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Original Article

Free Radical Scavenging Capacity and Antioxidant Activity of Methanolic Extracts of *Borago Officinalis, Teucrium Polium, Mentha Aquatica* and *Allium Taradox*

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Abstract

Background and Aim: Free radicals and the oxidative stress they generate are implicated in a wide range of disorders in the human body. One of the important approaches to eliminate free radicals and prevent their side effects is to use natural antioxidants. Plants contain significant amounts of phenolic compounds in all their parts that give them antioxidant properties. This study aims to evaluate the antioxidant activity and phenolic content of methanolic extracts from aerial parts of some medicinal plants.

Methods: Methanolic extracts from the leaves of Wild Borage (Borago officinalis), Teucrium (Teucrium polium), Water Mint (Mentha aquatica) and Wild Leek (Allium taradox) were prepared and evaluated for their free-radical scavenging capacity or their antioxidant activity. The amount of total phenolic content was determined using the folin-ciocalteu method and the amount of anti- radical activity was determined using the DPPH free radical scavenging method and determining inhibitory concentration (IC50). All experiments were performed in triplicate.

Results: The obtained results and statistical analyses showed that the methanolic extracts of Borago officinalis and Teucrium polium contain high amounts of phenolic compounds and also had more free radical inhibition due to the lower IC50 values. Also, Borago officinalis had significant antioxidant activity compared to other plants.

Conclusion: Our investigation indicates that Borago officinalis leaves are a promising source of natural antioxidants.

Keywords: Antioxidant activity; Natural antioxidants; Plant extracts; Polyphenols

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Introduction

The physiological role of antioxidants is to prevent damage to cell components caused by chemical reactions of free radicals. Free radicals cause the oxidation of macromolecules such as nucleic acids, lipids and proteins, which leads to cell damage and death (1). Many free radicals are highly reactive and can act as both electron donors and electron acceptors, so they undergo oxidation and reduction; Most free radicals in biological systems have short half-lives (106 seconds or less), although some species persist for long periods (2). Currently, the results of clinical studies show that oxidative stress caused by reactive oxygen and nitrogen species plays a role in a variety of disorders, including cancer and aging. Therefore, it seems plausible that antioxidants could be effective in preventing or suppressing such disorders through ameliorating free radical-induced damage. In fact, many studies show that antioxidants have a strong inhibitory effect against oxidative damage, and some epidemiological reports suggest that the consumption of foods with high antioxidant content have beneficial effects and lower the risk of several diseases (3). Antioxidants exert their beneficial effects through several mechanisms such as suppressing the production of active species by reducing hydroperoxides and hydrogen peroxide, scavenging reactive oxygen species or other free radicals, sequestering metal cations and repairing or clearing the damage caused by oxidative stress (4). Synthetic and chemical antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) have been used for many years as food additives to preserve and stabilize nutritional value in the food industry. However, research showed that these artificial antioxidants can cause cancer in laboratory animals (5). Therefore, many studies are being conducted on antioxidants, especially natural antioxidants that inhibit the formation of free radicals and the damage caused by them. Despite the presence of natural antioxidant systems such as superoxide dismutase and catalase enzymes, albumin, ceruloplasmin, ascorbic acid, alpha - tocopherol, and beta - carotene in the human body that can fight free radicals, additional antioxidants must be used through nutrition to eliminate free radicals in the body (6, 7). Recently, the role of antioxidants in the diet especially through the consumption of herbs has been noticed (9). The ongoing studies on medicinal plants show that they are suitable research subjects due to their strong antioxidant properties and cost-effectiveness (8). The antioxidant properties of plants are primarily due to the phenolic compounds present in all parts of plants such as leaves (10). These phenolic compounds are the secondary metabolites of plants that have anti-inflammatory, anti-tumor, and even anti-viral activities as well (11, 12). The antioxidant activity of these compounds is exerted through the hydroxyl groups in the aromatic ring, and unpaired electrons around the ring, which scavenge free radicals (13). The most important group of polyphenols is flavonoids, which neutralize free radicals by having hydroxyl and methoxyl groups (14). Flavonoids also inhibit enzymes such as cyclooxygenase and lipoxygenase which play a role in the production of reactive oxygen species (15). Despite this information, reports on the antioxidant properties of aerial parts of endemic plants are scarce. Therefore, this research aimed to evaluate the antioxidant activity and phenolic content of methanolic extracts from aerial parts of *Borago officinalis, Teucrium polium, Mentha aquatic* and *Allium taradox.*

Methods

Chemicals and reagents

2, 2- Diphenyl- 1- picrylhydrazyl (DPPH), Folin Ciocalteu's reagent, gallic acid, sodium carbonate, and methanol, all were purchased from Merck company representatives.

Collection of plant samples

The aerial parts of *Borago officinalis, Teucrium polium, Mentha aquatica* and *Allium taradox* (Figure 1 and Figure 2) were collected from the surrounding areas of Gorgan in spring and summer. Different parts (including leaves and stems) were completely separated and dried in the shade and ambient temperature. The dried samples were turned into powder by an electric mill. The obtained powders were stored in closed containers at a temperature of 5°C until extraction.

Figure 1. *Borago officinalis* (left) and *Teucrium polium* (right) (photographed by the author)

Figure 2. *Mentha aquatic* (left) and *Allium taradox* (right) (photographed by the author)

Preparation of plant extract

In order to prepare a methanolic extract, 10 g of plant powder was added to 100 ml of methanol-water solvent (80:20, v/v) and stirred for 12 h at room temperature. After this time, the mixture was filtered by filter paper (Whatman No. 2). The obtained liquid was concentrated by a rotary evaporator at a temperature of 40˚C. Then, for complete drying, extracts were spread on a watch glass and placed under a chemical hood at ambient temperature. The dried extracts were kept at 5°C until the next steps.

Determination of total phenolic content

The amount of total phenolic compounds was measured by Folin Ciocalteu's reagent method and according to Shahidi and Nachek with some modifications (19). One mg of each plant extract was dissolved in 10 ml of methanol to create a concentration of 100 ppm. 0.5 ml of the extract was poured into the test tube and 0.5 ml of Folin Ciocalteu's reagent was added and stirred for 2 minutes. Then 10 ml of sodium carbonate solution (7%) was added. The samples were kept at 45˚C for 60 min. Then the absorbance of the samples was measured at 765 nm. Total phenol content was expressed as mg of gallic acid per g of extract. The Folin Ciocalteu's is an important method for measuring the amount of phenol in the extract, the basis of this method is the reduction of the Folin Ciocalteu's reagent by the phenolic compounds in the extract and the formation of a blue complex that has a maximum absorption at 765 nm.

DPPH radical scavenging activity

Evaluation of the antioxidant properties of the extracts was done using the 2, 2- Diphenyl- 1- picrylhydrazyl (DPPH) free radical reduction method. The basis of this method is the trapping of DPPH radicals by the hydrogenation ability of the extract samples (17). DPPH as a stable free radical is purple, which turns yellow when reduced by electron-donating agents (antioxidant compounds such as phenols). In this test, the reducing power of the extracts is measured by the dimming or decolorization of DPPH by the extract compounds, and as the reaction solution becomes fainter, its absorption decreases, and this shows that the extract has high antioxidant properties (16). To investigate the antioxidant activity and DPPH free radical inhibition, Fu et al.'s method was used in this research (18). First, 100 µl of 0.1 mM DPPH solution (in methanol solvent) was prepared and then 100 microliters of different concentrations of the extract were added to it. This mixture was incubated for 30 minutes at 25°C. DPPH solution was used as a control. After 30 minutes, absorption was measured at 517 nm. Free radical scavenging activity (DPPH) was calculated using the following equation (1):

$$\text{DPPH scavenging activity (\%)} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

The capacity of the radical scavenging property of plants was also determined by calculating the IC_{50}. The IC_{50} is a concentration of the extract that inhibits 50% of free radicals.

Statistical analysis

The data obtained from the experiments in this research were analyzed using SAS software. The analysis of Variance (ANOVA) test was used to examine and compare the average of methanolic extracts. All the data obtained from the experiment were repeated three times and their values were reported as mean ± standard deviation.

Results

Figure 3, shows the amount of total phenolic contents of the methanolic extracts of *Borago officinalis*, *Teucrium polium*, *Mentha aquatic* and *Allium taradox*. Among the extracts, *Borago officinalis* had the highest total phenolic contents. At a concentration of 100 µg/ mL, the mean amount of total phenolic content was 38 ± 0.45, 31 ± 0.43, 23 ± 0.91, and 12 ± 0.21 mg of GAE/g for *Borago officinalis*, *Mentha aquatic*, *Teucrium polium*, and *Allium taradox*, respectively (Figure 3).

Among the medical plants, Borago officinalis showed the highest antiradical capacity which was not very different from *Mentha aquatic* (Figure 4A). The IC_{50} index was used to express the inhibition effects of free radicals in the DPPH method. In this way, the lower the IC_{50} value indicated, the higher the inhibition activity. According to Figure 4B, the lowest value of IC_{50} was observed with *Borago officinalis* 1620 ± 41.36 extract, and the highest IC_{50} was observed in *Allium taradox* 398 ± 36.8.

The results obtained from the analysis of variance of the data showed that the highest and the lowest amount of total phenolic compounds and anti-radical activity was observed with *Borago officinalis* and *Allium taradox*, respectively. The difference between the groups was significant at the probability level of P<0.01 for total phenolic content, DPPH and IC_{50} (Table 1).

Table 1. Analysis of variance among *Borago officinalis*, *Teucrium polium*, *Mentha aquatica* and *Allium taradox*

S.O.V	df	Mean Squares		
		Total phenolic (mg of GAE /g)	DPPH (%)	IC_{50} (ppm)
Plant	3	53.86 **	94.694 **	3.00 **
Error	22	28.37	650.63	20.20
CV %	-	4.42	8.05	6.8

** Significant at the probability level of < 0.01
S.O.V: Source of Variance

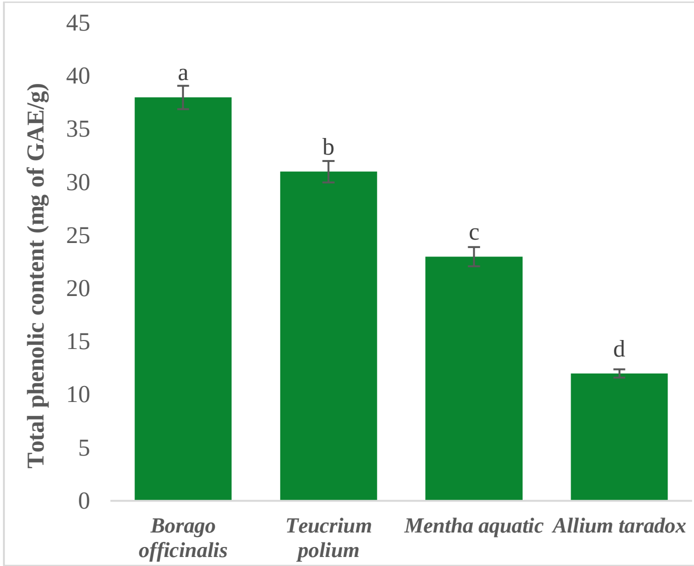

Figure 3. Determination of the total amount of phenolic compounds with methanolic extractives of *Borago officinalis, Teucrium polium, Mentha aquatic* and *Allium taradox.* All experiments were performed in triplicate. Data are expressed as mean ± SD (n= 3, ** P-value <0.01) for all tested dosages (different letters are used to mark significant differences).

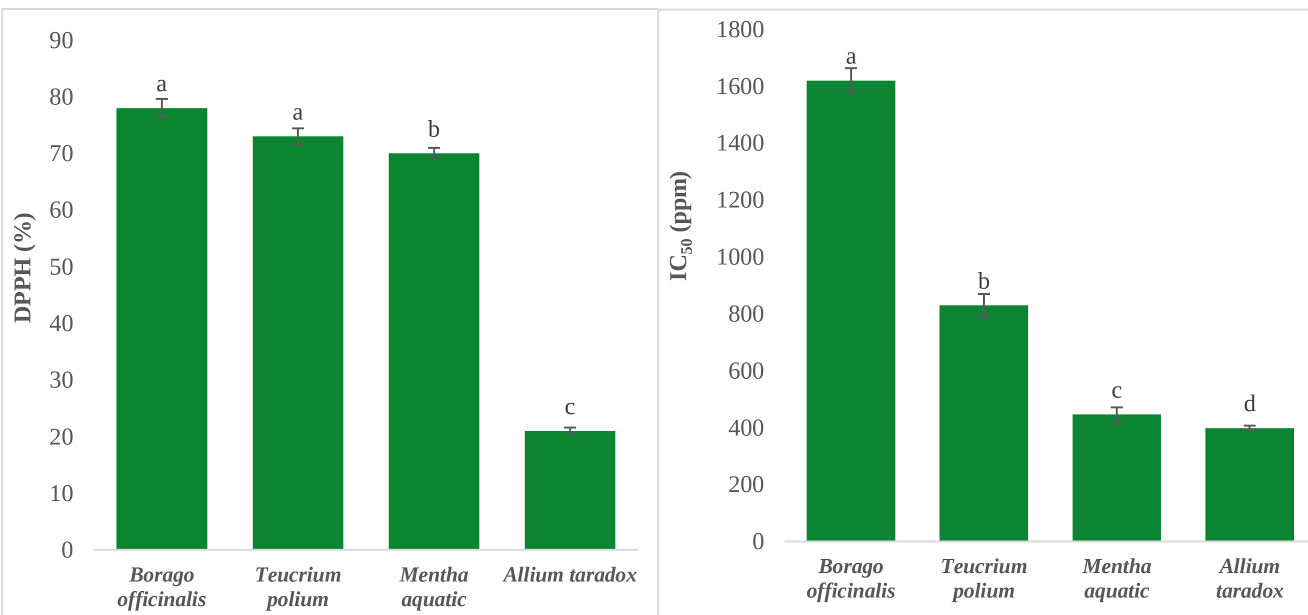

Figure 4. Radical scavenging activity of *Borago officinalis, Teucrium polium, Mentha aquatic* and *Allium taradox*. (A) DPPH radical scavenging activity of methanolic extracts (500 ppm concentration). (B) IC_{50} of methanolic extracts. All experiments were performed in triplicate. Data are expressed as mean ± SD (n= 3, ** P-value <0.01) for all tested dosages (different letters are used to mark significant differences).

Discussion

Natural antioxidants in plants mainly include phenolic compounds. Phenolic compounds have a high ability to scavenge free radicals and this ability mostly depends on the number of hydroxyl groups (20, 21). Plants of *Lamiaceae* (22), *Boraginaceae* (23) and *Amaryllidaceae* (24) have main polyphenolic, flavonoid and terpenoid compounds that have strong antioxidant properties. The phenolic content and compounds of plants depend on genetic and environmental factors (25, 26). It seems that these compounds can be obtained more through plant extracts compared to their essential oils; probably the reason for this difference is in various extraction methods (27). The yield of the extract depends on the type of solvent, the time and temperature of the extraction and the chemical nature of the sample, and under the same temperature and time, the solvent used and the chemical characteristics of the samples are two important factors (28). Methanol has the most efficiency among the common solvents for extracting plant materials, including polar and non-polar compounds; therefore, the measurement of antioxidant properties using methanol extract will be higher due to the extraction of the appropriate amount of polar and non-polar compounds (26). Due to the abundance of antioxidant compounds in plants, it is difficult to identify them individually, so the antioxidant capacity of the extracts is evaluated with several assays (29). The DPPH test is used to determine the antiradical capacity of compounds and indicates their ability to inhibit free radicals by donating hydrogen to the DPPH radical (30, 31). The present study is the first study on the antioxidant capacity of the methanolic extract of E. *labiosiformis*, so the results obtained are not comparable with the results of other studies. Therefore, the present study was compared with studies on *Borago officinalis, Teucrium polium*, *Mentha aquatica* and *Allium taradox* plants. The results showed that the antioxidant property of the methanol extract of the plants was dependent on the concentration. Therefore, the higher the concentration, the higher the antioxidant properties of the methanolic extract. In Hamedeyazdan et al.'s study (32), the antioxidant properties of *Marrabium persicum* extract from the *Lamiaceae* family were investigated, and IC_{50}=0.052 ppm was obtained. Kamkar et al. (33) evaluated the antioxidant capacity of Iranian mint essential oil and extract, and the IC_{50} value for the plant extract was 0.012 mg/ml. These studies had higher antioxidant properties compared to the methanolic extract of these researched plants. In other studies, conducted by Paduch et al. (34), Özgen et al. (35), Asadi Barbiha (36), Salehi Sardoei and Shahdadi (37), on the methanol extract of *Lamium Album*, *Salvia limbata, Eremostachys labiosiformis* and *Asperugo procumben*s, the IC_{50} values were 0.4659, 0.6195, 0.392 and 1500 ppm, respectively. A comparison of this research with the methanolic extract of *Borago officinalis* showed that this plant has a higher antioxidant property (IC_{50} = 1620 ± 41.36 ppm) than the mentioned plants.

Conclusion

Here, we examined leaves of *Borago officinalis, Teucrium polium, Mentha aquatic* and *Allium taradox* and found that the methanolic extract of *Borago officinalis* leaf, which contains large amounts of phenolic and flavonoid compounds, exhibited the highest antioxidant and free radical scavenging activity. These investigation assays indicate that *Borago officinalis*, *Teucrium polium*, *Mentha aquatic* and *Allium taradox* leaves are significant sources of natural antioxidants, which could help to prevent the progression of various diseases caused by free radicals, such as certain cancers.

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Note declared.

Conflict of Interest

The authors declared that they have no conflict of interest associated with this study.

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No company or organization paid for this study.

Ethics

The author did not use any human or animal samples for this study.

References

1. Speakman, J. R., and Selman, C., 2011. The free-radical damage theory: accumulating evidence against a simple link of oxidative stress to ageing and lifespan. Bioessays. 2011. 33(4): 255-259.

2. Halliwell, B., and Gutteridge, J. M., 2015. Free radicals in biology and medicine: Oxford University Press, USA.

3. Mishra, K., Ojha, H., and Chaudhury, N. K. Estimation of antiradical properties of antioxidants using DPPH assay: A critical review and results. Food chemistry. 2012. 130(4), PP: 1036-1043.

4. Noguchi, N., and Niki, E. Phenolic antioxidants: A rationale for design and evaluation of novel antioxidant drug for atherosclerosis. Free Radical Biology and Medicine. 2000. 28(10): 1538-1546.

5. Lundebye, A. K., Hove, H., Måge, A., Bohne, V., and Hamre, K., 2010. Levels of synthetic antioxidants (ethoxyquin, butylated hydroxytoluene and butylated hydroxyanisole) in fish feed and commercially farmed fish. Food Additives & Contaminants: Part A. 2010. 27(12): 1652-1657.

6. Jayaprakasha, G., and Patil, B. S., 2007. In vitro evaluation of the antioxidant activities in fruit extracts from citron and blood orange, Food Chemistry. 2007. 101(1): 410-418.

7. Waraho, T., McClements, D. J., and Decker, E. A., 2011. Mechanisms of lipid oxidation in food dispersions. Trends in Food Science & Technology. 2011. 22(1): 3-13.

8. Mathew, S., and Abraham, T. E., 2006. In vitro antioxidant activity and scavenging effects of Cinnamomum verum leaf extract assayed by different methodologies. Food and Chemical Toxicology. 2006. 44(2):198-206.

9. McDonald, S., Prenzler, P. D., Antolovich, M., and Robards, K., 2001. Phenolic content and antioxidant activity of olive extracts, Food chemistry. 2001. 73(1): 73-84.

10. Shah, M. A., Bosco, S. J. D., and Mir, S. A., 2014. Plant extracts as natural antioxidants in meat and meat products. Meat science. 2014. 98(1): 21-33.

11. Barbaro, B., Toietta, G., Maggio, R., Arciello, M., Tarocchi, M., Galli, A., and Balsano, C., 2014. Effects of the olive-derived polyphenol oleuropein on human health. International journal of molecular sciences. 2014. 15(10):18508-18524.

12. Alam, M. S., Kaur, G., Jabbar, Z., Javed, K., and Athar, M., 2007. Eruca sativa seeds possess antioxidant activity and exert a protective effect on mercuric chloride induced renal toxicity, Food and chemical toxicology. 2007. 45(6): 910-920.

13. Di Meo, F., Lemaur, V., Cornil Jrm, Lazzaroni, R., Duroux, J. L., Olivier, Y., and Trouillas, P., 2013. Free radical scavenging by natural polyphenols: atom versus electron transfer, The Journal of Physical Chemistry A. 2013. 117(10): 2082-2092.

14. Jiang, J., Xiong, Y. L., 2016. Natural antioxidants as food and feed additives to promote health benefits and quality of meat products: A review. Meat science. 2016. 120: 107-117.

15. Pietta, P. G., 2000. Flavonoids as antioxidants. Journal of natural products. 2000. 63(7):1035-1042.

16. Molyneux, P., 2004. The use of the stable free radical diphenylpicrylhydrazyl (DPPH) for estimating antioxidant activity. Songklanakarin J Sci Technol. 2004. 26(2): 211-219.

17. Chung, Y. C., Chien, C. T., Teng, K. Y., and Chou, S. T., 2006. Antioxidative and mutagenic properties of Zanthoxylum ailanthoides Sieb and zucc, Food Chemistry. 2006. 97(3): 418-425.

18. Fu, R., Zhang, Y., Guo, Y., and Chen, F., 2014. Antioxidant and tyrosinase inhibition activities of the ethanol-insoluble fraction of water extract of Sapium sebiferum (L.) Roxb. leaves. South African Journal of Botany. 2014. 93: 98-104.

19. Shahidi, F., and Naczk, M. Food phenolics: Technomic Pub. Co. 1955.

20. Sataei Mokhtari, T., F. Shahdadi, A. Salehi Sardoei. The effect of different solvents on the percentage of free radical scavenging DPPH and antioxidant activity of native medicinal plants in Jiroft city. Karafan Quarterly Scientific Journal. 2021. 18(4): 145-158.

21. Shahdadi, F.; Faryabi, M.; Khan, H.; Salehi Sardoei, A; Fazeli-Nasab,B.; Goh, B.H.; Goh, K.W.; Tan, C.S. Mentha longifolia Essential Oil and Pulegone in Edible Coatings of Alginate and Chitosan: Effects on Pathogenic Bacteria in Lactic Cheese. Molecules. 2023. 28: 4554.

22. Mardani, M, Motamedifar, M, Hosienipour, R, A Study of the Antiviral Effect of the Essential oil of Zararia Multiflora Boiss on Herpes Simplex TAntiviral Effect of the Essential oil of Zararia Multiflora Boiss on Herpes Simplex Type 1 in Vero Cell Culture, dentjods, 2012. 15: 414-420. [Persian]

23. Michalak, M.; Zagórska-Dziok, M.; Klimek-Szczykutowicz, M.; Szopa, A. Phenolic Profile and Comparison of the Antioxidant, Anti-Ageing, Anti-Inflammatory, and Protective Activities of Borago officinalis Extracts on Skin Cells. Molecules. 2023. 28: 868.

24. Kurnia D, Ajiati D, Heliawati L, Sumiarsa D. 2021. Antioxidant Properties and Structure-Antioxidant Activity Relationship of Allium Species Leaves. Molecules. 2021. 26(23):7175.

25. Vahabpour R, Shamsi S, Monavari S. H. R, Sajjadi S.E., Sajjadi N, Evaluation of Potential Antiviral Activity of the Hydroalcoholic Extract of Lemon Balm L, against Herpes Simplex Virus Type 1, Iranian Journal of Virology, 2010. 4(3-4): 52-57 [Persian]

26. Mirzaei, A, Mohammdi, J, Mirzaei, N, Mirzaei, M, The antioxidant capacities and total phenolic contents of some medicinal plants in iran, JFUMS 2011. 1(3): 160-167. [Persian]

27. Yang D, Wang Q, Ke L, Jiang J, Ayaing T, Antioxidant Activities of Various Extracts of Lous (NelumbonuficeraGaertn) Rhizome, Asia Pac J ClinNutr, 2007. 16(1): 158-163.

28. Shahdadi, F., M Payandeh, A Salehi Sardoei. Comparison of Antioxidant Activity of Dracocephalum polychaetum Bornm and Nepeta cataria L. and Their Effect on Probiotic Bacteria in a Simulated

Gastrointestinal Environment. Journal of Medical Microbiology and Infectious Diseases. 2021. 9(1): 5-11.

29. Ahvazi, M., F, Khalighi – Sigaroodi, H, Ebrahimzadeh, N, Rahimifard, Chemical Composition of the Essential Oil and Antioxidant Activities, Total Phenol and Flavonoid Content of the Extract of Nepetapogonosperma, JMP, 2013. 4(48): 185-198. [Persian]

30. Floegel, A, Kim, D, O, Chung, S, J, Koo, S, I, Chun, O, K, Comparison of ABTS/DPPH assays to measure antioxidant capacity in popular antioxidant-rich US foods, Journal of food composition and analysis, 2011. 24(7): 1043-1048.

31. Demir, T, Özen, M, Ö,Hameş-kocabaş, E, E, Antioxidant and Cytotoxic Activity of Physalisperuviana, JMPR, 2014. 4(3): 30-34.

32. Hamedeyazdan, S, Fathiazad, F, Sharifi, S, Nazemiyeh, H, Antiproliferative activity of Marrubiumpersicum extract in the MCF-7 human breast cancer cell line, APJCP, 2012. 13(11): 5843 – 8.

33. Kamkar, A, Asadi, F, JebelliJavan, A, Jamshidi, R, Antioxidant capacity of essential oil and extract of Iranian Menthaspicat, J VLR. 2009. 1(1): 69-77. [Persian]

34. Paduch, R, Matysik, G, Wójciak-Kosior, et al, Lamium album extracts express free radical scavenging and cytotoxic activities, PJOES, 2008. 17(4): 569-580.

35. Özgen, U, Mavi, A, Terzi, Z, Yilduim, A, Coşkun, M. Houghton, P, J, Antioxidant properties of some medicinal Lamiaceae (Labiatae) species, JPB, 2006. 44(2): 107-112.

36. Asadi barbariha S, Roudbari F, Mohajerani M, Mahmoudi Otaghvari A, Pourmoradi S, Kavoosian S et al . Evaluation of free radical scavenging capacity and cytotoxiceffect of methanol extracts of plant Eremostachys labiosiformis (Popov) Knorring on Vero cells . Journal of North Khorasan University of Medical sciences. 2017. 8(4):633-641.

37. Salehi Sardoei A, F Shahdadi. Antioxidant activities of traditional plants in Qaen by DPPH free radical-scavenging assay. Archives of Medical Laboratory Sciences. 2022. 8: 1-6.