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Comparative characteristics of polyphenols in extracts of wild and cultivated Bulgarian white oregano

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White oregano is a widespread aromatic plant with proven antioxidant properties due to its antioxidant content. The content of polyphenolic compounds in ethanol extracts of wild and cultivated Bulgarian white oregano was studied. It was found that wild oregano extracts are richer in polyphenolic compounds (2,78 – 4,17 mg/mL) than cultivated ones (1,44 – 1,78 mg/mL). Cinnamic acid derivatives are predominant in all white oregano extracts obtained with 70 % ethanol extractant. They occupy from 78.54 % to 84.57 % of the total phenolic content of wild oregano extracts and from 47.52 % to 59.92 % of cultivated oregano ones.

Keywords: *Origanum heracleoticum* L., ethanol extracts, phenolic acids, flavonoids, flavonoid glycosides

INTRODUCTION

Two species of the genus *Origanum* are disseminated in our country: the ordinary oregano (*Origanum vulgare* L.), which grows in mountainous and sub-mountainous areas throughout all the country and white oregano (*Origanum heracleoticum* L.), growing only on the rocky slopes of the Eastern Rhodopes, Belasitsa, South Struma Valley, Kresna Defile, Trakia Lowland, at an altitude of 250 to 700 m [1, 2]. In recent years, the interest in cultivating white oregano has been growing due to its widespread entry into the food industry [3].

Current nutrition trends are aimed at limiting the use of synthetic antioxidants and replacing them with natural ones. In this regard, a big part of the researches are focused on the use of the natural potential of herbs and spices [4-8].

Oregano extracts have been investigated to identify the antioxidant compounds contained in them. Solvents with increasing polarity and isolation of different fractions have been used. Their composition and effectiveness against free radicals and lipid oxidation have been determined [9]. The highest activity has been found in the fraction containing rosemary acid, which has significant antiradical properties and stronger activity than the synthetic antioxidants BHA, BHT, PG, OG, TBHQ [10-12].

Apigenin, eriodictyol dihydrokaempferol and dihydroquercetin have been identified by the study of flavonoid constituents in oregano extracts [9, 13].

It is considered the antioxidant activity of the essential oils of white oregano is due to the high content of volatile (carvacrol and thymol) and non-volatile (phenolic acids and flavonoids) compounds [9, 14-22]. It has been established that the effectiveness of the essential oils is higher than the activity of the individual components [23].

Oregano essential oil also exhibits strong antioxidant activity when added directly to chicken meat [24-26], beef [27], minced beef [28, 29], minced lamb [30] and fish fillets [31]. According to the authors, the oil can replace synthetic antioxidants in the meat products because it reduces lipid oxidation, improves the color stability of meat, and extends shelf life.

It is known that there is a good correlation between the total polyphenols content and the antioxidant activity of the plants. To our knowledge, there are no papers reporting the content of polyphenols in Bulgarian white oregano extracts. Such kind of information will be useful to food scientists and technologists for the development of functional foods rich in natural antioxidants. The results of the current study will enrich the national database of the white oregano, which is the aim of the research.

EXPERIMENTAL

Materials

The object of this research is Bulgarian white oregano (*Origanum heracleoticum* L.) – wild and cultivated. The wild oregano was collected at the end of July 2018 from the southern slopes of the Eastern Rhodopes (360 m altitude), the region of Ivaylovgrad, Haskovo. The cultivated oregano (dried crumbled leaf) was purchased from BulgarLuk Ltd. – Katunitsa, from the area of Parvomay, region Plovdiv, harvest year 2018. The raw material was in flowering phase.

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Preparation of extracts

The extraction was carried out with ethanol at a concentration of 70 % as a periodic process, without breaking, at two hydromodules (HM) - 1:8 and 1:10, 60 °C for 6 hours. The raw material was separated by filtration through a vacuum filter. A rotary vacuum evaporator with a water vacuum pump at a water bath temperature of 60-65 °C was used to separate the solvent. The resulting extracts were stored at 4 - 6 °C until analysis.

Determination of polyphenols

Sample preparation, μg/g. Dried samples were powdered by using laboratory homogenizer. Phenolic compounds were extracted from 0.5 g of powdered sample using 70 % methanol (Sigma) on a ultrasonic bath at 70 °C for 3 hours. The biomass was separated by filtration and then the extraction procedure was repeated another two times. The combined extract was evaporated to dryness on a rotary evaporator. The residue was dissolved in methanol and used for HPLC analyses after filtration with 0.45 μm syringe filter.

HPLC analysis. Qualitative and quantitative determinations of phenolic acids and flavonoids, were performed by using Waters 1525 Binary Pump HPLC systems (Waters, Milford, MA, USA), equipped with Waters 2484 dual absorbance detector (Waters, Milford, MA, USA) and Supelco Discovery HS C18 column (5 μm, 25 cm × 4,6 mm), operated under control of Breeze 3,30 software.

Analyses of phenolic acids

Gradient elution by using mobile phase of solvent A (2 % acetic acid) and solvent B (0.5 % acetic acid: acetonitrile (1:1)) was used. The gradients of the setup according to Marchev *et al.,* (2011) were as follows: 0-30 min solvent B increase from 5 % to 35 % at a flow rate of 0.8 mL/min; 30-45 min solvent B increase to 70 % at a flow rate of 0.4 mL/min; 45-50 solvent B increase to 80 % at a flow rate of 1.2 mL/min; 50-60 min solvent B increase to 100 % at the same flow rate; 60-65 min solvent B drop down to 5 % at a flow rate of 0.8 mL/min and hold on up to 70 min to equilibrate the column [32]. Gallic, protocatechuic, salicylic, chlorogenic, vanillic, caffeic, syringic, ferulic, sinapic, *p*-coumaric and cinnamic acids (Sigma) were used as standards to build calibration curves. The detection was carried out at 280 nm.

Analyses of flavonoids

Gradient elution by using mobile phase of solvent A (2 % acetic acid) and solvent B (methanol) was used.

The gradients of the setup according to Marchev *et al*., (2011) were as follows: 0-10 min solvent B increase from 30 % to 50 % at a flow rate of 1.0 mL/min; 10-15 min hold on at the same flow rate; 15-16 min solvent B increase to 52 % at a flow rate of 0.8 mL/min; 16-30 min solvent B increase to 80 % at the same flow rate; 30-35 min solvent B drop down to 30 % at a flow rate of 1.0 mL/min and hold on up to 40 min to equilibrate the column [32]. Myricetin, kaempferol, quercetin, hesperidine and apigenin (Sigma) were used as standards to build calibration curves. The detection was carried out at 380 nm.

The quercetin glycosides rutin and hyperoside were analyzed on the same HPLC system by using gradient elution by applying mobile phase of solvent A (2 % acetic acid) and solvent B (acetonitrile). The gradients of the setup according to Ivanov *et al*. (2014) were as follows: 0-15 min 20 % solvent B; 15-17 min 50 % solvent B; 17-20 min 20 % solvent B [33]. Rutin and hyperoside (Sigma-Aldrich) were used as standards to build calibration curves. The detection was carried out at 370 nm.

The reagents used were pure for analysis. All experiments were repeated three times. The data were expressed as means ± standard deviation (SD).

RESULTS AND DISCUSSION

Content of polyphenolic compounds in ethanol extracts of wild white oregano

The content of polyphenols was determined in the extracts obtained at 60 °C, duration 6 h, hydromodule 1:8 and 1:10 (Table 1).

In the analysis of the data it was found that the extracts obtained at HM 1:8 were richer in polyphenol compounds than those obtained at HM 1:10. Derivatives (mustard and ferulic acid in higher amounts, p-coumaric, caffeic, chlorogenic and cinnamic) predominated in the extracts of the phenolic acid, cinnamic acid. It is noteworthy that at HM 1:8 it is better to extract sinapic acid and at HM 1:10 - ferulic acid. 2-Hydroxy benzoic, vanilla, syringan and 3,4-dihydroxy benzoic acid derivatives were identified, with slightly higher amounts at HM 1:8. The flavonoids content in the extracts obtained at the two hydromodules was comparable. Flavone glycosides (hesperidin) and quercetin glycosides (rutin) predominated. Flavones (luteolin, apigenin) and flavonols (campferol, myricetin, quercetin) were identified.

Table 1. Content of polyphenolic compounds in ethanol extracts of wild white oregano

Type of structure	Compounds	Content, mg/mL (from the test sample)	
		Hydromodule 1:8	Hydromodule 1:10
Phenolic acids			
Derivatives of cinnamic acid	Caffeic acid	0,04±0,08	0,03±0,01
	Chlorogenic acid	0,03±0,06	0,02±0,08
	p- Coumaric acid	0,09±0,09	0,03±0,11
	Sinapic acid	2,98±0,11	0,81±0,03
	Ferulic acid	0,39±0,02	1,27±0,06
	Cinnamic acid	0,01±0,02	0,01±0,02
Derivatives of benzoic acid	3,4-dihydroxy Benzoic acid	0,01±0,04	0,01±0,11
	2- hydroxy Benzoic acid	0,07±0,05	0,05±0,01
	Vanillic acid	0,06±0,16	0,06±0,01
	Syringic acid	0,05±0,02	0,04±0,15
Flavonoids			
Flavonols	Myricetin	0,02±0,02	0,03±0,10
	Kaempferol	0,04±0,01	0,04±0,01
	Quercetin	0,01±0,06	0,01±0,02
Flavons	Apigenin	0,04±0,36	0,06±0,02
	Luteolin	0,06±0,08	0,06±0,03
Flavon glycosides	Hesperetin	0,18±0,04	0,17±0,68
Quercetin glycosides	Rutin	0,09±0,02	0,08±0,12
Total:		4,17	2,78

Data expressed as mean ± SD (n=3)

The content of polyphenols in ethanol extracts of wild white oregano cannot be compared with descriptions in the literature due to lack of data. The distribution of the polyphenol compounds by classes in the wild oregano extracts is shown in Fig. 1. For comparison of data, the amount of separate classes of polyphenols is recalculated as a percentage of the total polyphenols content of the relevant hydromodule.

In the extracts of the two variants, cinnamic acid derivatives predominated, their amount was higher in comparison with those obtained at HM 1:8 (84.57 %) compared to those at HM 1:10 (78.54 %). The amounts of the other classes of polyphenols were comparable in the extracts obtained at the two hydromodules: benzoic acid derivatives (4.88 % at HM 1:8 and 5.77 % at HM 1:10), flavonols (1.61 % at HM 1:8 and 2.51 % at HM 1:10), flavones (2.51 % at HM 1:8 and 4.16 % at HM 1:10), flavone glycosides (4.30 % at HM 1:8, and 6.22 % at HM 1:10), quercetin glycosides (2.14 % at HM 1:8 and 2.80 % at HM 1:10).

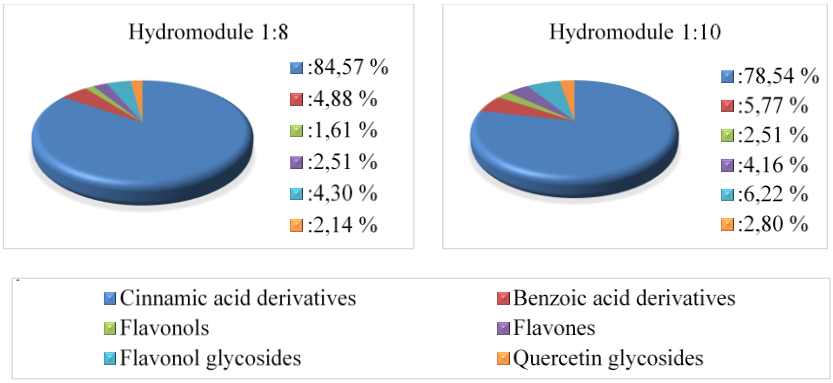

Figure 1. Classification of polyphenolic compounds in ethanol extracts of wild white oregano

Table 2. Content of polyphenolic compounds in ethanol extracts of cultivated white oregano

Type of structure	Compounds	Content, mg/mL (from the test sample)	
		Hydromodule 1:8	Hydromodule 1:10
Phenolic acids			
Derivatives of cinnamic acid	Caffeic acid	0,16±0,03	0,13±0,02
	Chlorogenic acid	0.04±0.01	0.03±0.05
	p- Coumaric acid	0.08±0.07	0.08±0.01
	Sinapic acid	0.37±0.11	0.34±0.07
	Ferulic acid	0.16±0.06	0.18±0.10
	Cinnamic acid	0.05±0.02	0.10±0.05
Derivatives of benzoic acid	2- hydroxy Benzoic acid	0.12±0.09	0.08±0.07
	Vanillic acid	0.08±0.10	0.07±0.04
	Syringic acid	0.04±0.05	0.04±0,09
Flavonoids			
Flavonols	Kaempferol	0.04±0.02	0.01±0.03
	Quercetin	–*	0.01±0.07
Flavons	Apigenin	0.06±0.08	0.04±0.11
	Luteolin	0.14±0.05	0.10±0.08
Flavon glycosides	Hesperetin	0.12±0.07	0.11±0.05
Quercetin glycosides	Rutin	0.13±0.02	0.12±0.07
	Hyperoside	0.19±0.01	–
Total:		1.78	1.44

* not identified; Data expressed as mean ± SD (n=3)

Content of polyphenolic compounds in ethanol extracts of cultivated white oregano

The content of polyphenols was determined in the extracts obtained at 60 ° C, duration 6 h, HM 1:8 and 1:10 (Table 2.).

Unlike the wild plant, in the cultivated one, relatively similar amounts of the polyphenolic compounds in the extracts, obtained at both hydromodules, were found. Cinnamic acid derivatives (mustard, caffeic, ferulic, p-coumaric, cinnamic and chlorogenic), predominated in the phenolic acids. The benzoic acid derivatives were represented by 2-hydroxy benzoic, vanillic and syringic acids. In cultivated white oregano extracts, unlike wild-growing, 3,4-dihydroxy benzoic acid was not detected. The flavonoids were dominated by quercetin glycosides (hyperoside – identified only in the extract obtained at HM 1:8) and rutin, and flavone glycosides (hesperitin). Flavones (luteolin and apigenin) and flavonols (caempferol and quercetin) were identified. The extracts of cultivated white oregano did not contain the flavonol myricetin that was found in the extract of the wild plant.

Polyphenols are a product of secondary metabolism. The richer polyphenolic composition in the wild white oregano is explained by the habitat of the plant and the lack of cultivation activities.

The correlation of the polyphenol compounds by classes in the cultivated white oregano extracts is shown in Fig. 2. For comparison of data, the amount of separate classes of polyphenols was recalculated as a percentage of the total polyphenols content of the relevant hydromodule.

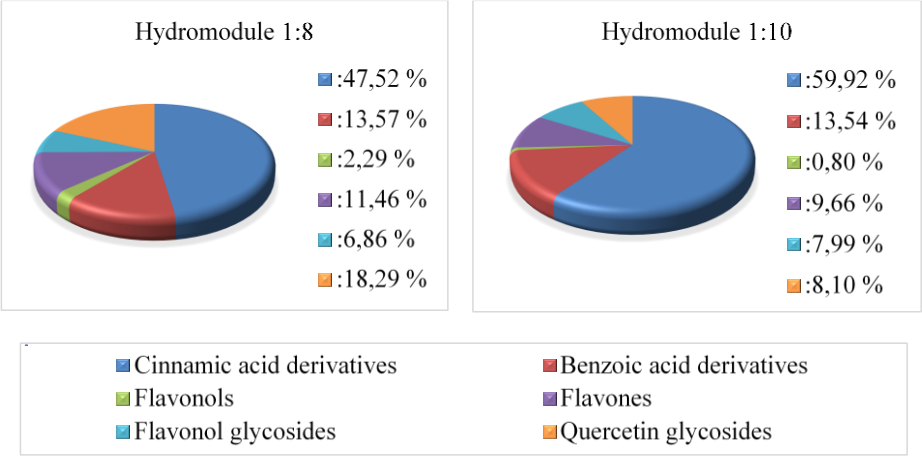

Figure 2. Classification of polyphenolic compounds in ethanol extracts of cultivated white oregano

Cinnamic acid derivatives also predominated in the cultivated white oregano extracts. Unlike the extracts obtained from the wild oregano, their amount is higher than those obtained at HM 1:10 (59.92 %), compared to obtained at HM 1:8 (47.52 %). Differences were observed also for quercetin glycosides (18.29 % at HM 1: 8 and 8.10 % at HM 1:10). The amounts of the other classes of polyphenols were comparable in the extracts obtained at both hydromodules: benzoic acid derivatives (13.57 % at HM 1:8 and 13.54 % at HM 1:10), flavonols (2.29 % at HM 1:8 and 0.80 % at HM 1:10), flavones (11.46 % at HM 1:8 and 9.66 % at HM 1:10), flavone glycosides (6.86 % at HM 1:8) and 7.99 % at HM 1:10.

The content of polyphenolic compounds in white oregano extracts is difficult to compare with the literature data because of the origin of the raw material, the various agro-meteorological conditions and the various methods of analysis.

CONCLUSIONS

In summary, the content of polyphenolic compounds in ethanol extracts of wild and cultivated Bulgarian white oregano was studied. The ethanol extracts of wild white oregano had higher contents of polyphenolic compounds. In both types of extracts cinnamic acid derivatives predominated. The content of polyphenols in Bulgarian white oregano is a prerequisite for antioxidant activity, which confirms scientific reports about white oregano of other origins.

Based on the proven relationship between the polyphenolic content and the antioxidant activity of Bulgarian herbs and spices, the results confirm that Bulgarian white oregano can be used as a natural source of antioxidants in food production. In addition, the effect of the antioxidant compounds in a food matrix (mixture) may be significantly different than the activity of a purified extract. This can be a subject to in-depth analysis in further research.

REFERENCES

1. M. Anchev, BAS Publishing House, **9**, 286 (1989).
2. http://www.naas.government.bg/bilki/doc/-otglezdane/1_raioni_uslovia_kultivirane_lechebni_rastenia.pdf (available on 18.10.2019), (2016).
3. http://susherbsbg.eu/wp-content/uploads/2015/04/Tehnologia-bial-rigan-Origanum-vulgare-spp-hirtum-EKO.pdf (available on 18.10.2019). (2015).
4. G. Nikolova, D. Ivanova, Y. Karamalakova, B. Grigorov, V. Gadjeva, A. Zheleva, *Biochemistry*, **71**, 780 (2018).
5. Y. Karamalakova. M. Adhikari, N. Kovacheva, V. Ivanov, G. Nikolova, V. Gadjeva, *Bulgarian Chemical Communications*, **49 (**Special Issue C), 14 (2017).
6. A. Zheleva, Y. Karamalakova, G. Nikolova, R. Kumar, R.Sharma, V.Gadjeva, *Biotechnol. & Biotechnol. Equipm.*, **26,** 1 (2012).
7. Y. Karamalakova, J. Sharma, G. Nikolova, S. Stanev, R. Arora, V. Gadjeva, A. Zheleva, *Biotechnology & Biotechnological Equipment*, **27**, 3861 (2013).
8. M. Adhikari, Y. Karamalakova, V. Ivanov, V. Gadjeva, R. Kumar, R. Sharma, A. Zheleva, R. Arora, *Trakia Journal of Sciences*, **10,** 17 (2012).
9. D. Tsimogiannis, M. Stavrakaki, V. Oreopoulou, *International Journal of Food Science and Technology*, **41,** 39 (2006).
10. V. Exarchou, N. Nenadis, M. Tsimidou, I. Gerothanassis, A. Troganis, D. Boskou, *Journal of Agricultural and Food Chemistry*, **50**, 5294 (2002).
11. M. Hossain, N. Brunton, C. Barry-Ryan, A. Martin-Diana, M. Wilkinson, *Rasayan Journal of Chemistry*, **1,** 751 (2008).
12. H. Hotta, S. Nagano, M. Ueda, Y. Tsujino, J. Koyama, T. Osakai, *Biochimica et Biophysica Acta*, **1572,** 123 (2002).
13. P. Bocchini, M. Russo, G. Galletti, *Rapid Communications in Mass Spectrometry*, **12,** 1555 (1998).
14. F. D'Antuono, G. Galletti, P. Bocchini, *Annals of Botany*, **86**, 471 (2000).
15. J. Karpouhtsis, E. Pardali, E. Feggou, S. Kokkini, Z. Seouras, P. Mavragani-Tsipidou. *Journal of Agricultural and Food Chemistry*, **46**, 1111 (1998).
16. T. Kulisic, A. Krisko, V. Dragovic-Uzelac, M. Milos, G. Pifat,. *International Journal of Food Sciences and Nutrition*, **58,** 87 (2007).
17. J. Mastelić, I. Jerković, I. Blazević, M. Poljak-Blazi, S. Borović, I. Ivancić-Baće, V. Smrecki, N. Zarković, K. Brcić-Kostic, D. Vikić-Topić, N. Müller, *Journal of Agricultural and Food Chemistry*, **56**, 3989 (2008).
18. A. Ocana-Fuentes, E. Arranz, F. Senorans, G. Reglero, *Food and Chemical Toxicology*, **48,** 1568 (2010).
19. B. Shan, Y. Cai, M. Sun, H. Corke, *Journal of Agricultural and Food Chemistry*, **53,** 7749 (2005).
20. M. Skoula, J. Harborne, Taylor and Francis, London, **67** (2002).
21. C. Timothy, V. Priya, R. Gayathri, *Drug Invention Today*, **10**, 1903 (2018).
22. W. Zheng, S. Wang, *Journal of Agricultural and Food Chemistry*, **49,** 5165 (2001).
23. M. Miloš, J. Mastelić, I. Jerković, *Food Chemistry*, **71**, 79 (2000).
24. E. Chouliara, A. Karatapanis, I. Savvaidis, M. Kontominas, *Food Microbiology*, **24,** 607 (2007).
25. M. Al-Hijazeen, E. Lee, A. Mendonca, D. Ahn. *Antioxidants (Basel)*, **5,** 18 (2016).
26. I. Čabarkapa, M. Škrinjar, N. Blagojev, J. Gubic, D. Plavšic, B. Kokic, T. Radušin, *Journal of Pure and Applied Microbiology*, **7,** 221 (2013).
27. S. Scramlin, R. Newman, R. Cox, H. Sepe, A. Alderton, J. O'Leary, W. Mikel, *Journal of Food Science*, **75,** 89 (2010).
28. P. Skandamis, G.-J. Nychas, *Journal of Applied Microbiology*, **91,** 1011 (2001).
29. K. Ünal, A. Babaoglu, M. Karakaya, *Journal of Essential Oil-Bearing Plants*, **17**, 797 (2014).
30. A. Govaris, N. Solomakos, A. Pexara, P. Chatzopoulou, *International Journal of Food Microbiology*, **137,** 175 (2010).
31. A. Goulas, M. Kontominas, *Food Chemistry*, **100,** 287 (2007).
32. A. Marchev, V. Georgiev, I. Ivanov, I. Badjakov, A. Pavlov, *Biotechnology Letters*, **33,** 1873 (2011).
33. I. Ivanov, R. Vrancheva, A. Marchev, N. Petkova, I. Aneva, P. Denev, V. Georgiev, A. Pavlov, *International Journal of Current Microbiology and Applied Science*, **3**, 296 (2014).