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Chemical Composition and Biological Activity of Jordanian Saffron

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Abstract: The chemical composition and active constituents of Jordanian planted saffron (*Crocus sativus* L) were studied, to evaluate a natural preservative effect of saffron on food. High Performance Liquid Chromatography (HPLC) studies using photodiode array analyses were performed on a waters HPLC system for the separation of several ingredients from alcoholic extracts of Jordanian saffron. Eight saffron peaks were identified by comparison of their retention times with those of known reference compounds and quantified from samples of saffron as follows: picrocrocin, picrocrocin acid, kaempferol, trans-crocin, trans-crocin-3, trans-crocin-4, safranal and cis-crocin-4. In this study the antibacterial effect of ethanolic extracts from petal of saffron against the most important foodborne pathogens *Salmonella typhimurium*, *Listeria monocytogenes*, *E. coli*, *Bacillus cereus* and *Staphylococcus aureus* were evaluated. The Minimum Inhibitory Concentration (MIC) of extracts were evaluated by agar dilution and broth micro dilution method. The most sensitive strain to extracts was *S. typhimurium* and the most resistant strains were *S. aureus* and *E. coli* MICs of all extracts were estimated against all bacteria using agar dilution method. The results of this study revealed that the studied extracts might be potential sources of natural antimicrobial agents and propose their potential application in food system as a natural preservative.

Key words: Saffron, petal, extract, antimicrobial activity, MIC, natural preservatives

INTRODUCTION

Saffron is the long orange-red dried stigma of *Crocus sativus* flower which is the most expensive spice in the world due to its production costs. About 68 kg of flowers are required to obtain one kg of saffron (Nasrabadi *et al*., 2012). Saffron (*Crocus sativus* L) is a source of plant polyphenols/carotenoids, used as important spice and food colorant in different parts of the world. It has also been used in traditional medicine for treatment of different types of illnesses, since, ancient times (Bukhari *et al*., 2018; Alonso *et al*., 2012; Veverka *et al*., 2013; D'Auria *et al*., 2004; Petrakis and Polissiou, 2017; Li *et al*., 1999; Garcia-Lafuente *et al*., 2009). Many of these medicinal properties of saffron can be attributed to a number of its compounds such as crocetin, crocins and other substances having strong antioxidant and radical scavenger properties against a variety of radical oxygen species and pro-inflammatory cytokine. The chemicals found in saffron may be classified according to their volatility or the absence of it. Its major non-volatile components include crocin, a-crocin, carotenoids that include lycopene, zeaxanthine and both α- and β-carotenes, crocetin and picrocrocin. Saffron contains more than 150 volatile and aroma-yielding compounds. Safranal is one of themain components of saffron essential oil. The major volatile components include terpene, terpene alcohol and terpene esters. Safranalis also a major volatile composite formed from picrocrocin as a result of the interaction of heat and enzymes during the drying process. Crocin from saffron has antioxidant activity stronger than α-tocopherol and can prevent the formation of peroxidized lipids and can partly restore Superoxide Dismutase (SOD) activity (Bukhari *et al*., 2018; Alonso *et al*., 2012; Veverka *et al*., 2013). During the processing of saffron stigma, a huge amount of petals and sepals called "tepal" are generated which is wasted as a bio-residue and useless material. Recently, phenolic compounds and radical scavenging activity of saffron tepal have been investigated (D'Auria *et al*., 2004; Petrakis and Polissiou, 2017). Also, the existence of kaempferol glycosides (84.0% of total flavonol content) anthocyanins and carotenoids in tepal of saffron was described (Li *et al*., 1999). Kaempferol as a tyrosinase inhibitor in the flower petals of *C. sativus* (Garcia-Lafuente *et al*., 2009). Tyrosinase is known as a Polyphenol Oxidase (PPO) which is responsible in browning of crustaceans (Bathaie and Mousavi, 2010). Many studies the anti-inflammatory effect of the saffron petal extracts (Ling *et al*., 2011; Arapceska *et al*., 2014; Fahim *et al*., 2012; Kafi *et al*., 2006; Lahmass *et al*., 2018; Husaini *et al*., 2010a, b).

MATERIALS AND METHODS

Plant materials: Plant materials were collected from South Jordanian Valley and cultivated at home.

Preparation of extracts: Approximately, 5 g of plant sample was weighed accurately and macerated with 250 mL of water at roomtemperature for 2 days using a laboratory-scale shaker. Then, the n-hexane phases were filtrated and evaporated undervacuum until dryness. The residue was dissolved again in 100 mL of n-hexane and the n-hexane phase was washed in aseparatory funnel with 2% NaOH solution to get rid of the impurity which is soluble in NaOH. After abandoning thealkali solution present in the lower layer, the upper solution was washed with distilled water several times until it wasneutralized. The extract, obtained after distillation under vacuum at 45°C in rotary evaporator was dissolved with 95% ethanol and then filtrated in 250 mL measuring flask. Then, 10 mL of filter liquor was transferredinto a 100 mL measuring flask. The 40 mL of 0.2% NaOH solution was added in the flask and then letit react at 50°C for 30 min. Afterthat, 0.08 mol/L acetic acid solution was filled up to the mark (Agha-Hosseini *et al*., 2008).

HPLC analysis: The analytical HPLC system employed consisted of a JASCO high performance liquid chromatography coupled with a diode array detector (MD910 JASCO, Tokyo, Japan). The analytical data were evaluated using a JASCO dataprocessing system (DP-L910/V). The separation was achieved on a Waters Spherisorb 5 μm ODS2 4.6×250 mm column (Milford, MA, USA) at ambient temperature. The mobile phase consisted of water with 1% glacial acetic acid (solvent A), water with 6% glacial acetic acid (solvent B) and water-acetonitrile (65:30 v/v) with 5% glacial acetic acid (solvent C). The gradient used was similar to that used for the determination of phenolics in wine (D'Auria *et al*., 2004) with some modifications.

The flow rate was 0.5 mL/min and the injection volume was 10 μL. The monitoring wavelength was 233. Theidentification of each compound was based on a combination of retention time. A mobile phase consisting of formicacid (% 0.2 v/v): acetonitrile (50:50) by isocratic elution was chosen to achieve maximum separation and sensitivity. Flow rate was 1.0 mL/min. Column temperature was set at 30°C. The samples were detected at 254 nm using photodiode array detector.

Antimicrobial activity

Minimum Inhibitory Concentration (MIC) tests: The samples were tested for their antimicrobial testing *in vitro* by the agar dilution technique. All samples were dissolved in Dimethyl Sulphoxide Solvent (DMSO) for the antimicrobial test and the solutions were sterilized by membrane filtration. Aliquots of samples were diluted with melted typtic Soy agar, tryptone, soytone, sodium chloride and agar to give concentrations of 2000, 1500, 1000, 500, 250, 125, 62.5 and 31.3 g/mL.

The saffron extracts were tested against microorganisms including *Salomonella typhimuririum*, *Listeria monocytogenes*, *E. coli*, *Bacillus cereus* and *Staphylo coccusaureus* and bacterial strains were cultured overnight in Nutrient Broth (NB) at 37°C.

RESULTS AND DISCUSSION

Essential oils extracted by ethanol from dry saffron analysis revealed the following compounds: picrocrocin, acid from picrocrocin, kaempferol, trans-crocin, trans-crocin-4, trans-crocin-3, safranal and cis-crocin-4 (Fig. 1 and 2 and Table 1 and 2). The Minimum Inhibitory Concentration (MIC) of extracts were evaluated by agar dilution and broth micro dilution method. The most sensitive strain to extracts was *S. typhimurium* (31.3 μg/mL) and the most resistant strains were *S. aureus* and *E. coli* (125 μg/mL) MICs of all extracts were estimated against all bacteria using agar dilution method.

Table 1: Chemical composition of the saffron

Compound	RT (min)	Yield (%)
Picrocrocin	22	4.00
Picrocrocin acid	26	15.3
Kaempferol	29	14.1
Trans-crocin	30	28.8
Trans-crocin-4	33	26.2
Trans-crocin-3	35	3.90
Safranal-1	37	3.70
Cis-crocin-4	39	4.00

Table 2: Antimicrobial activity of the saffron extracts MIC (μg/ml)

Microorganism	MIC (μg/mL)
S. typhimurium	31.3
L. monocytogenes	62.0
E. coli	125.0
B. cereus	62.0
S. aureus	125.0

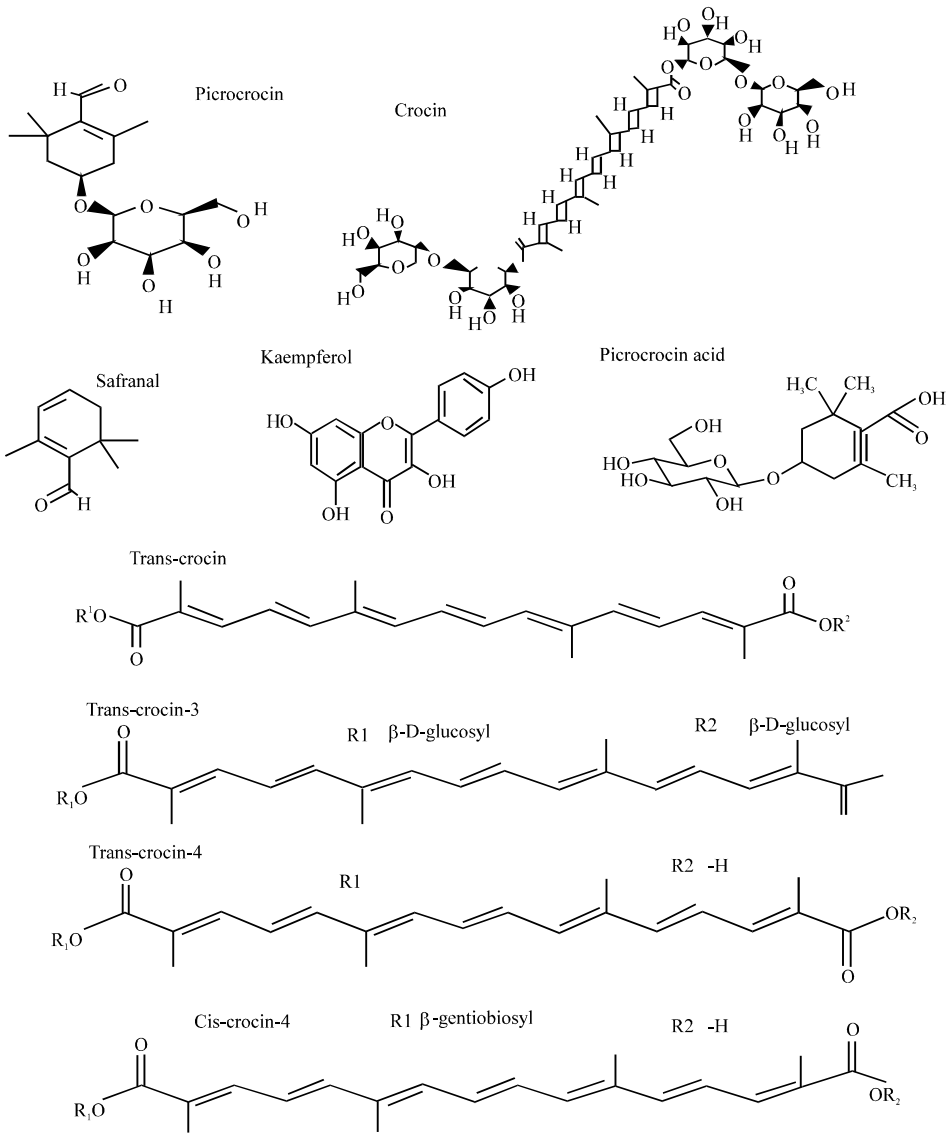

Fig 1: Chemical composition of the most active constituents of saffron

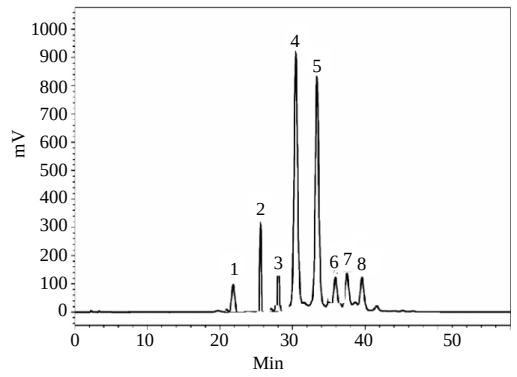

Fig. 2: HPLC chromatographic profiles for compounds from saffron petals

CONCLUSION

In this study the Minimum Inhibitory Concentration (MIC) of extracts were evaluated in food system as a natural preservative According to these finding, it can be concluded that saffron petals represents a very important dietary source of phytosterols. The saffron petals are a good source of phytosterols. It was demonstrated that it is possible to extract a considerable quantity of phytosterols from saffron petals. Nowadays, phytosterols are prepared from expensive sources. Given that each year, several tons of saffron petals are produced which are discarded and considered as agricultural waste. So, a rich source of phytosterols could be very beneficial in food and pharmaceutical industries.

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