J. Agr. Sci. Tech. (2019) Vol. 21(6): 1459-1471

Total Phenol/Flavonoid Content, Antibacterial and DPPH Free Radical Scavenging Activities of Medicinal Plants

L. Fahmideh[1], A. Mazaraie[1], and M. Tavakoli [2*]

ABSTRACT

The general desire to replace antibiotics and synthetic antioxidants with natural plants extracts has gained importance in recent years. This approach may be associated with the negative health effects of synthetic antioxidants and antibiotic resistance. Due to these controversial issues, in this study, free radical scavenging activity, Total Phenolic Content (TPC), Total Flavonoid Content (TFC), and antimicrobial activity of Methanolic Extracts (ME) from *Teucrium polium*, *Smyrnium cordifolium Boiss*, *Mentha longifolia*, and *Nectaroscordum tripedale* leaves were compared with *Crocus sativus* tepals. The antioxidant activity of these extracts was investigated in comparison with BHA, BHT, and α-tocopherol by DPPH method. Antimicrobial activities were determined by paper disc agar diffusion method against *S. aureus* (Gram positive) and *E. coli O157: H7* (Gram negative). Results showed that *C. sativus* tepals contained the highest TPC (37.36 mg GAE g^{-1}) and TFC (138.52 mg Q g^{-1}). Also, radical scavenging activity of *C. sativus* tepals ME (87.33%) was significantly higher than the other extracts; and it was the same as BHA and α-tocopherol statistically. In addition, a significant relationship between radical scavenging activity and TPC (R= 0.964) and TFC (R= 0.806) was found, illustrating the major role of these compounds in antioxidant activity of the mentioned plants. Antibacterial activity of *N. tripedale* leaf extract and *C. sativus* tepal extract against the two abovementioned pathogens were the highest among all the studied herbal extracts (P< 0.05). Moreover, Results of antimicrobial activities were also strongly correlated to free radical scavenging activity and TPC, which indicates the importance of these factors on antimicrobial properties of the five studied medicinal herbs.

Keywords: *C. sativus*, *Nectaroscordum tripedale*, Paper disc agar diffusion method, Total phenolic content.

INTRODUCTION

In the last decade, great efforts have been devoted to the study of medicinal plants properties like antioxidant and antibacterial activities (Mishra *et al*., 2018a). Antioxidants are compounds that can reduce cell damage by inactivation of free radicals which cause reduction in cardiovascular disease and cancer (Mishra *et al*., 2018b; Salehi *et al*., 2018; Vali Aftari *et al*., 2017). Although artificial antioxidants are extensively used in foods and drinks, experts have raised concerns about their negative side effects. Therefore, replacement of synthetic antioxidants with natural ones is an important issue for scientists (Dolek *et al*., 2018; Mianabadi *et al*., 2015; Yang *et al*., 2018). In the last few years, there has been a growing interest in chemical and microbial characteristics of medicinal plants as a perfected replacement for artificial antioxidants (Sharifi-Rad *et al*., 2018).

[1] Department of Plant Breeding and Biotechnology, Faculty of Agriculture, University of Zabol, Zabol, Islamic Republic of Iran.
[2] Department of Food Science and Technology, Faculty of Agriculture, University of Zabol, Zabol, Islamic Republic of Iran.
[*] Corresponding authors; e-mail: mtavakoli@uoz.ac.ir

[DOR: 20.1001.1.16807073.2019.21.6.21.0]

On the other hand, quite recently, considerable attention has been paid to antibiotic resistance among plant and human pathogens, which is drastically increased because of the growing interest in the use of commercial antimicrobial drugs for the treatment of infectious diseases (Tavakoli *et al*., 2017).

Current researches on spices and aromatic vegetable materials, which have long been used as flavoring agents and preservatives for foodstuffs, are focused on their important role in extending the shelf life of food products (Sethi *et al*., 2013). *Saffron* (*Crocus sativus*) is an expensive spice that has been used as food colorant and flavorant. Apart from that, several publications have appeared in recent years documenting the functional properties of *C. sativus* like antioxidant (Menghini *et al*., 2018; Rahaiee *et al*., 2015), antidepressant (Ghasemi *et al*., 2014), and anti-hyperlipidemic activity (Lee *et al*., 2005). For several years, great effort has been devoted to study of *C. sativus* stigmas, but little attention has been paid to the other parts of saffron flower such as tepals. *C. sativus* tepals are important by-products, since researchers reported that 350 kg tepals are discarded to produce 1 kg of *saffron* spice (Lotfi *et al*., 2013). On the other hand, Iran ranks worldwide as the first saffron producer and provide more than 80% of the world's saffron supply, especially Ghaenat City (Khorasan Province) (Azadi *et al*., 2017; Rahaiee *et al*., 2015). However, most of the previous studies did not take into account the Ghaenat *C. sativus* tepal despite its widespread use around the world.

In addition to *C. sativus*; *Teucrium polium*, *Smyrnium cordifolium Boiss* (SCB), *Mentha longifolia* and *Nectaroscordum tripedale* are examples of wild growing medicinal plants that have been commonly used in traditional medicine. *T. polium* is a gramineous, aromatic, perennial plant species that belongs to laminaceae family. This plant is wildly growing in southwestern Asia, Europe, and North Africa (Hasani *et al*., 2007). Belmekki *et al*. (2013) studied *T.polium* and showed that tannins, terpenoids, saponins, sterols, flavonoids and leucoantocianines compounds are responsible for its medical effects. However, most of the previous studies did not take into account antimicrobial properties of different parts of *T. polium*plant. Another species from this family, namely, *M . Longifolia*is, is widely spread in the world (de Sousa Barros *et al*., 2015). Quite recently, considerable attention has been paid to antioxidant and antimicrobial activities of various extracts of *Mentha* Spp. However, more studies are needed to identify its bioactive compounds and their properties (Gulluce *et al*., 2007).

SCB, which belongs to the umbeliferae family, can be found in Zagros Mountains of Iran, specifically Ilam Province (Mehrabi and Mehrabi, 2011). Recently, some authors have proposed that the SCB leaves could be a good source of natural antioxidants for industrial purposes (Sodeifian *et al*., 2014). However, to the authors' knowledge, leaf extract of SCB plant have been scarcely investigated from the point of view of antioxidant and antimicrobial activities.

N. tripedale is a species of the Alliaceae family that wildly grows in the west part of Iran (Yasuj region). Root, leaves, bark, and fruit of *N. tripedale* have been used traditionally for medical treatments like rheumatic disease, joint pains, and kidney and bladder stones (Mahmoudvand *et al*., 2016). However, to the author's best knowledge, very few publications can be found in the literature that discuss antioxidant and antimicrobial activities of *N. tripedale*.

Based on the above approaches, the purpose of this study was to investigate and compare, for the first time, the potential application of the methanolic extract of Ghaenat *C. Sativus* tepals and the ME of *M. longifolia*, *N. tripedale*, *T. polium* and SCB leaves extracts as natural food preservatives. Indeed, we aimed to compare TPC and TFC, antioxidant (DPPH assay) and antimicrobial (Disc-diffusion) activity of ME of *M. longifolia*, *N. tripedale*, *T. polium* and SCB leaves with *C. sativus* tepals extract; and compare free

[DOR: 20.1001.1.16807073.2019.21.6.21.0]

radical scavenging capacity of the 5 mentioned medicinal plants extracts with 3 commercial synthetic antioxidants (BHA, BHT and α-Tocopherol). In this paper, authors also explore the possible relationships between different assays.

MATERIALS AND METHODS

Materials

Five types of wild medicinal plants, namely, *C. sativus*, *T. polium*, *N. tripedale*, *M. longifolia* and SCB were collected from different parts of Iran. Folin-Ciocalteu reagent, gallic acid, sodium carbonate, aluminium chloride, potassium acetate, quercetin, Butylated HydroxyAnisole (BHA), Butylated HydroxyToluene (BHT) and α- tocopherol were purchased from Sigma and Merck Chemical Companies. Gentamicin disc were obtained from PatanTeb Co. Iran.

Sample Preparation

Sun dried medical herbs were powdered and sieved. Powder samples (5 grams) were used for methanolic extraction with methanol %76 (1:10) at room temperature under agitation (48 hours). Then, the mixtures were filtered in a Buchner funnel over Whatman No. 1 paper. The filtered methanolic extracts were concentrated in a rotary evaporator followed by oven distillation to constant weight. Finally, the dried residual materials were collected and stored at 4°C in a dark-glass container. Further analysis on the crude extracts was performed by using a concentration of 90 µg mL^{-1}.

Determination of Total Phenol Content

The amount of total phenolic was determined by using the Folin–Ciocalteu method (Chang *et al*., 2002). A volume of 0.5 mL from each of ME solutions was mixed with 25 mL of Folin-Ciocalteu reagent (0.2 normal). The reaction mixture was vigorously shaken for 5 minutes and 2 mL sodium carbonate (20% w/v) was added. The suspensions were maintained at room temperature for 2 hours, then, the absorbance was read at 760 nm. The TPC was expressed as mg of gallic acid equivalent per gram of extract dry powder based on a standard calibration curve.

Determination of Flavonoid Content

The colorimetric method was performed in order to estimate TFC (Chang *et al*., 2002). Half mL of extract solution was dissolved in 1.5 mL methanol and 0.1 mL aluminium chloride (10%), 0.1 mL of potassium acetate (1M) and 2.8 mL distilled water were added to the mixture and kept at room temperature for 30 minutes. The prepared samples absorbance was read spectrophotometrically at 415 nm. TFC of samples were calculated as quercetin equivalent (mg quercetin g^{-1} extract powder) from a calibration curve.

Determination of Antiradical Activity

Free radical-scavenging activity was examined by (1,1-diphenyl-2-picryl hydrazyl radical) DPPH assay (Burits and Bucar, 2000). Samples of 0.1 mL of plant extracts and synthetic antioxidant (α-Tocopherol, BHA and BHT) solutions were added to 3.9 mL DPPH (0.004%) in methanol (A_s). A control sample containing 0.1 mL methanol in the DPPH solution was prepared (A_c). After 30 minutes incubation at room temperature in dark, absorbance of samples were determined spectrophotometrically at 517 nm. The equation that describes radical Scavenging activity is as follows:

$$Sc\ (\%) = [(A_c - A_s)/A_c] \times 100$$

Where,Sc was DPPH scavenging activity (%), A_c was the Absorbance of the control

[DOR: 20.1001.1.16807073.2019.21.6.21.0]

and A_s was the Absorbance of the herbal extract.

Antibacterial Activity Determination

Disc diffusion inhibitory method on Mueller-Hinton agar was carried out to find out antimicrobial activity of the 5 studied herbal extracts against *Escherichia coli* O157: H7 and *Staphylococcus aureus* ATCC25923. Microbial suspension containing 10^6 cfu mL^{-1} of the mentioned bacteria was used for plate inoculation by sterile swabs. Disc (6 mm diameter) was placed on each plate. The distance between two discs was approximately 25 mm and the distance between a disc and the plate edge was at least 5 mm. Eighteen microliters of herbal extract suspension was placed onto the center of the discs using micropipette. Gentamycin (GM10) was used as positive control. Finally, the plates were incubated at 37°C for 18 to 24 hours. The inhibition zone diameters were measured using a caliper (Jahan *et al*., 2013).

Statistical Analysis

Experimental treatments were carried out using full factorial design with tree replications. Analysis of variance was performed by ANOVA procedures (SAS 9.1 for Windows). Tukey's tests were performed to analyze for mean differences. *P* values (< 0.05) were regarded as significant level. Correlations between data obtained were calculated by statistical coefficient correlation option in the MS Excel software.

RESULTS AND DISCUSSION

Total Phenolic Analysis

In this study, the Folin–Ciocalteu assay was performed in order to estimate the TPC of 5 medicinal plants. Phenolic compounds in plants play an important role in antioxidant properties. Analysis of variance showed that *C. sativus* tepals extract contained the highest amount of TPC (37.36 mg GAE g^{-1} of extract) despite SCB extract (13.86) which had the lowest level of this

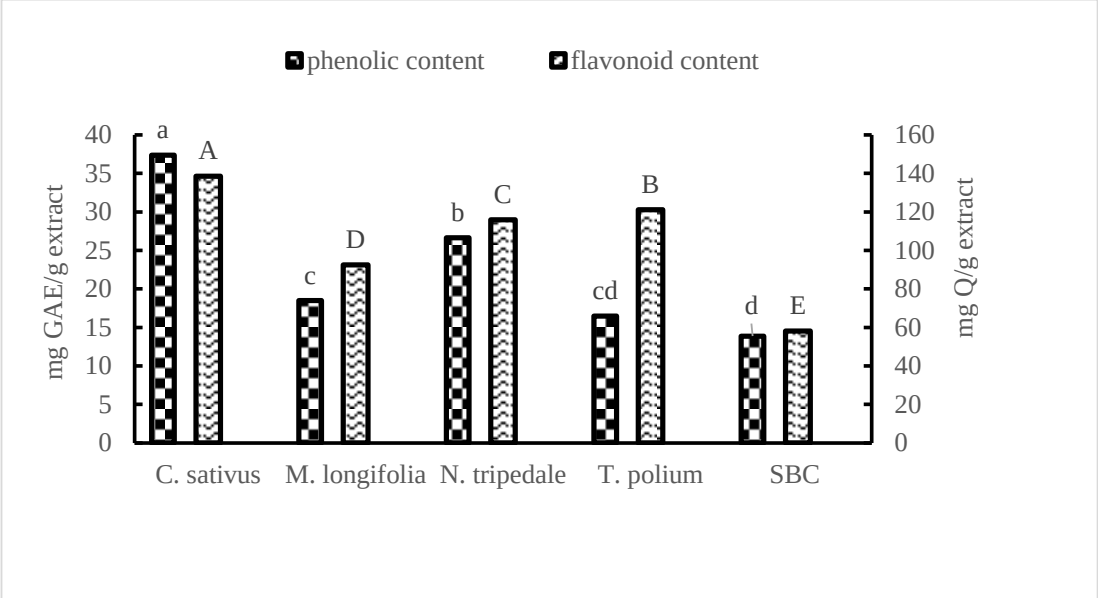

Figure 1. Total phenolic content (mg gallic acid g^{-1}) (▪) and Total flavonoid content (mg quercetin g^{-1}) (▨) of methanolic extracts from *C. sativus* tepals and *Teucrium polium*, *Smyrnium cordifolium Boiss*, *Mentha longifolia*,*Nectaroscordum tripedale* leaves. (▪) Columns followed by different letters (a–d) are significantly different (P< 0.05). (▨) Columns followed by different letters (A–E) are significantly different (P< 0.05).

[DOR: 20.1001.1.16807073.2019.21.6.21.0]

factor (P< 0.05) (Figure 1). In this way, the TPC (mg GAE g^{-1}) of *N. tripedale* (26.63), *M. longifolia* (18.47), and *T. polium* (16.46) were determined (P< 0.05). Sariri *et al*. (2011) studied *C. sativus* flower waste and proposed 86.65 mg GAE g^{-1} of TPC for its ME. Similar to the current study, Abbasvali *et al*. (2016) obtained a TPC value of 64.18 mg GAE g^{-1} for *C. sativus* tepals. In addition, previous researches have documented the different TPC values for the ME of *M. longifolia* (67.05 mg GAE g^{-1}) (Bahadori *et al*., 2018) and (89.1 mg GAE g^{-1}) (Hajlaoui *et al*., 2009). Several authors have proposed that the antioxidant activity of *M. longifolia* extract is related to its phenolic compounds (Asekun *et al*., 2007; Tepe *et al*., 2004). In similar studies, the amount of TPC in ME from *T. polium* and SCB leaves was 157.84 and 22.26±0.18 mg GAE g^{-1} (Stanković *et al*., 2012; Tabaraki and Ghadiri, 2013). More researches on *T. polium* extract have revealed that phenylpropanoid, glycosides, iridoid glycosides, and flavonoids compounds play a key role in the antioxidant activity of this plant (De Marino *et al*., 2012). Further analysis of essential oils obtained from SCB leaves indicated the presence of curzerenone (22.8%), hexadecanoic acid (18.7%), curzerene (16.9%), and spathulenol (7.7%) with potential antioxidant activities (Amiri *et al*., 2006). In this context, to the author's knowledge, this is the first study to investigate the TPC of *N. tripedale*.

From the above section, it could be recognized that the experimental data of the present study are not compatible with the other researchers' data. This may be due to the differences in environmental factors, climate, cultivars, time of collection, and variation in the sample preparation, solvent extraction methods, extraction times, and storage condition.

Total Flavonoids Analysis

Figure 1 represents the TFC of the 5 medicinal plants extracts. Among all the herbal extracts, *C. sativus* tepals had the highest TFC (mg Q g^{-1} extract) (138.52), followed by *T. polium* (121.06), *N. tripedale* (115.81), *M. longifolia* (92.4), and SCB (58.06) (P< 0.05).

The anthocyanins belong to the flavonoid family, which is mostly responsible for the *C. sativus* tepals color. *C. sativus* tepals contain different types of flavononls (Kaempferol, quercetin, isorhamnetin) and anthocyanins (delphinidin, petunidin, malvidin). Several studies have suggested that there are relationships between flavonoids and antioxidant activity and health effects (Bagherzade *et al*., 2017). Menghini *et al*. (2018) compared the TFC of the ME of *C. sativus* tepals (27±4 mg Q g^{-1}) and stigma (162±8 mg Q g^{-1}) as well.

In addition to *C. sativus*, scientists found considerable TFC for the ME of *M. longifolia* (63.93 mg Q g^{-1}) (Hajlaoui *et al*., 2009). Lately described in the literature, *Mentha* plant contains a variety of natural antioxidants such as flavonoids and phenolic acids (Bahadori *et al*., 2018). Also, several publications have appeared in recent years documenting a variety of flavonoids in *T. polium* such as cirsimaritin, cirsilineol, 5-hydroxy-6, 7, 3', 4'-tetramethoxyle flavone, salvigenin, apigenin 5-galloylglucoside, apigenin-7-glucoside, vicenin-2 and luteolin-7-glucoside (Hasani *et al*., 2007).

Previous research on chemical structure of *SCB* species has demonstrated the presence of flavonoid glycosides, essential oils and sesquiterpenelactones (Amiri *et al*., 2006). Contrary to the 4 abovementioned plant extracts, very few publications are available in the literature about *N. Tripedale* ingredients.

Antioxidant Activity

Radical scavenging activity of *C. sativus* tepals, *T. polium, N. tripedale, M. longifolia* and SCB were tested against the DPPH (2, 2-Diphenyl-1-Picryl-Hydrazyl-Hydrate) free-radical (Figure 2). ANOVA Results indicated that radical scavenging activity of

[DOR: 20.1001.1.16807073.2019.21.6.21.0]

ME of *C. sativus* tepals (87.33%) was significantly higher than the rest of the herbal ME (P< 0.05). On the other hand, although the capacity to scavenge DPPH by *C. sativus* tepals extract was lower than BHA and α-tocopherol standards, the differences were not statistically significant. These results are consistent with previous studies which have found out that tepals of *C. sativus* contain active constituents with antioxidant potential (Abbasvali *et al*., 2016; Sanchez-Vioque *et al*., 2012). Ochiai *et al*. (2004) studied Crocin, isolated carotenoid from saffron, and showed that the lipid peroxidation-inhibiting properties and antioxidant activity against neuronal oxidative stress of crocin was higher than α-tocopherol. According to the phytochemical analysis of *C. sativus* flower, it is rich in antioxidant ingredients like flavonols, flavanones, crocin and crocetin (Zeka *et al*., 2015).

It can be understood from the ANOVA analysis that *N. tripedale* (64.22%) had the second highest level of antiradical activity among 5 herbal extracts comparable with that of BHT and much lower than that of α-tocopherol and BHA. Although some researchers have investigated the important substances (flavonoids, terpenoids, tannins and fatty acids) in *N. tripedale* (Mahmoudvand *et al*., 2016), very few publications can be found in the literature that discuss its antioxidant activity.

As plotted in Figure 2, the free radical-scavenging activities of *T. polium* (37%), *M. longifolia* (23.88%) and SCB (22.5%) were statistically similar and lower than synthetic antioxidants (BHA, BHT and α-Tocopherol). Numerous articles have appeared recently documenting the antioxidant activity of the abovementioned herbal medicines as well (Bahadori *et al.*, 2018; De Marino *et al*., 2012; Hajlaoui *et al*., 2009; Sodeifian *et al*., 2014; Stanković *et al*., 2012; Tabaraki and Ghadiri, 2013;Tepe *et al*., 2004;). In related references, it was observed that compounds such as iridoid glycosides, phenyl propanoid glycoside and flavonoids were the most potent DPPH scavengers in *T. polium* extracts (De Marino *et al*., 2012).

As mentioned above in sections 1 and 2, *C. sativus* tepals represented the highest TPC and TFC. These findings could be good reasons for high antiradical activity of *C. sativus* tepals extracts. On the other hands, our results described for the first time that *N.*

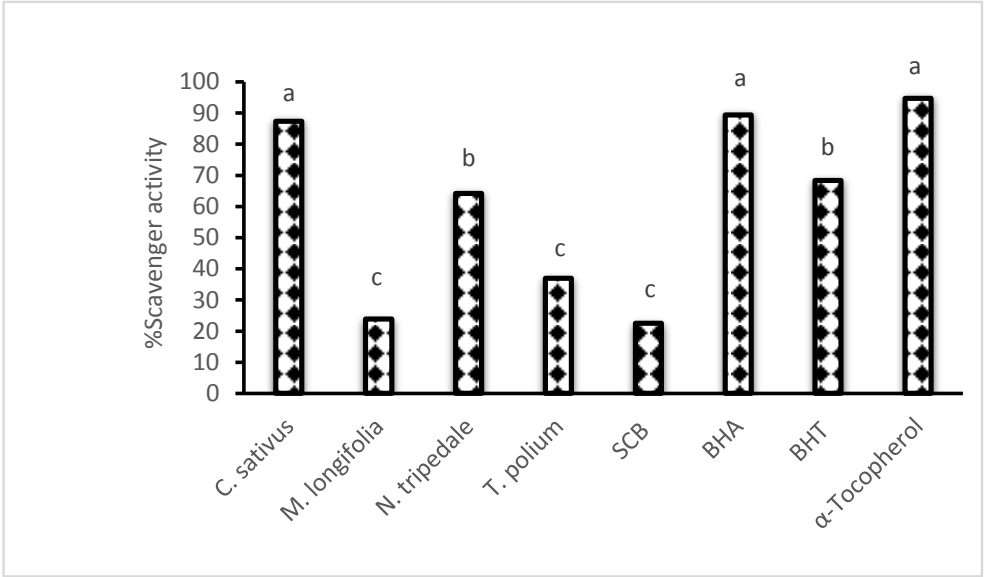

Figure 2. DPPH radical scavenging activity (%) of methanolic extracts obtained from *C. sativus* tepals and leaves of *Teucrium polium*, *Smyrnium cordifolium Boiss*, *Mentha longifolia*, and *Nectaroscordum tripedale*. Columns with unlike superscript letters are significantly different (P< 0.05).

[DOR: 20.1001.1.16807073.2019.21.6.21.0]

tripedale has a high level of antioxidant activity comparable with *C. sativus* tepals. There is a good match between antioxidant activity of *N. tripedale* and its TFC and TFC as well.

Antimicrobial Analysis

Quantitative evaluation of antibacterial activities of 5 herbal ME against *S. aureus* and *E. coli* O157: H7 are given in Table 1. All of the 5 studied MEs exerted considerable inhibitory activity against the two foodborne pathogens. As determined by ANOVA test (Table 1), the inhibition zones diameters of *C. sativus* tepals and *N. tripedale* extracts were significantly higher than *T. polium, M. longifolia* and SCB extracts; and were mostly identical ($P< 0.05$). Results showed that the antimicrobial activity of all 5 studied medicinal plants were higher against *S. aureus* than *E. coli*, in good agreement with previous studies (Gandomi Nasrabadi *et al*., 2012; Shan *et al*., 2007). In similar approach, Carmona *et al*. (2007) studied the antibacterial ingredients of *C. sativus* and indicated that crocin and safranal were the most effective components against the tested strains. Khanahmadi *et al*. (2010) reported that Gram-negative bacteria seemed to be more resistant to ME of SCB than Gram-positive bacteria. Also, they claimed that antimicrobial activity of SCB was due to the presence of hydrocarbons and sesquiterpenes compounds like germacrone, curzerenone and curzerene (Sodeifian *et al*., 2014). Similarly, scientists suggested that antimicrobial activity of *N. tripedale* is due to its terpenoids compositions. The mostly proposed mechanism to explain the antibacterial activity of terpenoids is damaging microbial cell membrane and DNA molecule and inhibiting the fatty acid synthesis in pathogens (Mahmoudvand *et al*., 2016).

The presence of antimicrobial compounds in plants tissues may occur because of the host defense mechanism against pathogens. Phenolic compounds are examples of these antimicrobial substances. In this regard, results of present research demonstrated that all 5 methanolic herbal extracts contained high level of TPC as well (Figure 1). These materials damage bacteria cell membranes integrity and interfere with cell functions, ultimately disrupt cell membranes with the release of cellular content. Effects of antimicrobial agents on microorganisms mostly depend on microbial cell membrane structures and components. For instance, gram-positive bacteria are more sensitive to plant extracts than gram-negative bacteria (Karaman *et al*., 2003; Sahin *et al*., 2003).

Furthermore, to the best of our knowledge, this is the first time that the in-vitro antimicrobial activity of *T. polium* is

Table 1. Growth inhabitation of *Staphylococcus aureus* ATCC25923 and *Escherichia coli* O157: H7 bacteria by methanolic extracts from *C. sativus* tepals and *Teucrium polium, Smyrnium cordifolium Boiss, Mentha longifolia, Nectaroscordum tripedale* leaves.

	Inhibition zone diameters (mm)	
Medicinal plants	*S. aureus*	*E. coli*
Crocus sativus	$22.35 \pm 0.25^{a*}$	15.00 ± 0.50^{AB}**
M. longifolia	7.55 ± 0.10^{b}	7.50 ± 0.25^{BC}
N. tripedale	20.00 ± 0.12^{a}	16.65 ± 0.15^{A}
T. polium	6.25 ± 0.50^{b}	5.50 ± 0.30^{C}
SCB	11.30 ± 0.37^{ab}	6.65 ± 0.25^{BC}
Gentamicin (Control)	24.95 ± 0.19	21.20 ± 0.46

* Mean values±standard deviation in first column followed by different superscripts letters 'a–b' are significantly different ($P< 0.05$). ** Mean values±standard deviation in second column followed by different superscripts letters 'A-C' are significantly different ($P< 0.05$).

[DOR: 20.1001.1.16807073.2019.21.6.21.0]

Table 2. Correlation coefficient (R) between Assays.

	Phenolic content	Flavonoids content	Antimicrobial effects against *S. aureus*	Antimicrobial effects against *E. coli*
Flavonoids compounds	0.759			
Antimicrobial effects against *S. aureus*	0.877	0.470		
Antimicrobial effects against *E. coli*	0.850	0.553		
Antioxidant activity	0.964	0.806	0.892	0.86

reported in the literature.

Correlation between Assays

Table 2 outlines a regression analysis to correlate the results of different analyses [correlation coefficient (R)]. Significant correlations were observed between the antioxidant activity, TPC, TFC and antimicrobial activity ($P< 0.05$). It has been found that the correlation between DPPH assays and TPC was the highest (R= 0.964, Figure 3-A). These findings indicate that TPC plays a major role in the antioxidant activity of these herbal medicines. Similarly,

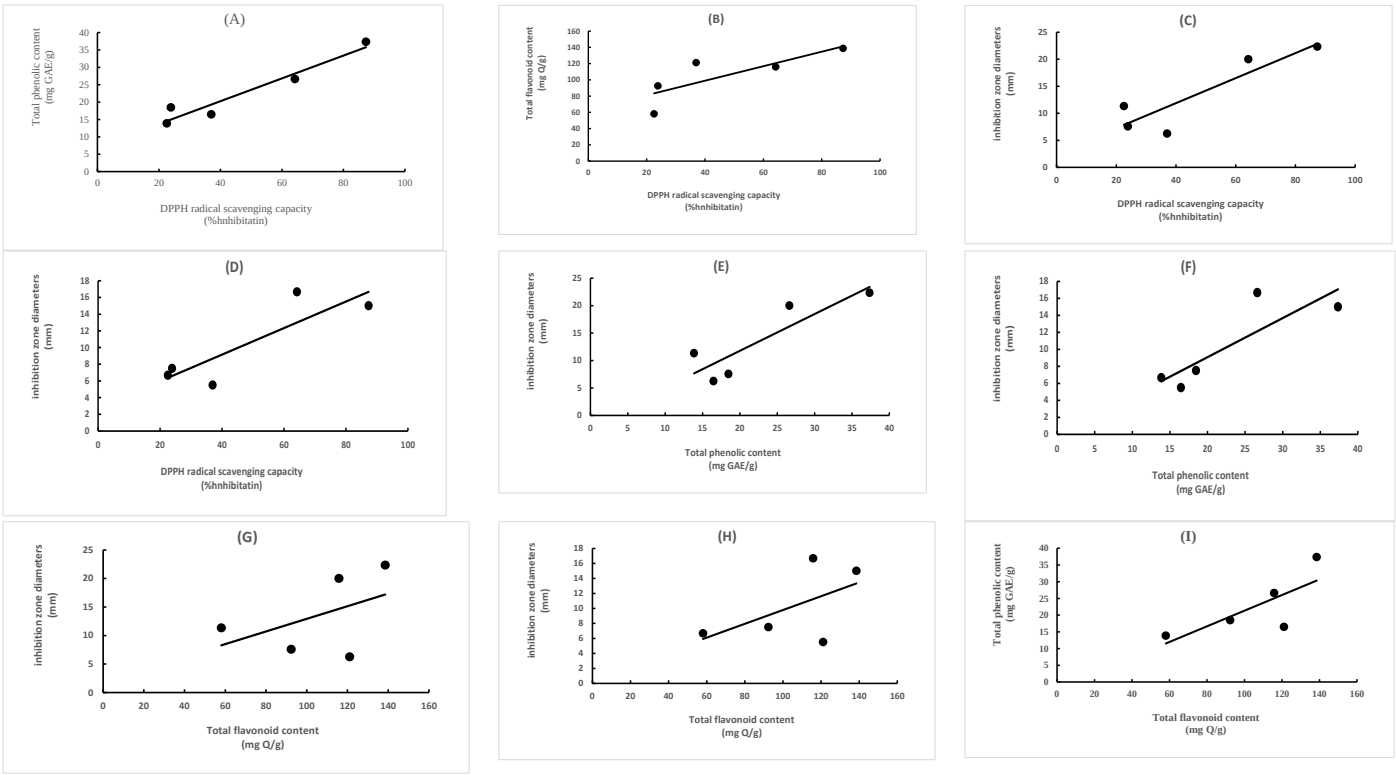

Figure 3. Correlation between: (A) DPPH assay and Total phenolic contents (R= 0.964), (B) DPPH assay and Total flavonoid contents (R= 0.806), (C) DPPH assay and antimicrobial activity against *S. aureus* (R= 0.892), (D) DPPH assay and antimicrobial activity against *E. coli* (R=0. 863), (E) Total phenolic contents and antimicrobial activity against *S. aureus* (R= 0.877), (F) Total phenolic contents and antimicrobial activity against *E. coli* (R= 0. 850), (G) Total flavonoid contents and antimicrobial activity against *S. aureus* (R= 0.470), (H) Total flavonoid contents and antimicrobial activity against *E. coli* (R= 0.553), and (I) Total flavonoid contents and Total phenolic contents (R= 0.759).

[DOR: 20.1001.1.16807073.2019.21.6.21.0]

results of antiradical activity were also correlated to TFC (R= 0.806, Figure 3-B). These strong correlations are in good agreement with the viewpoint that describes the potential of antioxidant activity of medicinal plants as a function of their TPC and TFC. Likewise, results obtained with DPPH and TPC assays can be related significantly with results obtained in antimicrobial assays against gram positive (*S. aureus*) (R= 0.892, Figure 3-C and R= 0.877, Figure 3-E) and gram negative bacteria (*E. coli*) (R=0.86, Figure 3-D and R= 0.850, Figure 3-F). All these data clearly demonstrated that the correlation between antioxidants and gram- positive bacteria was higher than their correlations with gram-negative bacteria. Moreover, all these results may be able to support the idea of antimicrobial properties of medicinal herbal extracts as a function of their antioxidant capacities. The lowest correlations were found between TFC and antimicrobial assays (R= 0.553, Figure 3-H and R= 0.470, Figure 3-G against *E.coli* and *S. aureus,* respectively).On the contrary, results showed a moderate correlations between TFC and TPC (R= 0.759, Figure 1).

CONCLUSIONS

The application of antioxidants and antimicrobials of natural origin in human food have drawn more attention in recent years. In this research, we studied the TPC, TFC, antioxidant and antimicrobial activities of 5 Iranian herbal medicine (*T. polium*, SCB, *M. longifolia*, *C. sativus*, *N. tripedale*). This paper has clearly shown that *C. sativus* tepals contain the highest amount of phenolics (37.36 mg GAE g^{-1}) and flavonoids (138.52 mg Q g^{-1}) and also the highest antiradical activity (87.33%); and antimicrobial activity against *E. coli* and *S. aureus*. Based on the results, it can be concluded that the antioxidant activity of the 5 mentioned extracts had significant correlations with TPC (R= 0.964) and TFC (R= 0.806). From this, we deduce that TPC and TFC are two most effective compounds on antioxidant activity of the abovementioned medicinal plants. Likewise, significant correlation was found between antimicrobial activity of these plants and DPPH assay (R= 0.892, against *S. aureus* and R= 0.86, against *E. coli*). In the same way, results of antimicrobial properties were also correlated to TPC (R= 0.877, against *S. aureus* and R= 0.850, against *E. coli*). These results indicated a high significant relationship between antioxidant concentrations in medicinal plant extracts and their antimicrobial capacities against pathogens. The findings suggested that the tepals of *C. sativus*, as a by-product, have great potential for industrial applications such as food preservatives and pharmaceutical formulations. Regression analysis indicated that the antioxidant properties were significantly correlated with TPC and TFC. The next stage of our research will study more medicinal plants extracts to find a mathematical model to predict the effects of their major compounds on their antioxidant and antibacterial activities. Further, in our future research, we intend to concentrate on the potential use of these medicinal plants extracts in foods and drugs formulations.

ACKNOWLEDGEMENTS

The authors would like to extend their thanks to Zabol University for its support in implementing the project. This work was supported by grants from the University of Zabol (UOZ-GR-9517-118).

REFERENCES

1. Abbasvali, M., Ranaei A., Shekarforoush, S. S. and Moshtaghi, H. 2016. The Effects of Aqueous and Alcoholic Saffron (*Crocus sativus*) Tepal Extracts on Quality and Shelf-Life of Pacific White Shrimp (*Litopeneousvannamei*) during Iced Storage. *J. Food Quality*, **39**: 732-742.

[DOR: 20.1001.1.16807073.2019.21.6.21.0]

2. Amiri, H., Khavari-Nejad, R. A., Masoud, S. H., Chalabian, F. and Rustaiyan, A. 2006. Composition and Antimicrobial Activity of the Essential Oil from Stems, Leaves, Fruits and Roots of *Smyrnium cordifolium Boiss*. From Iran. *J. Essent. Oil Res*., **18**: 574-577.
3. Asekun, O. T., Grierson, D. S. and Afolayan A. J. 2007. Effects of Drying Methods on the Quality and Quantity of the Essential Oil of *Mentha longifolia* L. subsp. *Capensis*. *Food Chem*., **101**: 995-998.
4. Azadi, P., Bagheri, K., Gholami, M., Mirmasoumi, M., Moradi, A. and Sharafi, A. 2017. Thin Cell Layer, a Suitable Explant for *In vitro* Regeneration of Saffron (*Crocus sativus* L.). *J. Agr. Sci. Tech*., **19***:* 1429-1435.
5. Bagherzade, G., Tavakoli, M. M. and Namaei, M. H. 2017. Green Synthesis of Silver Nanoparticles Using Aqueous Extract of Saffron (*Crocus sativus* L.) Wastages and Its Antibacterial Activity against Six Bacteria. *Asian Pac. J. Trop. Biomed*., **7**: 227-233.
6. Bahadori, M. B., Zengin, G., Bahadori, S., Dinparast, L. and Movahhedin, N. 2018. Phenolic Composition and Functional Properties of Wild Mint [*Mentha longifolia* var. *calliantha* (Stapf) Briq.]. *Int. J. Food Prop*., **21**: 183-193.
7. Belmekki, N., Bendimerad, N., Bekhechi, C. and Fernandez, X. 2013. Chemical Analysis and Antimicrobial Activity of *Teucrium polium* L. Essential Oil from Western Algeria. *J. Med. Plants Res*., **7**: 897-902.
8. Burits, M. and Bucar, F. 2000. Antioxidant Activity of *Nigella sativa* Essential Oil. *Phytother. Res*., **14**: 323-328.
9. Carmona, M., Zalacain, A., Salinas, M. R. and Alonso, G. L. 2007. A New Approach to Saffron Aroma. *Crit. Rev. Food Sci*., **47**: 145-159.
10. Chang, Y. L., Kim, D. O., Lee, K. W., Lee, H. J. and Lee, C. Y. 2002. Vitamin C Equivalent Antioxidant Capacity (VCEAC) of Phenolic Phytochemicals. *J. Agr. Food Chem*., **50**:3713-3717.
11. De Marino, S., Festa, C., Zollo, F., Incollingo, F., Raimo, G., Evangelista, G. and Iorizzi, M. 2012. Antioxidant Activity of Phenolic and Phenylethanoid Glycosides from *Teucriumpolium* L. *Food Chem*., **133**: 21-28.
12. de Sousa Barros, A., de Morais, S. M., Ferreira, P. A. T., Vieira, Í. G. P., Craveiro, A. A., dos Santos Fontenelle, R. O., de Menezes, J. E. S. A., da Silva, F. W. F. and de Sousa, H. A. 2015. Chemical Composition and Functional Properties of Essential Oils from *Mentha* Species. *Ind. Crops Prod*., **76**: 557-564.
13. Dolek, U., Gunes, M., N. Genc, N. and Elmastas, M. 2018. Total Phenolic Compound and Antioxidant Activity Changes in Rosehip (*Rosa* sp.) during Ripening. *J. Agr. Sci. Tech.,* **20:** 817-828.
14. Gandomi Nasrabadi, H., Azami Sarokelaei, L., Misaghi, A., Abbaszadeh, S.,Shariatifar, N. and TayyarHashtjin, N. 2012. Antibacterial Effect of Aqueous and Alcoholic Extracts from Petal of Saffron (*Crocus sativus* L.) on Some Foodborne Bacterial Pathogens. *J. Med. Plants***, 2**: 189-196.
15. Ghasemi, T., Abnous, K., Vahdati, F., Mehri, S., Razavi, B. M. and Hosseinzadeh, H. 2014. Antidepressant Effect of *Crocus sativus* Aqueous Extract and Its Effect on CREB, BDNF, and VGF Transcript and Protein Levels in Rat Hippocampus. *Drug Res. (Stuttg)*, **65**: 337-343.
16. Gulluce, M., Sahin, F., Sokmen, M., Ozer, H., Daferera, D., Sokmen, A., Polissiou, M., Adiguzel, A. and Ozkan, H. 2007. Antimicrobial and Antioxidant Properties of the Essential Oils and Methanol Extract from *Mentha longifolia* L. ssp. *Longifolia*. *Food Chem*., **103**: 1449-1456.
17. Hajlaoui, H., Trabelsi, N., Noumi, E., Snoussi, M., Fallah, H., Ksouri R. and Bakhrouf, A. 2009. Biological Activities of the Essential Oils and Methanol Extract of Tow Cultivated *Mint* Species (*Mentha longifolia* and *Mentha pulegium*) Used in the Tunisian Folkloric Medicine. *World J. Microbiol. Biotechnol*., **25**: 2227-2238.
18. Hasani, P., Yasa, N., Vosough-Ghanbari, S., Mohammadirad, A., Dehghan, G. and Abdollahi, M. 2007. *In Vivo* Antioxidant Potential of *Teucrium polium*, as Compared to α-Tocopherol. *Acta Pharm*., **57**: 123-129.
19. Jahan, N., Khatoon, R., Shahzad, A., Shahid, M. and Ahmad, S. 2013. Comparison of Antibacterial Activity of Parent Plant of *Tylophora indica Merr*. with Its *In Vitro* Raised Plant and Leaf Callus. *Afr. J. Biotechnol*., **12**: 4891-4896.
20. Karaman, I., Sahin, F. and Gulluce M. 2003. Antimicrobial Activity of Aqueous and Methanol Extracts of *Juniperus oxycedrus* L. *J Ethnopharmacol*., **85**:231-235
21. Khanahmadi, M., Rezazadeh, S. H. and Taran, M. 2010. *In Vitro* Antimicrobial and Antioxidant Properties of *Smyrnium*

[DOR: 20.1001.1.16807073.2019.21.6.21.0]

cordifolium Boiss, (Umbelliferae) Extract. *Asian J. Plant Sci*., **9**:99-103.
22. Lee, I. A. H., Lee, J. H., Baek, N. I. and Kim, D. H. 2005. Antihyperlipidemic Effect of Crocin Isolated from the Fractus of *Gardenia jasminoides* and Its Metabolite Crocetin. *Biol. Pharm. Bull*., 28: 2106-2110.
23. Lotfi, L., Kalbasi-Ashtari, A. and Hamedi M-Ghorbani, F. 2013. Effects of Sulfur Water Extraction on Anthocyanins Properties of Tepals in Flower of Saffron (*Crocus sativus* L). *J. Food Sci. Tech*., **52**: 813-821.
24. Mahmoudvand, H., Ezatpour, B., Rashidipour, M., Jahanbakhsh, S. and Mahmoudvand, H. 2016. Evaluation of the Scolicidal Effects of *Nectaroscordum tripedale* Extract and Its Acute Toxicity in Mice Model. *Pak. J. Pharm. Sci*., **29**: 2125-2128.
25. Mehrabi, Y. and Mehrabi, N. 2011. Determination of Feed Nutritive Value of *Smyrnium cordifolium Boiss* in Animal Nutrition. *Middle-East J. Sci. Res*., **5**: 659-663.
26. Menghini, L., Leporini, L.,Vecchiotti, G., Locatelli, M.,Carradori, S., Ferrante, C., Zengin, G., Recinella, L., Chiavaroli A., Leone, S., Brunetti, L. and Orlando, G. 2018. *Crocus sativus* L. Stigmas and Byproducts: Qualitative Fingerprint, Antioxidant Potentials and Enzyme Inhibitory Activities. *Food Res. Int*., **109**: 91-98.
27. Mianabadi, M., Hoshani, M. and Salmanian, S. 2015. Antimicrobial and Anti-Oxidative Effects of Methanolic Extract of *Doremaaucheri* Boiss. *J. Agr. Sci. Tech*., **17**: 623-34
28. Mishra, A. P., Sharifi-Rad, M., Shariati, M. A., Mabkhot, Y. N., Al-Showiman, S. S., Rauf, A., Salehi, B., Župunski, M., Sharifi-Rad, M., Gusain, P., Sharifi-Rad, J., Suleria, H. A. R., Iriti, M. 2018a. Bioactive Compounds and Health Benefits of Edible *Rumex* Species: A Review. *Cell. Mol. Biol.* (Noisy-le-Grand France). **64**: 27-34.
29. Mishra, A. P., Saklani, S., Salehi, B., Parcha, V., Sharifi-Rad, M., Milella, L., Iriti, M., Sharifi-Rad, J. and Srivastava, M. 2018b. *Satyrium nepalense*, a High Altitude Medicinal Orchid of Indian Himalayan Region: Chemical Profile and Biological Activities of Tuber Extracts. *Cell. Mol. Biol.* (Noisy-le-Grand France), **64**: 35-43.
30. Ochiai, T., Ohno, S., Soeda, S., Tanaka., H., Shoyama, Y. and Shimeno, H. 2004. Crocin Prevents the Death of Rat Pheochromocytoma (PC-12) Cells by Its Antioxidant Effects Stronger than Those of α-Tocopherol. *Neurosci. Lett.*, **362**: 61-64.
31. Rahaiee, S., Moini, S., Hashemi, M. and Shojaosadati, S. A. 2015. Evaluation of Antioxidant Activities of Bioactive Compounds and Various Extracts Obtained from Saffron (*Crocus sativus* L.): A Review. *J. Food Sci. Tech*., **52**: 1881-1888.
32. Sahin, F., Karaman, I., Gulluce, M., Ogutcu, H., Sengul, M., Adiguzel, A., Ozturk, S. and Kotan, R. 2003. Evaluation of Antimicrobial Activities of *Satureja hortensis* L. *J. Ethnopharmacol*, **87**: 61-65.
33. Salehi, B., Valussi, M., Jugran, A.K., Martorell, M., Ramírez-Alarcón, K., Stojanovi´c-Radi´c, Z. Z., Antolak, H., Kręgiel, D., Mileski, K. S.; Sharifi-Rad, M.; Setzer, W. N., CádizGurrea, M .L., Segura-Carretero, A., Şener, B., Sharifi-Rad, J. 2018. Nepeta Species: From Farm to Food Applications and Phytotherapy. *Trends Food Sci. Technol.*, **80**: 104-122.
34. Sanchez-Vioque, R., Rodriguez-Conde, M. F., Reina-Urena, J. V., Escolano-Tercero, M. A., Herraiz-Penalver, D., Santana-Méridas, O. 2012. In Vitro Antioxidant and Metal Chelating Properties of Corm, Tepal and Leaf from Saffron (*Crocus sativu L*.). *Ind. Crop Prod*. **39**: 149-153.
35. Sariri, R., Sabbaghzadeh, R., Poumohamad, F. 2011. In-vitro Antioxidant and Anti-tyrosinase Activity of Methanol Extracts from *Crocus sativus* flowers. *Pharmacologyonline*. **3**: 1-11.
36. Sethi, S., Dutta, A., Gupta, B. L. and Gupta, S. 2013. Antimicrobial Activity of Spices against Isolated Food Borne Pathogens. *Int. J. Pharm. Sci*., **5**: 260-262.
37. Shan, B., Cai Y. Z., Brooks, J. D. and Corke, H. 2007. The *In Vitro* Antibacterial Activity of Dietary Spice and Medicinal Herb Extracts. *Int. J. Food Microbiol*., **117**: 112-119.
38. Sharifi-Rad, J., Sharifi-Rad, M., Salehi, B., Iriti, M., Roointan, A., Mnayer, D., Soltani-Nejad, A. and Afshari, A. 2018. *In Vitro* and *In Vivo* Assessment of Free Radical Scavenging and Antioxidant Activities of *Veronica persica Poir*. *Cell. Mol. Biol.* (Noisy-le-Grand France), **64**: 57–64.
39. Sodeifian, G. H., Azizi, J. and Ghoreishi, S. M. 2014. Response Surface Optimization of *Smyrnium Cordifolium Boiss* (SCB) Oil Extraction via Supercritical Carbon Dioxide. *J Supercrit Fluid*., **95**: 1-7.

[DOR: 20.1001.1.16807073.2019.21.6.21.0]

40. Stanković, S. M., Nićiforović, N., Mihailović, V., Topuzović, M. and Solujić, S. 2012. Antioxidant Activity, Total Phenolic Content and Flavonoid Concentrations of Different Plant Parts of *Teucrium polium* L. subsp. *Polium*. *Acta Soc. Bot. Pol*., **81**:117-122.
41. Tabaraki, R. and Ghadiri, F. 2013. *In Vitro* antioxidant Activities of Aqueous and Methanolic Extracts of *Smyrnium cordifolium Boiss* and *Sinapis arvensis* L. *Int. Food Res. J*., **20**: 2111-2115.
42. Tavakoli, M.,Hamidi-esfahani, Z., Hejazi, M. A., Azizi, M. H. and Abbasi, S. 2017. Characterization of Probiotic Abilities of Lactobacilli Isolated from Iranian Koozeh Traditional Cheese. *Pol. J. Food Nutr. Sci*., **67**:41-8.
43. Tepe, B., Donmez, E., Unlu, M., Candan, F., Daferera, D., Vardar-Unlu, G., Polissiou, M. and Sokmen, A. 2004. Antimicrobial and Antioxidative Activities of the Essential Oils and Methanol Extracts of *Salvia cryptantha* (MontbretetAucher ex Benth.) and *Salvia multicaulis* (Vahl). *Food Chem*., **84**: 519-525.
44. Vali Aftari, R., Rezaei, K., Bandani, A. R. and Mortazavi, A. 2017. Antioxidant Activity Optimization of *Spirulina platensis* C-Phycocyanin Obtained by Freeze-Thaw, Microwave-Assisted and Ultrasound-Assisted Extraction Methods. *Qual. Assur. Saf. Crop Foods*, **9**: 1-9.
45. Yang, C. S., Ho, C. T., Zhang, J., Wan, X., Zhang, K. and Lim, J. 2018. Antioxidants: Differing Meanings in Food Science and Health Science. *J. Agric. Food Chem*., **66**: 3063-3068.
46. Zeka, K., Ruparelia, K. C., Continenza, M. A., Stagos, D., Vegliò, F. and Arroo, R. R. J. 2015. Petals of *Crocus sativus* L. as a Potential Source of the Antioxidants Crocin and Kaempferol. *Fitoterapia*, **107**: 128-134.

مقادیر فنل/ فلاونوئید کل، فعالیت ضد باکتری و مهار رادیکال آزاد به روش DPPH گیاهان دارویی

ل. فهمیده، ا. مزارعی، و م. توکلی

چکیده

در سال های اخیر تمایل عمومی برای جایگزینی آنتی بیوتیک ها و آنتی اکسیدان های مصنوعی با عصاره های گیاهی طبیعی اهمیت فراوانی یافته است. این رویکرد به دلیل تاثیر منفی آنتی اکسیدان های سنتزی بر سلامت و مقاومت نسبت به آنتی بیوتیک ها می باشد. لذا به دلیل این مشکلات بحث برانگیز، در این تحقیق مهار رادیکال آزاد، مقدار فنل و فلاونویید کل و فعالیت ضدمیکروبی عصاره متانولی گلپوره، اندول، پونه و انشک با گلبرگ زعفران مقایسه شد. فعالیت آنتی اکسیدانی این عصاره ها با BHA، BHT و آلفا-توکوفرول به روش DPPH مقایسه شد. فعالیت ضد میکروبی عصاره ها به روش انتشار دیسک کاغذی در آگار روی *S.aureus* (گرم مثبت) و *E. coli* O157:H7 (گرم منفی) اندازه گیری شد. نتایج نشان داد که گلبرگ زعفران بالاترین مقدار فنل کل (۳۷/۳۶ mg GAE/g) و فلاونویید کل (۱۳۸/۵۲ mg Q/g) می باشد. همچنین فعالیت مهار رادیکال آزاد عصاره متانولی گلبرگ زعفران (۸۷/۳۳ ٪) از سایر عصاره ها بیشتر و از نظر آماری با BHA و آلفا-توکوفرول مشابه بود. به علاوه، رابطه معنی دار بین فعالیت مهار رادیکال آزاد و مقدار فنل کل (R۰/۹۶۴ =) و مقدار فلاونویید کل (R۰/۸۰۶ =) بدست آمد که اثر مهم این

[DOR: 20.1001.1.16807073.2019.21.6.21.0]

ترکیبات روی فعالیت آنتی اکسیدانی گیاهان دارویی تحقیق حاضر را نشان می دهد. عصاره برگ انشک و عصاره گلبرگ زعفران از بین سایر عصاره گیاهان دارویی تحقیق بالاترین فعالیت ضد میکروبی بر علیه میکروارگانیسم های استفاده شده نشان داد ($P<0.05$). همچنین نتایج فعالیت ضد میکروبی ارتباط قوی با فعالیت مهار رادیکال آزاد و مقدار فنل کل نشان داد که این ارتباط اهمیت این فاکتورها در فعالیت ضد میکروبی ۵ عصاره گیاه دارویی تحقیق را مشخص می نماید.

[DOR: 20.1001.1.16807073.2019.21.6.21.0]