (*Oncorhynchus mykiss*) at different stages of farming. Fish & Shellfish Immunology 127:965-974. *https://doi.org/10.1016/j.fsi.2022.07.028*

Adegbaju OD, Otunola GA, Afolayan AJ (2020). Effects of growth stage and seasons on the phytochemical content and antioxidant activities of crude extracts of *Celosia argentea* L. Heliyon 6:04086. *https://doi.org/10.1016/j.heliyon.2020.e04086*

Adnan M, Patel M, Deshpande S, Alreshidi M, Siddiqui AJ, Reddy MN (2020). Effect of *Adiantum philippense* extract on biofilm formation, adhesion with its antibacterial activities against foodborne pathogens, and characterization of bioactive metabolites: an in vitro-in silico approach. Front in Microbiology 11:823. *https://doi.org/10.3389/fmicb.2020.00823*

Afraze Z, Bolandi M, Khorshidi M, Mohammadi Nafchi A (2014). Evaluation of antioxidant activity of aqueous and alcoholic extracts (methanol, ethanol) saffron petals. Saffron Agronomy and Technology 2:231-236. *https://doi.org/10.22048/jsat.2014.7867*

AL-Hmadi H, El Mokni R, Joshi RK, Ashour ML, Hammami S (2021). The impact of geographical location on the chemical compositions of *Pimpinella lutea* Desf. growing in Tunisia. Applied Sciences 11:7739. *https://doi.org/10.3390/app11167739*

Alizadeh Behbahani B, Shahidi F, Tabatabaei Yazdi F, Mohebbi M (2013). Antifungal effect of aqueous and ethanolic mangrove plant extract on pathogenic fungus "*in vitro*". International Journal of Agronomy and Plant Production 4:1652-1658. *https://doi.org/10.17795/zjrms-5992*

Arsene MMJ, Davares AKL, Viktorovna PI, Andreevna SL, Sarra S, Khelifi I, Sergueïevna DM (2022). The public health issue of antibiotic residues in food and feed: Causes, consequences, and potential solutions. Veterinary World 15:662-671. *https://doi.org/10.14202/vetworld.2022.662-671*

Asgarpanaha J, Darabi-Mahbouba E, Mahboubib A, Mehrabb R, Hakemivalac M (2013). *In-vitro* evaluation of *Crocus sativus* petals and stamens as natural antibacterial agents against food-borne bacterial strains. Iranian Journal of Pharmaceutical Sciences 9:69- 82. *https://doi.org/10.22037/ijps.v9.40861*

Askary M, Behdani MA, Mollaei H, Fallahi HR (2023). Evaluation of the effects of organic and conventional cultivation practices on phytochemical and anti-cancer activities of saffron (*Crocus sativus*). Journal of Agricultural Science and Technology 25:139-154. *https://doi.org/10.22037/ijps.v9.40861*

Azami L, Babapour A, Garechahi M (2012). Antimicrobial effect of aqueous extract of saffron petals on some of food-borne bacterial pathogen. Food Hygiene 2:63-73.

Bouhanna I, Boussaa A, Boumaza A, Rigano D, Maisto M, Basile A (2021). Characterization and antibacterial activity of gelatin-based film incorporated with *Arbutus unedo* L. fruit extract on *Sardina pilchardus*. Journal of Food Processing Preservation 45:15424. *https://doi.org/10.1111/jfpp.15424*

Cejudo Bastante C, Casas Cardoso L, Fernández-Ponce MT, Mantell Serrano C, Martínez de la Ossa EJ (2019). Supercritical impregnation of olive leaf extract to obtain bioactive films effective in cherry tomato preservation. Food Packag. Shelf Life 21:100338. *https://doi.org/10.1016/j.fpsl.2019.100338*

Chen J, Liao C, Ouyang X, Kahramanoglu I, Gan Y, Li M (2020). Antimicrobial activity of pomegranate peel and its applications on food preservation. Journal of Food Quality 2020:8850339. *https://doi.org/10.1155/2020/8850339*

Elisha IL, Botha FS, McGaw LJ, Eloff JN (2017). The antibacterial activity of extracts of nine plant species with good activity against *Escherichia coli* against five other bacteria and cytotoxicity of extracts. BMC Complementary and Alternative Medicine 2017:17-133. *https://doi.org/10.1186/s12906-017-1645-z*

Farahmandfar R, Esmaeilzadeh Kenari R, Asnaashari M, Shahrampour D, Bakhshandeh T (2019). Bioactive compounds, antioxidant and antimicrobial activities of *Arum maculatum* leaves extracts as affected by various solvents and extraction methods. Food science and nutrition 7:465-475. *https://doi.org/10.1002/fsn3.815*

Gandomi Nasrabadi H, Azami Sarokelaei L, Misaghi A, Abbaszadeh S, Shariatifar N, Tayyar Hashtjin N (2012). Antibacterial effect of aqueous and alcoholic extracts from petal of saffron (*Crocus sativus* L.) on some foodborne bacterial pathogens. Journal of Medicinal Plants 11:189-196.

Gebrechristos HY, Ma X, Xiao F, He Y, Zheng S, Oyungerel G, Chen W (2020). Potato peel extracts as an antimicrobial and potential antioxidant in active edible film. Food Science and Nutrition 8:6338-6345. *https://doi.org/10.1002/fsn3.1119*

Ghimpețeanu OM, Pogurschi EN, Popa DC, Dragomir N, Drăgotoiu T, Mihai OD, Petcu CD (2022). Antibiotic use in livestock and residues in food- a public health threat: A Review. Foods 11:1430. *https://doi.org/10.3390/foods11101430*

Gohari AR, Saeidnia S, Mahmoodabadi MK (2013). An overview on saffron, phytochemicals, and medicinal properties. Pharmacognosy Reviews 7:61-66. *https://doi.org/10.4103/0973-7847.112850*

Goli SAH, Mokhtari F, Rahimmalek M (2012). Phenolic compounds and antioxidant activity from saffron (*Crocus sativus* L.) petal. Journal of Agriculture Science 4:175-81. *https://doi.org/10.5539/jas.v4n10p175*

Gong S, Fei P, Sun Q, Guo L, Jiang L, Duo K (2021). Action mode of cranberry anthocyanin on physiological and morphological properties of *Staphylococcus aureus* and its application in cooked meat. Food Microbiology 94:103632. *https://doi.org/10.1016/j.fm.2020.103632*

Hashemi SM, Maassoumi SM, Gasempour HR (2018). The antimicrobial properties of extracts in *Crocus sativus* var. haussknechtii Boiss. & Reut. Ex Maw. Saffron Agronomy and Technology 5:407-412. *https://doi.org/10.22048/jsat.2017.55499.1171*

Hosseini A, Razavi BM, Hosseinzadeh H (2018). Saffron (*Crocus sativus*) petal as a new pharmacological target: a review. Iranian Journal of Basic Medical Sciences 21: 1091-1099. *https://doi.org/10.22038/IJBMS.2018.31243.7529*

Jadouali SM, Atifi H, Bouzoubaa Z, Majourhat K, Gharby S, Achemchem F, Elmoslih A, Laknifli A, Mamouni, R (2018). Chemical characterization, antioxidant and antibacterial activity of Moroccan *Crocus sativus* L petals and leaves. Journal of Materials and Environmental Science 9: 113-118. *https://doi.org/10.26872/jmes.2018.9.1.14*

Jafari-Sales A, Pashazadeh M (2020). Antibacterial effect of methanolic extract of saffron petal (*Crocus sativus* L.) on some standard gram positive and gram-negative pathogenic bacteria *in vitro*. Current Perspectives on Medicinal and Aromatic Plants 3:1-7. *https://doi.org/10.38093/cupmap.692879*

Jomehpour N, Ghazvini K, Jomehpour M (2019). Antibacterial activity of aqueous and methanolic extracts of *Crocus sativus* stigma and *Cinnamomum cassia* against clinical isolates of some gram-positive and gram-negative pathogenic bacteria. Medical Laboratory Journal 13:31-34. *http://dx.doi.org/10.29252/mlj.13.3.31*

Lee H, Yoon Y (2021). Etiological agents implicated in foodborne illness worldwide. Food Science of Animal Resources 41:1-7. *https://doi.org/10.5851%2Fkosfa.2020.e75*

Makarova K, Sajkowska-Kozielewicz JJ, Zawada K, Olchowik-Grabarek E, Ciach MA, Gogolewski K, ... Gambin A (2021). Harvest time affects antioxidant capacity, total polyphenol and flavonoid content of Polish St John's wort's (*Hypericum perforatum* L.) flowers. Scientific Reports 11:1-12. *https://doi.org/10.1038/s41598-021-83409-4*.

Marquez-Rodriguez AS, Nevárez-Baca S, Lerma-Hernández JC, Hernández-Ochoa LR, Nevárez-Moorillon GV, Gutiérrez-Méndez N (2020). *In vitro* antibacterial activity of *Hibiscus sabdariffa* L. phenolic extract and its *in situ* application on shelf-life of beef meat. Foods 9:1080. *https://doi.org/10.3390/foods9081080*

Mirheidar H (2005). Maaref giahi (Plant Knowledge). Tehran, Iran, Daftare Nashre Farhange Eslami 6:172-173.

Muzaffar S, Rather SA, Khan KZ (2016). In vitro bactericidal and fungicidal activities of various extracts of saffron (*Crocus sativus* L.) stigmas from Jammu & Kashmir, India. Cogent Food and Agriculture 2:1158999. *http://dx.doi.org/10.1080/23311932.2016.1158999*

Nekkaa A, Benaissa A, Lalaouna AE, Mutelet F, Canabady-Rochelle L (2021). Optimization of the extraction process of bioactive compounds from Rhamnus alaternus leaves using Box-Behnken experimental design. Journal of Applied Research on Medicinal and Aromatic Plants 25:100345. *https://doi.org/10.1016/j.jarmap.2021.100345*

Okmen G, Kardas S, Bayrak D, Arslan A Cakar H (2016). The antibacterial activities of *Crocus sativus* against mastitis pathogens and its antioxidant activities. World Journal of Pharmacy and Pharmaceutical Sciences 5:146-156

Patnala S, Kanfer I (2021). Quality control, extraction methods, and standardization: Interface between traditional use and scientific investigation. Herbal Medicine in Andrology 8:175-187. *http://dx.doi.org/10.1016/B978-0-12-815565-3.00006-0*

Pintado C, Miguel A, Acevedo O, Nozal L, Novella JL, Rotger R (2011). Bactericidal effect of saffron (*Crocus sativus* L.) on *Salmonella enterica* during storage. Food Control 22:638-642. *http://dx.doi.org/10.1016/j.foodcont.2010.09.031*

Rahnemoon P, Sarabi-Jama M, Bostan A, Mansouri E (2021). Nano-encapsulation of pomegranate (*Punica granatum* L.) peel extract and evaluation of its antimicrobial properties on coated chicken meat. Food Bioscience 43:101331. *https://doi.org/10.1016/j.fbio.2021.101331*

Rubab M, Chelliah R, Saravanakumar K, Kim JR, Yoo D, Wang MH (2020). Phytochemical characterization, and antioxidant and antimicrobial activities of white cabbage extract on the quality and shelf life of raw beef during refrigerated storage. RSC Advances 10:41430-41442. *https://doi.org/10.1039/D0RA06727J*

Saquib SA, AlQahtani NA, Ahmad I, Kader MA, Al Shahrani SS, Asiri EA (2019). Evaluation and comparison of antibacterial efficacy of herbal extracts in combination with antibiotics on periodontal pathobionts: An *in vitro* microbiological study. Antibiotics 8:89. *https://doi.org/10.3390/antibiotics8030089*

Serrano-Díaz J, Sánchez AM, Maggi L, Martínez-Tomé M, García-Diz L, Murcia MA, Alonso GL (2012). Increasing the applications of *Crocus sativus* flowers as natural antioxidants. Journal of Food Science 77:1162-1168. *https://doi.org/10.1111/j.1750-3841.2012.02926.x*

Seyfried EE, Newton RJ, Rubert KF, Pedersen JA, McMahon KD (2010). Occurrence of tetracycline resistance genes in aquaculture. Facilities with varying use of oxytetracycline. Microbial Ecology 59:799-807. *https://doi.org/10.1007/s00248-009-9624-7*

Shahid A, Ali MA, Muzammil S, Aslam B, Shahid M, Saqalein M, ... Khurshid M (2021). Antibiotic residues in food chains; impact on the environment and human health: A Review. Applied Ecology and Environmental Research 1:3959-3977. *http://dx.doi.org/10.15666/aeer/1905_39593977*

Shahnia M, Khaksar R (2013). Antimicrobial effects and determination of minimum inhibitory concentration (MIC) methods of essential oils against pathogenic bacteria. Iranian Journal of Nutrition Sciences and Food Technology 7:949-955.

Shehata MG, Awad TS, Asker D, El Sohaimy SA, Abd El-Aziz NM, Youssef MM (2021). Antioxidant and antimicrobial activities and UPLC-ESI-MS/MS polyphenolic profile of sweet orange peel extracts. Current Research in Food Science 4:326-335. *https://doi.org/10.1016/j.crfs.2021.05.001*

Sidiq S, Shrivastava P (2020). Antimicrobial, antioxidant, and anticancer activities of saffron (*Crocus sativus*): a review. Journal of Emerging Technology and Innovative Research 7:41-48

Silhavy TJ, Kahne D, Walker S (2010). The bacterial cell envelope. Cold Spring Harbor Perspectives in Biology 2:a000414. *https://doi.org/10.1101%2Fcshperspect.a000414*

Srivastava R, Ahmed H, Dixit RK Saraf SA (2010). *Crocus sativus* L.: a comprehensive review. Pharmacognosy Reviews 4:200-208. *https://doi.org/10.4103%2F0973-7847.70919*

Tayel AA, El-Tras WF (2009). Possibility of fighting food borne bacteria by Egyptian folk medicinal herbs and spices extracts. Journal of the Egyptian Public Health Association 84:21-32.

Terreni M, Taccani M, Pregnolato M (2021). New antibiotics for multidrug-resistant bacterial strains: latest research developments and future perspectives. Molecules 26:2671. *https://doi.org/10.3390/molecules26092671*

Thery T, Arendt EK (2018). Antifungal activity of synthetic cowpea defensin Cp-thionin II and its application in dough. Food Microbiology 73:111-121. *https://doi.org/10.1016/j.fm.2018.01.006*

Valizadeh S, Moosavy MH, Ebrahimi A, Akhondzadeh Basti A, Mahmoudi R, Khatibi SA (2022). The effect of aqueous extract of saffron (*Crocus sativus* L. Stigma) on the behavior of *Salmonella typhimurium* in a food model during storage at different temperatures. Journal of Nutrition and Food Security 7:37-45. *http://dx.doi.org/10.18502/jnfs.v7i1.8534*

Wali AF, Alchamat HAA, Hariri HK, Hariri BK, Menezes GA, Zehra U, Rehman MU, Ahmad P (2020). Antioxidant, antimicrobial, antidiabetic and cytotoxic activity of *Crocus sativus* L. petals. Applied Sciences 10:1519. *https://doi.org/10.3390/app10041519*

World Health Organization (2022). Key facts- food safety. Retrieved 2002 March 19 from: *https://www.who.int/news-room/fact-sheets/detail/food-safety*

Zambrano C, Kerekesa EB, Kotogán A, Papp T, Vágvölgyi C, Krisch J (2019). Antimicrobial activity of grape, apple and pitahaya residue extracts after carbohydrase treatment against food-related bacteria. LWT Food Science and Technology 100:416-425. *http://dx.doi.org/10.1016/j.lwt.2018.10.044*

Zhao Y, Kong H, Zhang X, Hu X, Wang M (2019). The effect of perilla (*Perilla frutescens*) leaf extracts on the quality of surimi fish balls. Food Science and Nutrition 7:2083-2090. *https://doi.org/10.1002/fsn3.1049*