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Antibacterial Effect of Methanolic Extract of Saffron Petal (*Crocus sativus* L.) on Some Standard Gram Positive and Gram Negative Pathogenic Bacteria *In vitro*

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Abstract

Background: Nowadays, increasing antibiotic resistance of bacteria has provided the opportunity to replace herbal remedies with fewer side effects than conventional medicines; therefore, in this study, the antibacterial effects of methanolic extract of saffron petal against *Staphylococcus aureus*, *Bacillus cereus*, *Pseudomonas aeruginosa* and *Escherichia coli* strains were evaluated. **Materials and Methods:** In this in vitro study, after collecting the saffron plant and confirming its scientific name, the saffron petal extract was prepared in concentrations of 20 mg/ml to 400 mg/ml. Then the antimicrobial effects of this extract were investigated by well diffusion and tube dilution method. **Results:** The results showed that the methanolic extract of saffron petal had antibacterial effects on the tested bacteria in both tube dilution and well diffusion methods. The highest effect was observed in *S. aureus* and the least in *P. aeruginosa.* **Conclusion:** Based on the above results, it can be hoped that Saffron petals extract can be used in the treatment of bacterial infections and can be a suitable replacement for conventional chemical drugs in the treatment of infections.

Key Words: Medicinal Plants, Antimicrobial Effects, Extract, Pathogenic Bacteria

1. Introduction

Increasing use of chemical drugs leading to development of antibiotic resistance to bacterial strains (VandenBergh et al., 1999). The medicinal plant is herbs with organs contain substances that affect living things (Singh et al., 2007). There has always been a close relationship between people and plants during the development of all human civilizations. Although most plant species are known to date, there is still much time left to discover new and valuable plant resources (Digrak et al., 2001; Skaltsa et al., 1999; Jafari-Sales et al., 2019a) which are only partially identified so far. These chemicals can be used as a drug but also as a unique starting point for the manufacture of pharmaceutical analogs, as well as an interesting tool to better understanding biological phenomena (Mobaiyen et al., 2016; Sales, 2014; Jafari-Sales et al., 2019b). One of the most important therapeutic challenges is coping with infectious diseases due to their high prevalence resulting with expansion of the

clinical use of synthetic antibiotics. The overuse of these antimicrobial drugs has led to increased drug resistance against different antibiotics in most bacteria (Sales et al., 2017; Sales et al., 2015; Jafari-Sales et al., 2019b). This has been one of the reasons for the growing use of herbs as low-risk, affordable and inexpensive natural ingredients in the treatment of bacterial infections compared to synthetic antibiotics. leading to increasing number of worldwide studies concerning antibacterial effects of various plants in recent years (Jafari-Sales et al., 2015; Jafari-Sales et al., 2019a).

The saffron plant, scientifically named Crocus sativus L. is a small, perennial plant of the Iridaceae family, which is a traditional medicinal plant that grows in different parts of the world. The dried stigma of this plant is used as saffron in the food industry (as a fragrant spice and for coloring food) and in the pharmaceutical industry (as a sedative and analgesic for asthma, black cough and inflammation) (Mirheidar, 2005). It is officially listed on the Chinese Medicines List and has been used in traditional Chinese medicine to treat hematomas, depression and seizures as a sedative (Tang and Eisenbrand, 2013). Recent studies have shown that this plant has the potential to reduce the risk of various diseases (Poma et al., 2012).

Some metabolites derived from saffron stigma due to hypolipidemic, antiparasitic, antioxidant, and diabetes mellitus function. Many therapeutic effects have shown themselves. The aqueous and alcoholic extracts of saffron are protective of the heart and counteract neurodegenerative disorders. Numerous medicinal properties of saffron are related to its various components such as Crocetin, Crocin and other substances that have potent antioxidant properties and accumulate oxygen free radicals and proinflammatory cytokines (Hepsø et al., 1988). Studies have shown that there are more than 151 different substances in saffron stigma. The strongest components of saffron are carotenoids and monoterpene aldehydes. Studies on the relationship between the function and structure of the molecule have shown that some properties of saffron are due to its deglycosylated derivatives, and others are related to glycosylated derivatives (Kandil et al., 1994).

Saffron petals contain strong antioxidant flavonoids that are bound to albumin in the blood serum and interact with this protein. On the other hand, the different effects of flavonoids on cholesterol-lowering and its anti-radical properties have been proven many times (Catoni et al., 2008). It is native to the Middle East and Southwest Asia, including Iran. Iran is one of the most important saffron production hubs in the world, so the value of saffron exports exceeds 300 billion rials per year. In the process of saffron production, stigma and cream are used as commercial saffron, and other parts of the flower, including petals, are discarded as having a high volume, so that the annual figure of 7257625 kg of saffron petals is obtained as a by-product which is expected to increase even in the coming years due to increasing production (Hemmati Kakhki and Rahimi, 1994).

Therefore, finding a solution to recover this large volume of waste is of great importance. One of these solutions could be the use of saffron petals as a natural antimicrobial agent in the treatment of bacterial infections. The root of this plant has beneficial medicinal properties such as anti-inflammatory, antiviral, antimicrobial and anti-cancer activity along with immune-enhancing effects, cough control and detoxification of the liver. It is also used in Addison's disease, asthma, bronchitis, cough, peptic ulcer and arthritis (Gupta et al., 2008; Gezici, 2019).

Therefore, this study aims to investigate the antibacterial effects of methanolic extract of saffron petal on some standard pathogenic bacteria.

2. Material and Method

In this descriptive *in vitro* study, saffron petals were collected from a field around the town of Benabe Marand in East Azarbaijan province. The petals were placed at ambient temperature for drying in the dark and were subjected to several steps until complete drying. After the samples were completely dried, the petals were prepared for grinding. Soxhlet method was used to extract 60 grams of petal powder with 300 ml of methanol as solvent for 8 hours in Soxhlet extractor. This solvent was slowly evaporated at 40 °C using rotary apparatus.

The concentrated extract was obtained from it. Extracts of solvent concentrated 5% DMSO (dimethylsulfoxide) at concentrations of 20, 30, 50 and 400 mg/ml for use in Minimum Inhibitory Concentration (MIC), Minimum Bactericidal Concentration (MBC) and Agar Well Diffusion experiments was prepared. The microorganisms studied in this study were: *S. aureus* ATCC 25923, *B. cereus* ATCC 1052, *E. coli* ATCC 25922, and *P. aeruginosa* ATCC 27853 (Microbial collection from University of Tehran). A separate culture was performed on the Mueller Hinton Agar medium to allow emerging colonies to be prepared with a half-McFarland turbidity solution (1.5×10^6 cfu/ml). For this purpose, for preparation of microbial suspension, 4-5 colonies of bacterial culture were transferred to Mueller Hinton Broth (MHB) to adjust the microbial suspension turbidity according to standard 0.5 McFarland tube. To reach a concentration of 1.5×10^6 cfu/ml, the microbial suspension was diluted to 0.01. In order to evaluate the antibacterial effect of methanol extract 4 concentrations of 20, 30, 50 and 400 mg/ml of methanolic extract were prepared in 5% DMSO solvent.

In this study, the antimicrobial effect of methanolic extract was investigated by agar well diffusion and dilution test. In the agar well diffusion method, 500 ml of microbial suspension was transferred onto Mueller Hinton Agar (MHA) medium and cultured by sterile swab in 3 directions. Then wells were prepared at 6 mm in diameter and 2.5 cm apart on agar surface. Then 100 µl of 20, 30, 50 and 400 mg/ml concentrations of methanol extract were injected into each well. Negative control was obtained using a solution used to dissolve the extracts (5% DMSO) and chloramphenicol antibiotic was used as positive control. Plates were then incubated at 37 ° C for 24 hours and microbial cultures were measured for the presence or absence of growth zone in millimeters.

The MIC and MBC of methanol extract were determined by tube dilution method. In this method, to determine the MIC of methanolic extract prepared by dilution serial dilutions of 6.25, 12.5, 25, 50, 100 and 200 mg/ml in MHB. Then, 1 ml of 1.5 × 106 cfu/ml active bacterial suspension was added to each dilution. Positive control (culture medium containing no bacterial extract) and negative control (culture medium without bacterium) were added to the tubes. Finally, the tubes were incubated at 37 °C for 24 hours.

After incubation, the tubes were examined for turbidity caused by inoculated bacterial growth and the last dilution in which no turbidity was observed (non-growth) was considered as MIC. Samples were then taken from all tubes in which bacterial growth was observed and MBC was determined by plate culture. Plates were then incubated for 24 hours at 37 °C. The tube containing the lowest concentration of the extract that had no visible bacterial growth on the plate was considered MBC of that material.

Each experiment was repeated 5 times to reduce the error of the experiment. SPSS software version 18 was used for data analysis. Analysis of variance and chi-square test were used to investigate the significant differences between the two groups and the significance level was set at $p < 0.05$.

3. Results

According to Table 1, the antibacterial activity of methanol extract of saffron petal in quantitative and qualitative methods showed that this extract showed a significant inhibitory effect on *S. aureus* and *B. cereus* bacteria. As the concentration of methanolic extract increased, the inhibitory effect increased. This study showed that the inhibitory effects of methanolic extract of saffron petal on Gram-positive bacteria was significantly higher compared to Gram-negative bacteria. MIC and MBC values of methanol extract of saffron petal against the tested bacteria showed that, like the well diffusion method, Saffron petal extract on Gram-positive bacteria has higher bactericidal power than Gram-negative bacteria (Table 2). These results indicated that there was a significant difference between the tested bacteria in the sensitivity of to saffron petal extract ($p < 0.05$).

Table 1. Mean diameter of non-growth zone of methanolic extract of saffron petal against selected bacteria in millimeters (mean ±standard deviation)

Well diffusion method (mm)						
	Concentration of extract (mg/ml)					
Bacterial strain	20 mg/ml	30 mg/ml	50 mg/ml	400 mg/ml	Negative control	Positive control
S. aureus	11.24±1.12	18±1.14	21.8±0.83	27.6±1.14	--	21
B. cereus	9±1.12	12.4±0.83	15.6±0.54	19.2±1.3	--	20
E. coli	0	0	11.6±1.14	16.5±0.83	--	27
P. aeruginosa	0	0	7.8±1.14	14.5±0.83	--	22

Table 2. MIC and MBC values of methanol extract of saffron petal (mg/ml)

Bacterial strain	**Concentration of extract (mg/ml)**	
	MIC	**MBC**
S. aureus	6.25	12.5
B. cereus	12.5	25
E. coli	50	100
P. aeruginosa	100	200

4. Discussion

The rise of pathogenic microorganisms and their resistance to a wide range of antibiotics along with the economic and social problems resulting from them have led to the expansion of studies on the production of herbal medicines. Therefore, screening such plants may lead to the discovery of new effective compounds that are able to inhibit pathogenic microorganisms. Compounds that can inhibit the growth of pathogenic microorganisms or kill them without toxicity to host cells are considered as candidates for the production of new antimicrobial drugs. As a result, there is a critical need for

research on novel antimicrobial agents with promising natural activities to provide alternatives to common antibiotics (Zhang et al., 2016). The results of this study showed that the saffron petal extract has an inhibitory effect on the gram-positive bacteria *S. aureus* and *B. cereus.* By increasing the concentration to 400 mg/ml, it is also effective on Gram-negative bacteria *P. aeruginosa* and *E. coli.* Vahid et al. (2002) examined the antimicrobial effects of the extract of different parts of saffron against E. coli, S. aureus, S. epidermidis and micrococcus showed that the extract of all parts of saffron except for leaves had antimicrobial activity (Vahidi et al., 2010). The results of this study showed that S. aureus and *B. cereus* were the most susceptible bacteria and *E. coli* and *P. aeruginosa* were the most resistant bacteria to the saffron petal extract, which was in agreement with the results of the study by Tayel and El-Tras (2009). Pintado et al. (2011) reported that safranal, crocin, and their associated chemicals are involved in the antimicrobial activity of saffron. Other researchers have pointed to the different susceptibilities of bacterial species to various antimicrobial agents. The difference in susceptibility of different microorganisms to antimicrobial agents is probably due to the different structure of the microorganisms. B. subtilis is one of the Gram-positive bacteria that, unlike Gram-negative bacteria, does not have an outer membrane on its wall, which can cause the active compounds to have a better penetration. Gram-negative bacteria are more impermeable due to the fat layer in the outer layer (Ordog et al., 2004). In a 2003 study by Razaghi et al., the antimicrobial effects of saffron stigma on three microbial strains of *E. coli, S. aureus* and *P. aeruginosa* were investigated and the results showed that safranal in saffron inhibited the growth of *E. coli* and *S. aureus* strains. Vahidi et al., (2010) investigated the antimicrobial effects of different parts of saffron extract including leaves, gynoecium and coralla against *E. coli, S. epidermidis,* micrococcus and fungi and reported their antimicrobial effects. Tajalli (2008), investigated the antioxidant effects of methanolic extract of saffron petal. The results showed that saffron petals were a natural and easy source of antioxidant with the highest concentration of inhibitory extract at 300pM. Islam et al., (2008) showed that plant extracts against Gram-positive bacteria were more effective than Gram-negative bacteria (Islam et al., 2008). Also, differences in the methods of evaluation of antibacterial properties of the extracts can lead to different results in the calculated MIC in different studies. Motamedi (2010.) by examining the antibacterial effect of ethanolic and methanolic extracts of saffron against some pathogenic bacteria showed that *S. aureus, B. anthracis, B. cereus, L. monocytogenes* and *B. melitensis* are the most susceptible species. *P. mirabilis* and *S. typhi* showed resistance to these extracts. In 2012, Azami et al., showed that *S. typhimurium* is the most sensitive bacterium to the petal of saffron petals. Gandomi Nasrabadi et al. (2012.) studying the antibacterial properties of saffron petal extract showed that methanol extract of saffron was effective on *S. typhimurium, B. cereus* and *L. monocytogenes*.

5. Conclusions

One of the reasons for the difference in MIC in different studies is the differences in the composition of the extracts. The composition of the extracts of a plant species can vary based on the region's geography, harvest season, plant age, growth stage, and the method of drying and extraction. In general, the plant extract has the highest antimicrobial activity during flowering or immediately after flowering. In addition, the extracts obtained from different parts of a particular plant have different antimicrobial activity. Also, the sensitivity of different bacteria to different extracts is different. In general, the results of this study showed that the extract of saffron extract has antibacterial

activity against the standard bacteria studied. However, clinical trials on patients after using saffron extract are recommended to confirm this data so that it can eventually be made available to patients in the category of medicinal plants.

Conflict of Interest

The authors have declared that they have no conflict of interest.

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