Evaluation of Four Extraction Methods on Antioxidant Compounds of Two Medicinal Vegetables, *Froriepia subpinnata* and *Eryngium planum*

Edris Shabani*, Mohammad Mahmoudisourestani and Mohammad Younis Mahen

Department of Horticultural Science, Faculty of Agriculture, Shahid Chamran University of Ahvaz, Ahvaz, Iran

Abstract

In this experiment, the effect of four extraction methods including percolation, maceration, ultrasound-assisted extraction, and microwave were investigated on the amount of polyphenolic compounds and antioxidant activity of *Froriepia subpinnata* and *Eryngium planum*. The highest level of total phenols in *F. subpinnata* and *E. planum* was observed in the microwave method and the highest levels of flavonoids was observed in the maceration and percolation methods, respectively. The highest antioxidant activity of *F. subpinnata* (98.48%) was recorded in the microwave method. Antioxidant activity data of *E. planum* showed that percolation method with 96.54% and microwave method with 90.82% were specially more efficient in in comparison with the other methods. Therefore, it was concluded that microwave method can lead to better extraction of *F. Subpinnata* and *E. planum*.

Keywords: Antioxidant activity, *Eryngium planum*, flavonoid; *Froriepia subpinnata*, phenol

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Introduction

Froriepia subpinnata (Ledeb.) Baill (Apiaceae) known as Anarijeh and *Eryngium planum* (Apiaceae) known as Zolang are mainly distributed in the forests of northern Iran, in Mazandaran, Golestan, and Guilan provinces (Khoshbakht et al., 2007; Akhani, 2003; Vaseghi et al., 2018). These medicinal plants are used by indigenous people as a local spices and flavor along with fish, rice, and yogurt. Zolang and Anarijeh are often widespread on the ground as herbs in early spring. They are also rarely seen in wet forests in the fall, when the weather is favorable.

The aerial parts of these plants have applications not only in folk medicine but also in modern pharmaceuticals (Flamini et al., 2007). *E. planum* is traditionally consumed to treat pertussis and urinary tract infections to increase urination and eliminate kidney stones (Flamini et al., 2007). The essential oil of *E. planum* contains phenylpropanoid methyl derivatives, eugenol, methyl isoeugenol, and benzaldehyde (Flamini et al., 2007). In addition, the anti-cancer properties of *F. subpinnata* essential oil compounds and the antioxidant activity of aerial parts have been reported in previous findings (Morteza-Semnani et al., 2009; Ebrahimzadeh et al., 2010).

** Corresponding Author*
E-mail Address: edris.shabani@scu.ac.ir
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Mohammadzadeh et al. (2018) showed that the major components of *F. subpinnata* essential oil before flowering were kuminol (42.05 %) and phenol (28 %) while after flowering its prominent compounds were sabinene (25.96 %), thymol (22.68 %), and cyclohexene (17.21 %). Phytochemical studies of *E. planum* and *F. subpinnata* showed that both have a high total phenolic and flavonoid content (Nabavi et al., 2008).

Despite the studies on the composition of *F. subpinnata* essential oil and the antioxidant properties of both plants, the effect of different extraction methods on secondary metabolites of these medicinal vegetables has not been investigated. In the percolation method, like the maceration, the powdered plant materials are placed in a closed system containing the solvent, with the difference that the solvent is slowly added to the plant materials in a few days (Alara et al., 2021). But both are time consuming and need large volume of solvent (Alara et al., 2021). Ultrasound releases phenolic and polyphenolic compounds through cell wall decomposition and leaching of intracellular contents (Kamran Khan et al., 2010). Many studies show that ultrasonic procedure could significantly reduce processing time, decrease solvent consumption, and improve extraction efficiency (Jamshidi et al., 2014). Microwave method has also been reported by researchers (Kaufmann and Christen, 2002; Mandal et al., 2007; Alara et al., 2021) in which the sample is heated in order to extract plant compounds in a closed system. In addition to needing shorter extraction time, microwave extraction method practically results in no heat loss (Mandal et al., 2007).

This study is an attempt to compare extraction of secondary metabolites as well antioxidant activities of *F. Subpinnata* and *E. planum* leaves using ultrasonically assisted extraction, percolation, maceration, and microwave methods. In fact, the study attempts to find a method with the highest extraction efficiency for the use of these plants in traditional and modern medicine.

Materials and Methods

Plant material and preparation

Eryngium planum and *Froriepia subpinnata* aerial parts were collected from Sangtarashan forest, Sari, Iran in December 2020. The plant materials were dried in an oven at 35 ℃ for 72 hours. The dry materials were milled, obtaining 2-3 mm particles. Each sample was extracted by percolation, maceration, ultrasonic and microwave methods using methanol. Briefly, in percolation and maceration methods, 5 and 1 g of powdered samples were infused in 50 and 10 ml of methanol 70% for 96 and 24 h at room temperature, respectively. The extract was then separated from the sample residue by filtration through filter paper. The extracts were kept at 4 ℃ until further use. In ultrasonic method, 1 g of the dry powdered material and 10 ml of methanol 70% were exposed to a sonicator at the rate of 20 KHz at 40 ℃ for 10 minutes, and then filtered through Whatman filter paper no.42. The procedure was repeated twice and the extract was stored in the same condition as described before. Finally, in microwave assisted extraction, 1 g of the dry powdered material and 10 ml of methanol 70% were extracted with a household automatic microwave oven at 300 w irradiation for 30 second. The mixture was filtered through Whatman filter paper no. 42. The extract was stored in the same manner as mentioned above.

Total phenolic compounds

Total phenolic compound contents were determined by the Folin-Ciocalteau method (Wojdylo et al., 2007). The extract samples (100 µl) were mixed with 200 µl of 50% Folin-Ciocalteau reagent and 2.0 mL distil water. After 3 min, 1.0 mL of 20% sodium carbonate was added. After 1 h of incubation at room temperature and under dark condition, the absorbance of the reaction was measured at 765 nm by a spectrophotometer (Shimadzu-UV 1201, Kyoto, Japan). The results were expressed as gallic acid equivalents (GAE) in milligrams (mg) per g dry weight (DW) (mg GAE g^{-1} DW).

Table 1
Descriptive statistics of total phenols, total flavonoid, and antioxidant activity of *Froriepia subpinnata* (Anarijeh)

Variable	Maximum	Minimum	Mean	Standard deviation	Standard error	Range
Total phenol	96.99	12.91	58.94	30.77	8.88	84.08
Total flavonoid	68.81	9.05	38.61	23.10	6.66	59.76
Antioxidant activity	98.67	19.58	64.93	32.94	9.51	79.09

Table 2
Correlation coefficient between total phenol, total flavonoids, and antioxidant activity of *Froriepia subpinnata* (Anarijeh)

	Total phenol	Total flavonoid	Antioxidant activity
Total phenol	1.00		
Total flavonoid	0.43	1.00	
Antioxidant activity	0.86 ***	0.29	1.00

*** Significance at p≤0.001.

Total flavonoid contents

Total flavonoids were estimated using the aluminum chloride colorimetric procedure with minor modifications (Menichini et al., 2009). Briefly, 500 µl of extracts was mixed with 150 µl of sodium nitrite (5% w/v) and allowed to stand for 5 min, and then 300 µl of 10% aluminum chloride was added. After 6 min, 2 mL of NaOH (0.1 N) was added and the volume was increased to 5.0 ml by adding distilled water. The absorbance of the reaction mixture at 510 nm was measured spectrophotometrically (Shimadzu-UV 1201, Kyoto, Japan). Quercetin was used for making the calibration curve. The results were expressed as mg quercetin equivalents (QE) per g DW (mg QE g^{-1} DW).

Total antioxidant activity

Stable 1,1-diphenyl-2-picryl hydrazyl radical (DPPH) with minor modifications was used for determination of free radical-scavenging activity of the extracts (Oke et al., 2009). The extract samples (25 µl) were mixed with 2.5 mL DPPH (0.008 g per 100 cc of methanol). Then it was stirred vigorously for 30 seconds and incubated in dark condition for 30 minutes. The absorbance was recorded at 517 nm. Methanol and DPPH were used as blank.

Total antioxidant activity was calculated by the following ratio:

$$\text{Antioxidant activity}=\frac{\text{A control}-\text{A sample}}{\text{Acontrol}}\times 100$$

($A_{control}$: absorbance of the DPPH solution; A_{sample}: absorbance of the DPPH solution after the addition of the sample).

Statistical Analysis

The experimental design for this research was a completely randomized design with three replicates. Treatments were defined by a factorial combination of four extraction method and two medicinal vegetables. Analysis of variance (ANOVA) of the experimental data was evaluated using SAS 9.1 software. Data analysis was performed using GLM procedure and means were compared using Duncan's multiple range tests at p≤0.05 on each of the significant variables measured.

Result

Secondary metabolites and antioxidant activity of F. Subpinnata

Total phenol compounds were reported as gallic acid equivalents (GAE)/g of extract powder by reference to standard curve (y = 0.0026x + 0.0369, r^2 = 0.9877). The total flavonoid contents were reported as mg quercetin equivalent (QUE)/g of extract powder, by reference to standard curve (y = 0.0008x - 0.0303, r^2 = 0.9684). The descriptive statistics of the antioxidant compounds of the *F. Subpinnata* are shown in Table 1. The correlation coefficient table shows a positive and significant correlation (r= 0.86) between total phenol content and antioxidant activity in *F. Subpinnata* (Table 2). Compared to *E. planum*, the highest levels of

Table 3
Descriptive statistics of total phenol, total flavonoid, and antioxidant activity of *Eryngium planum* (Zolang)

Variable	Maximum	Minimum	Mean	Standard Deviation	Standard Error	Range
Total phenol	53.20	5.27	26.18	15.55	4.48	47.93
Total flavonoid	24.88	2.49	14.97	8.22	2.37	22.39
Antioxidant activity	97.68	20.96	67.15	31.31	9.04	76.72

Table 4
Correlation coefficient between total phenol, total flavonoids and antioxidant activity of *Eryngium planum* (Zolang)

	Total phenol	Total flavonoid	Antioxidant activity
Total phenol	1.00		
Total flavonoid	0.74 **	1.00	
Antioxidant activity	0.84 ***	0.97 ***	1.00

** and *** Significant at p≤ 0.01 and 0.001, respectively.

Table 5
Analysis of variance of the effects of different extraction methods on the amounts of total phenols, total flavonoids, and antioxidant activity in *Froriepia subpinnata* (Anarijeh) and *Eryngium planum* (Zolang)

		Mean square		
Source	df	Total phenol	Total flavonoid	Antioxidant activity
Plant	1	6437.34 ***	3350.49 ***	29.57 ***
Method	3	3832.45 ***	1499.25 ***	7490.66 ***
Plant×Method	3	476.27 ***	688.55 ***	83.46 ***
Error	16	9.32	3.29	0.48
CV (%)	-	7.17	6.77	1.05

*** Significant at p≤0.001%.

Table 6
Main effects of different extraction methods and plant type on total phenols, total flavonoids, and antioxidant activity of *Froriepia subpinnata* (Anarijeh) and *Eryngium planum* (Zolang)

Treatment		Total phenol (mg GAE/g DW)	Total flavonoid (mg QUE/g DW)	Antioxidant activity (%)
Plant	Anarijeh	58.94 a	38.61 a	64.93 b
	Zolang	26.18 b	14.97 b	67.15 a
Extraction method	Percolation	44.44 b	37.64 b	93.46 b
	Maceration	44.75 b	40.58 a	55.45 c
	Ultrasonic	9.71 c	6.16 d	20.62 d
	Microwave	71.35 a	22.78 c	94.65 a

Different letters within each column indicate significant differences according to Duncan's multiple-range test (p≤0.05).

phenol and flavonoids were observed with 58.94 (mg GAE/g DW) and 38.61 (mg QUE/g DW) in *F. Subpinnata*, respectively (Table 6). Analysis of variance indicated that plant type and extraction methods and their interactions were effective (p≤0.01) on the level of secondary metabolites and antioxidant capacity of *F. Subpinnata* and *E. planum* (Table 5). Comparison of the main effects of extraction methods showed that the highest amount of total phenol (71.35 mg GAE/g DW) and antioxidant capacity (94.65%) was observed in the microwave method and the highest amount of total flavonoids (40.58 mg QUE/g DW) was observed in the maceration method (Table 6). As shown in Table 1, total phenol contents ranged from 9.71 to 71.35 mg GAE/g of extraction powder. Also, total flavonoid contents ranged from 6.16 to 40.58 mg QUE/g of extraction powder. The percolation method showed no significant difference with the maceration method and the lowest amount of total phenol (9.71 mg GAE/g of extract powder) was observed in the ultrasonic extraction method (Table 6). This information shows that the microwave method has played a more important role in creating antioxidant capacity than other methods by affecting the extraction of more phenolic compounds (Table 6). The interaction of plant type and extraction methods showed that the amount of total phenols in *F. Subpinnata* and microwave method was higher than other methods (Fig. I) while the highest levels of total flavonoids were observed in *F. Subpinnata* and in traditional methods such as maceration. Also, the highest antioxidant capacity was observed in *F. Subpinnata* and microwave method (Fig. III). DPPH radical-scavenging activity was in order of: microwave extraction > percolation extract > maceration extract > ultrasonic extraction (Table 6). Therefore, the above findings showed that the microwave method had a more effective role in increasing the antioxidant activity of *F. Subpinnata* by affecting a better extraction of antioxidant compounds such as phenolic compounds (Table 6).

Secondary metabolites and antioxidant activity of E. planum

The descriptive statistics of the antioxidant compounds of the *E. planum* are shown in Table 3. The correlation coefficient table showed that there is a high correlation between total phenol content and antioxidant capacity (r= 0.84) such as *F. Subpinnata* of *E. planum*, but the relationship between flavonoids and antioxidant capacity was higher (r= 0.97) (Table 4). There was also a significant relationship between flavonoid levels and total phenol of *E. planum* ($p \leq 0.01$) (Table 4). The main effects of plant type showed that although there was a significant difference between the phenols and flavonoids of *E. planum* and *F. Subpinnata*, the antioxidant capacity of *E. planum* was higher than that of *F. Subpinnata* (Table 4). This indicates the importance of other secondary metabolites such as terpenoids and alkaloids.

The amounts of both phenol and flavonoid compounds in *F. Subpinnata* were higher than those in *E. planum* (Figs. I and II). Although Fig. (I) illustrated lower levels of total phenols in *E. planum* compared to *F. Subpinnata*, it still showed that the microwave method had an effective role in extracting secondary metabolites compared to other methods. Total phenol content was in order of: microwave extraction > percolation method > maceration method > ultrasonic assisted extraction (Fig. I). Although the total flavonoid values of *E. planum* have been less than *F. Subpinnata*, percolation and microwave methods resulted in the highest amount of flavonoid extraction in this plant (Fig. II). Total flavonoid

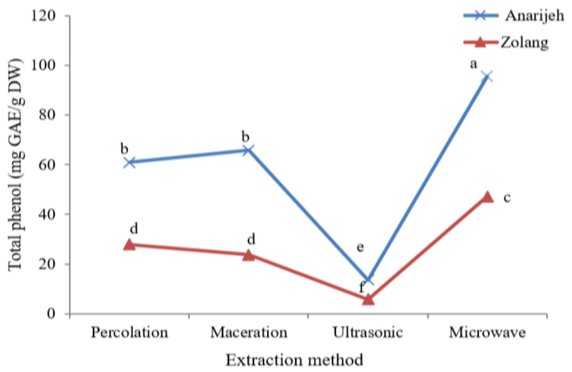

Fig. I. The effect of different extraction methods on the total phenol content of *Froriepia subpinnata* (Anarijeh) and *Eryngium planum* (Zolang)

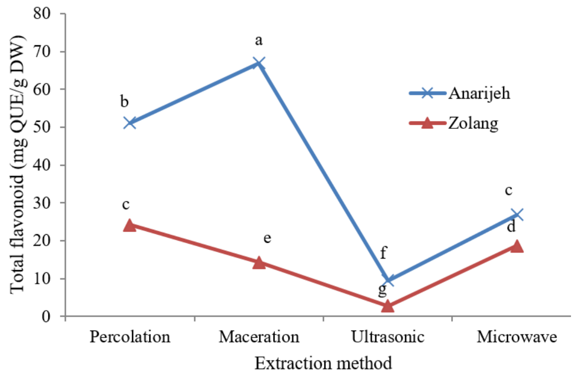

Fig. II. The effect of different extraction methods on the total flavonoid content of *Froriepia subpinnata* (Anarijeh) and *Eryngium planum* (Zolang)

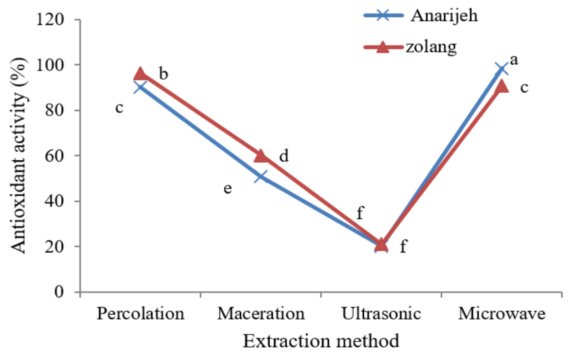

Fig. III. The effect of different extraction methods on the antioxidant activity of *Froriepia subpinnata* (Anarijeh) and *Eryngium planum* (Zolang) content was in order of: percolation method > microwave extraction > maceration method > ultrasonic assisted extraction (Fig. II). Antioxidant activity data of *E. planum* showed that percolation method with 96.54% and microwave method with 90.82% played a special role in increasing this trait in comparison with other methods (Fig. III). After microwave method with *F. Subpinnata* plant, the highest antioxidant capacity was observed in *E. planum* plant and through percolation method. Although the microwave method in *E. planum* resulted in more phenol extraction compared to other methods, interaction effect of data showed that only percolation method resulted in more flavonoid extraction compared with other methods such as microwave, ultrasonic, and maceration (Fig. III). Therefore, it seems that flavonoids in comparison with phenols have played a more effective role in the antioxidant capacity of *E. planum*.

Discussion

Secondary metabolites and antioxidant activity of F. Subpinnata

Previous reports have shown that the importance of extraction method in assaying antioxidant capacity and contents (James and Alewo, 2014; Yasmeen and Hassnain, 2016). Various reports have shown that plants rich in phenols and polyphenolic compounds such as flavonoids have high antioxidant activity (Nabavi et al., 2008; Dehpour et al., 2009). The results of this experiment showed that the difference in the levels of antioxidant compounds, in addition to the geographical area of growth, cultivar, plant age, environmental and seasonal conditions, type of cultivation, harvest time, and genetic diversity (Mohammadzadeh et al., 2018), can be influenced by extraction method. The microwave method in this study was more efficient in extraction of antioxidants. Yasmeen and Hassnain (2016) reported that the phytochemical contents differed independently with different extraction techniques, which is consistent with the results of this experiment. The activities that lead to the inhibition of free radicals are different in both extracts and extraction methods (Yasmeen and Hassnain, 2016). Therefore, the microwave method has probably been able to show more inhibitory activity by releasing more antioxidant compounds.

Secondary metabolites and antioxidant activity of E. planum

Jamshidi et al. (2014) investigated the effect of three extraction methods including ultrasonic assisted extraction, percolation method and polyphenol fraction on *Lythrum salicaria*. They reported the highest levels of flavonoids and antioxidant activity in classic extraction methods such as percolation. The effect of flavonoid compounds on increasing the antioxidant activity of *E. planum* was more than phenolic compounds, which was affected by percolation extraction method. In medicinal plants, compounds such as flavonoids, phenols, and tannins are the major contributor to the antioxidant activity (Dehpour et al., 2009). Phenol and flavonoids have various biological properties like anti-carcinogenic and anti-inflammatory activities. It seems that such activities are related to their antioxidant activity (Ebrahimzadeh et al., 2010). The results of this experiment showed that inexpensive traditional methods such as percolation, e.g. 24 h extraction time and consumption of 80 mL of solvent (Yasmeen and Hassnain, 2016), can still be an option for extracting medicinal plants. However, microwave method due to the fast extraction time (It only takes 30 seconds) and very low solvent consumption (10 mL) can be a more effective tool for extracting antioxidant compounds in the chemical and pharmaceutical industries. In microwave method heat is produced by affecting the polar materials/solvents through the two phenomena of ionic conductivity and bipolar rotation, which leads to better extraction of phenolic and flavonoid compounds. But since this heat is in a closed system, it does not pose any hazard to the environment (Mandal et al., 2007).

The comparison of extraction methods in the two plants showed that the *F. Subpinnata* plants contained higher levels of phenolic and flavonoid compounds and antioxidant activity compared to *E. planum*. Also, in both plants, the highest levels of phenolic compounds were observed in the microwave method, which shows the positive effect of this method in the extraction of secondary metabolites in native and wild species.

Conclusion

According to the results of this experiment, it can be concluded that the amount of secondary metabolites varies depending on the plant type. The findings of this study shows that although the levels of phenol and flavonoids in *F. Subpinnata* were higher than those in *E. planum*, the contribution of these compounds in creating antioxidant capacity was not the same. It seems that the microwave method with extraction of more phenol in *F. Subpinnata* and the percolation and microwave methods with extraction of more flavonoids in *E. planum* have played a significant role in increasing the antioxidant capacity of these plants.

References

Alara, O.R., N.H. Abdurahman and **Ch.I. Ukaegbu.** 2021. 'Extraction of phenolic compounds: A review'. *Curr. Res. Food Sci*, 4: 200-214.

Akhani, H. 2003. 'Notes on the flora of Iran: 3. Two new records and synopsis of the new data on Iranian Cruciferae since Flora Iranica'. *Candollea*, 58(2): 369-85.

Dehpour, A.A., M.A. Ebrahimzadeh, S.F. Nabavi and **S.M. Nabavi.** 2009. 'Antioxidant activity of the methanol extract of *Ferula assafoetida* and its essential oil composition'. *Grasas Y Aceites*, 60 (4): 405-412.

Ebrahimzadeh, M.A., S.F. Nabavi, S.M. Nabavi and **B. Eslami.** 2010. 'Antihemolytic and antioxidant activities of *Allium paradoxum*'. *Cent. Eur. J. Biol*, 5: 338-345.

Ebrahimzadeh, M.A., S.M. Nabavi, S.F. Nabavi, S. Eslami and **A.R. Bekhradnia.** 2010. 'Mineral elements and antioxidant activity of three locally edible and medicinal plants in Iran'.
Asian J. Chem, 22(8): 6257.

Flamini, G., M. Tebano and **P.L. Cion.** 2007. 'Composition of the essential oils from leafy parts of the shoots, flowers and fruits of *Eryngium amethystinum* from Amiata Mount (Tuscany, Italy) '. *Food Chem*, 107(2): 671 - 674.

James, O. and **I.M. Alewo.** 2014. 'In vitro antihemolytic activity of *gymnema sylvestre* extracts against hydrogen peroxide (H_2O_2) induced haemolysis in human erythrocytes'. *AJPCT*, 2: 861-869.

Jamshidi, M., E. Shabani, Z. Hashemi and **M.A. Ebrahimzadeh.** 2014. 'Evaluation of three methods for the extraction of antioxidants from leaf and aerial parts of *Lythrum salicaria* L. (Lythraceae) '. *Int. Food Res. J*, 21(2): 783-788.

Kamran Khan, M., M. Abert-Vian, A.S. Fabiano-Tixier, O. Dangles and **F. Chemat.** 2010. 'Ultrasound-assisted extraction of polyphenols (flavanone glycosides) from orange (*Citrus sinensis* L.) peel'. *Food Chem*, 119. 851-858.

Kaufmann, B. and **P. Christen.** 2002. 'Recent extraction techniques for natural products: microwave-assisted extraction and pressurised solvent extraction'. *Phytochem. Anal*, 13: 105-113.

Khoshbakht, K., K. Hammer and **K. Pistrick.** 2007. '*Eryngium caucasicum* Trautv. cultivated as a vegetable in the Elburz Mountains (Northern Iran) '. *Genet. Resour. Crop Evol*, 54(2): 445-448.

Mohammadzadeh, M., R. Mahmoudi and **P. Ghajarbeygi.** 2018. 'Evaluation of Chemical Composition and Antibacterial Properties of *Froriepia subpinnta* Essential Oils from Guilan Region: Before and After Flowering'. *J. Essent. Oil-Bear. Plants*, 21(4): 1119-1127.

Mandal, V., Y. Mohan and **S. Hemalatha.** 2007. 'Microwave assisted extraction–an innovative and promising extraction tool for medicinal plant research'. *Pharmacogn Rev*, 1(1): 7-18.

Menichini, F., R. Tundis, M. Bonesi, M.R. Loizzo, F. Conforti, G. Statti, B. Di Cindi, P.J. Houghton and **F. Menichini.** 2009. 'The influence of fruit ripening on the phytochemical content and biological activity of *Capsicum chinense* Jacq. cv. Habanero'. *Food Chem*, 114 (2): 553-560.

Morteza-Semnani, K., M. Saeedi and **M. Akbarzadeh.** 2009. 'The Essential Oil Composition of *Froriepia subpinnata* (Ledeb.) Baill'. *J. Essent. Oil Res*, 21(2): 127-128.

Nabavi, S., M. Ebrahimzadeh, S. Nabavi and **M. Jafari.** 2008. 'Free radical scavenging activity and antioxidant capacity of *Eryngium caucasicum* Trautv and *Froripia subpinnata*'. *Pharmacologyonline*, 3: 19-25.

Oke, F., B. Aslim, S. Ozturk and **S. Altundag.** 2009. 'Essential oil composition, antimicrobial and antioxidant activities of *Satureja cuneifolia* Ten'. *Food Chem*, 112 (4): 874-879.

Vaseghi, Z., O. Tavakoli and **A. Nematollahzadeh.** 2018. 'Rapid biosynthesis of novel Cu/ Cr/Ni trimetallic oxide nanoparticles with antimicrobial activity'. *J. Environ. Chem. Eng*, 6(2): 1898-1911.

Wojdylo, A., J. Oszmianski and **R. Czemerys.** 2007. 'Antioxidant activity and phenolic compound in 32 selected herbs'. *Food Chem*, 105 (3): 940-949.

Yasmeen, H. and **Sh. Hassnain.** 2016. 'Comparative analysis of different bioactivities of *Curcuma longa, Nigella sativa* seeds, and *Camellia sinensis* extracted by four different methods: a green way to reduce oxidative stress'. *Food Sci. Biotechnol*, 25(3): 811-819.