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DETERMINATION OF COMPOUNDS WITH POTENTIAL ANTIOXIDANT AND ANTIRADICAL CAPACITY IN DIFFERENT OREGANO EXTRACTS

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Abstract: *Oregano is an aromatic plant used both as a condiment and for medicinal purposes, with a great number of antioxidant compounds (43). The study analyzes the main groups of antioxidant compounds – flavonoids, polyphenolcarboxilic acids and polyphenols and the antiradical capacity of this plant. The determinations are done spectrophotometrically, based on the reactions with $AlCl_3$ – for the flavonoids, with Arnow reagent – for the polyphenolcarboxilic acids and Folin – Ciocâlteau reagent for total polyphenols.*
Because it is very important to know the way the observed antioxidant compounds can be extracted from plants eight extractions from two samples of dried oregano – condiment and medicinal plant were performed: with water, with methanol solution 50% and ethanol, hot and cold, and two mixtures: of methanol – water – acetic acid and methanol – acetone – water – formic acid, cold.
Water and methanolic solution had the capacity to extract the compounds both from the condiment and the medicinal plant, the temperature influencing in a positive way the extractability of the antioxidant compounds. A valuable extract was obtained at low temperature, too, with the mixture of solvents methanol – acetone - water – formic acid (S_5). Its scavenger activity against the free radicals, determined by the capacity of elimination of the radical 2,2-diphenyl-1-picrylhydrazyl (DPPH), was very good in the majority of the extracts. At the opposite end, there was the extract with ethanol, at low temperature.

Keywords: *flavonoids, polyphenolcarboxilic acids, total polyphenols, solvents, temperature influence*

1. Introduction

Herbs are used both with culinary and medicinal purposes, mainly due to their special antioxidant potential [1].
From the multitude of herbs with high antioxidant capacity, oregano, which is the best selling product in the world, was selected for analysis. Under this name there are sold dried leaves and flowers of *Origanum vulgare L.* subsp. *Hirtum* or *Origanum onites L.* or a combination of both. According to Franz [2], there are at least 68 species of plants sold worldwide with the name of oregano. In kitchen, oregano has been used for a long time to spice sauces, salads, egg dishes, stew, soups, vegetable or meat courses – beef, poultry, fish [3] and also pizza, sausages, especially Italian, Greek, Mexican recipes. It is also a good substitute for salt in products which contain tomatoes. From a medical point of view, oregano has antispasmodic action on smooth muscles, sedative effect on the central nervous system (and especially on respiratory centers), tonic and slightly astringent, due to tannins and bitter substances, according to [4], anti-aging effect (due to its components with antioxidant potential)

etc., being recommended as a tincture or infusion.

Oregano is among the plants with a high number of antioxidants (34 were identified), according to the database of USDA [5]. The main groups of compounds with antioxidant potential in oregano are the flavonoids, polyphenolcarboxilic acids and polyphenols. The main antioxidant compound identified in many studies was the rosmarinic acid [6]. The study aims to analyze the main groups of antioxidant compounds from two samples of oregano – condiment and medicinal plant – dried, and the way in which they are extracted in different solvents, in certain conditions.

The type of plant used and the method of extraction of the useful compounds are very important.

2. Materials and methods

For the research, condiment and medicinal plant were used, bought dried.

In order to analyze the way in which the type of solvent and the extraction temperature influence the extraction yields of the main compounds with antioxidant potential, eight extractions were carried out, in parallel, for oregano as condiment (K) and medicinal plant (M) – (Table 1): five extracts at low temperature and three at high temperature. For this, 2 g from the air part of the dried plant were extracted, using 20 ml of solvent for each. The extractive solution was filtered through filter paper and a volume of 20 ml was obtained with the solvent for each test.

Filtered extractive solutions were analyzed in terms of dry weight content (d.w.) and flavonoids (F), polyphenolcarboxilic acids (Ac. pf.), total polyphenols (Pf) and antiradical capacity.

Table 1

Extractions realized - working conditions

Type of extraction	Extracts obtained		Working temperature	Working conditions
	Symbol	Solvent		
Extraction at cold / low temperature	S_{1R}	water	room temperature	Extraction with agitation, for 30 minutes, then filtration
	S_{2R}	methanol solution 50% (50:50, v/v)		
	S_{3R}	ethanol 96^0		
	S_4	methanol – water – acetic acid, mixture (90:9:1, v/v/v)		
	S_5	methanol– acetone – water – formic acid, mixture (40:40:19,9:0,1, v/v/v/v)		
Extraction at hot / high temperature	S_{1C}	water	$85\ ^0C$	Extraction for 30 minutes, then filtration
	S_{2C}	methanol solution 50% (50:50, v/v)	$65\ ^0C$	
	S_{3C}	ethanol 96^0	$70\ ^0C$	

a. The content of dried substance (the extracting substance) was determined on the principle of mass loss by heating in an oven, at the temperature of 103±2°C, till constant mass, of a quantity of analysed sample.

b. The chemical study aimed to quantify the flavonoids, polyphenolcarboxilic acids and total polyphenols, being known that aromatic plants contribute significantly to the enhancement of the antioxidant activity [7].

b_1. To determine *flavonoids*, the spectrophotometrical method was used, based on the reaction with $AlCl_3$. The content of flavonoids of the analysed samples was expressed in g rutoside / 100 g dry plant (d.p.).

b_2. The determination of polyphenolcarboxilic acids is done spectrophotometrically and it is based on

colour reaction with Arnow reactive, in alkaline medium. The content of polyphenolcarboxilic acids from the analysed samples is expressed in g rosmarinic acid / 100 g dry plant (d.p.).

b_3. The polyphenols were determined by treating the extracts with Folin – Ciocâlteau reactive, when a blue complex (whose colorimetry is at λ= 760 nm) is formed, using as a reference gallic acid (GAE). The total content of polyphenols is expressed in g GAE / 100 g d.p. and g GAE / 100 g dry substance (d.w.).

c. The evaluation of the antiradical action was done by measuring the capacity of elimination of the radical 2,2-diphenyl-1-picrylhydrazyl (DPPH), and its conversion into its reduced form, by the analysed plant extracts. The antiradical activity was expressed as a percentage.

3. Results and Discussion

The working process was led both at room temperature and reflux temperature, with several types of extraction solvents. Some of them are mentioned in and recommended by specialised literature (especially the methanol solution 50%), as well as Other extraction solvents can offer information about the yields of extraction of aimed compounds in catering (water and alcohol), but also in combinations which are used in obtaining better compound extraction yields.

a. In determining the extract (Table 2) one can observe that most extractions were done at a high temperature, with methanol solution, for K sample (1.89 g / 100 ml) and with water, at high temperature, for M sample. The lowest values were obtained for extracts with ethanol, performed at low temperatures (0.568 g / 100 ml for the condiment and 0.24 g / 100 ml for the medicinal plant).

A general analysis of all the values leads to the conclusion that the extraction at low temperature is less efficient, except for the extract S_5, with a complex mixture, especially in the case of the medicinal plant.

Taking into account the way of obtaining the extract, the efficiency of the extraction was determined, as the ratio of dry substance from the extract and the mass of dry substance from the plant material, according to Materska [8].

Table 2
Dry substance from the extracts used in analysis and the efficiency of the extraction

No.	Sample (extract)	Dry substance from the extracts (g/ 100 ml)		Efficiency of the extraction (%)	
		K	M	K	M
1.	S_{1R}	1.322	1.01	14.46	11.07
2.	S_{1C}	1.753	**1.45**	19.18	**15.90**
3.	S_{2R}	1.230	1.29	13.46	14.14
4.	S_{2C}	**1.890**	1.38	**20.68**	15.13
5.	S_{3R}	**0.568**	**0.24**	**6.21**	**2.63**
6.	S_{3C}	1.384	0.60	15.14	6.58
7.	S_4	1.295	0.88	14.17	9.65
8.	S_5	1.285	1.40	14.06	15.35

K–spice (condiment); M–medicinal plant

For both samples, the difference between the efficiency values in the case of the extraction realised with water or methanol, in the same conditions, does not rise above 3.07%, so the two solvents have almost the same capacity of extracting the compounds from both the analysed condiment and the medicinal plant.

b. The concentration value in the compounds with antioxidant potential – the flavonoids (F), polyphenolcarboxilic acids (Ac. pf.), total polyphenols (Pf) - in the extracts of the same plant material, obtained with different solvents, varies a lot (Table 3).

b_1. When determining *the flavonoids*, one can observe that the extraction with water at high temperature is more complete (0.73

g rutoside / 100 g d.p. – for oregano K and 0,92 g rutoside / 100 g d.p. for oregano M). On the opposite end, the extraction with ethanol at cold is placed, in both cases the values being 8.1 and 7.7 or lower than the maximum values for oregano K, respectively M.

When comparing the recorded values at the determination of the flavonoids from tinctures with the values obtained under reflux, with the same solvent, one can observe that the former are much smaller, especially in the case of water and ethanol.

Table 3

The quantity of antioxidant compounds

Solvent (sample)	Flavonoids (g rutoside / 100 g d.p.)		Polyphenolcarboxilic acids (g rosmarinic acid /100 g d.p.)		Total polyphenols (g GAE / 100 g d.p.)		Pf – F – Ac. pf. ((g/ 100 g d.p.)	
	K	M	K	M	K	M	K	M
1	2	3	4	5	6	7	8	9
S_{1R}	0.38	0.54	0.4	0.66	3.58	5	2.8	3.8
S_{1C}	**0.73**	**0.92**	**1.18**	**1.9**	**4.72**	9.13	2.81	6.31
S_{2R}	0.4	0.8	0.71	1.85	4.56	9.33	3.45	6.68
S_{2C}	0.58	0.85	1.02	1.91	5.39	10.27	3.79	7.51
S_{3R}	**0.09**	**0.12**	**0.09**	**0.17**	**0.78**	**1.81**	0.6	1.52
S_{3C}	0.64	0.29	0.92	0.62	5.15	4.44	3.59	3.53
S_4	0.36	0.35	0.51	0.96	2.75	7.04	1.88	5.73
S_5	0.67	0.69	0.96	2.35	4.48	**11.73**	2.85	8.69

Thus, for water, the ratio of the content of the flavonoids from the extract obtained under reflux (S_{1C}) and the extract obtained at room temperature (S_{1R}) is 1.70 for oregano M and 1.91 for oregano K. This proves that by rising the temperature, the flavonoids have a better extractability. Very good results were obtained also when the extraction was done with the mixture S_5 (methanol – acetone – water – formic acid), these being comparable with the maximum values.

Comparing the two samples (K, M) one can observe higher values for the medicinal plant rather than for the condiment.

The comparison between the data obtained from this study and the values from other studies is difficult, due mainly to the difference between solvents used at extraction and the reference compound used to express the results, but also due to different types of plant and extraction conditions (time, temperature etc). For example, at the extraction of the flavonoids from basil, Grayer and collaborators [9] use diethyl ether and Goze [3] uses hexane and dichloromethane. The most commonly used solvents are water, methanol, or the combinations of these two. There are many ways of expressing the results and some studies use as reference [10]: quercetin [3], catechin, and moreover rutoside.

b_2. Polyphenolcarboxilic acids (Table 3, columns 4, 5) are extracted better at high temperature and the solvents with higher efficacy are water (S_{1C}), methanol (S_{2C}) and the mixture S_5. The lowest values were obtained from the extracts with alcohol 96^0, at low temperature (0.09 g rosmarinic acid / 100 g d.p. for oregano K and 0.17 for oregano M).

In comparison, the average ratio of the values condiment / medicinal plant is 0.63 – and this shows higher values for the medicinal plant.

It can also be observed that there is a correlation between the content of flavonoids and the content of polyphenolcarboxilic acids. There are limited studies on the measurement of the polyphenolcarboxilic acids in aromatic plants (the majority are related to the total content of polyphenols and flavonoids). In a study about phenolic compounds from oregano and thyme, [11] we reach the conclusion that the extracts from oregano have a high content of rosmarinic acid (0.1248 ~ 0.1546 g/100 g fresh plant), this being the main polyphenolcarboxilic acid in the analysed aromatic plant.

b_3. The content of polyphenols from the extracts (Table 3, columns 6, 7) decreased the order: $S_{2C} > S_{3C} > S_{1C} > S_{2R} > S_5 > S_{1R} > S_4 > S_{3R}$, for oregano K and $S_5 > S_{2C} > S_{2R} > S_{1C} > S_4 > S_{1R} > S_{3C} > S_{3R}$, for oregano M. From the used solvents, methanol proved to contribute very well to recover the polyphenols from aromatic plants, both at high and low temperatures. Higher values were obtained from extractions with water, at high temperature. The complex solvents extract selectively the polyphenols from plants, the values vary in the extract with methanol mixture – acetone – water – formic acid (S_5) between 11.73 g GAE/ 100 g d.p., for oregano M and 2.75 g GAE/ 100 g d.p., for condiment, with the solvent S_4.

Similar values were obtained by [12] for oregano (7.282 g GAE /100 g), the extraction being done with a mixture of acetone – water – acetic acid (70:29,5:0,5). The content of polyphenols relative to the dry substance (fig. 1) varied from 0.78 g GAE / 100 g d.w. (in the extract with ethanol, at low temperature) to 5.39 g GAE / 100 g d.w (in the extract with methanol at high temperature), with an average of 4.35 g GAE / 100 g d.w. for the condiment. For the medicinal plant the values are 1.81 (in the extract with ethanol, at low temperature) and 11.73 g GAE / 100 g d.w. (in the extract S_5), with an average of 8.15 g GAE / 100 g d.w.

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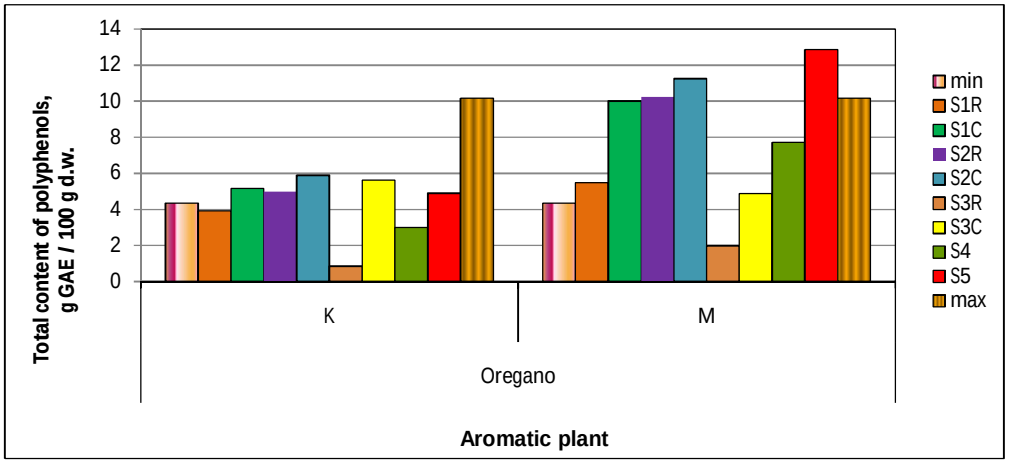

Figure 1 – The content of polyphenols for analysed condiments compared with values from the literature

A comparison between the minimum values obtained by [13] – 4.351 g GAE /100 g d.w., for the extract obtained with a mixture 80:20 acetone–perchloric acid 5% and the highest values obtained by [14] - 10.17 g GAE /100 g d.w., for the extract obtained with methanolic solution 80% demonstrates a higher capacity of extraction for water, methanol and the mixture of solvents S_5.

Taking into account the difference between total polyphenols and polyphenolcarboxilic acids and flavonoids (Table 3, columns 8, 9), it can be observed that higher values are recorded for the medicinal plant (with an average, for all the extractions, of 5.47 g useful compound / 100 g d.p., compared to the condiment, with an average of 2.72 g useful compound / 100 g d.p.). So, the conclusion might be that in these aromatic plants there are significant quantities of other types of polyphenols that were not dosed.

c. Antiradical capacity – it can be observed that both the medicinal plant and the condiment have very high values of scavenger capacity against the free radicals (Table 4). The ethanol solution is remarkable, at reflux temperature (92.43%) for the condiment and complex mixture S_5 (94.09%), for the medicinal plant, but the differences from the extractions with methanol and water, at high temperature are relatively low.

The two samples of oregano have similar values for the majority of the extracts, higher differences being found in the case of complex solvents (81.12%, in S_4, for sample K and 22.42% in S_5, for sample M).

Table 4
The antiradical capacity of the aromatic plants samples

No.	Solvent (sample)	Antiradical capacity, %	
		K	M
1.	S_{1R}	81,16	83,80
2.	S_{1C}	86,47	85,73
3.	S_{2R}	88,09	90,37
4.	S_{2C}	90,85	91,73
5.	S_{3R}	**40,12**	51,41
6.	S_{3C}	**92,43**	89,09
7.	S_4	84,87	**3,75**
8.	S_5	71,67	**94,09**

The obtained values are consistent with those obtained in other studies: the extract obtained with methanol solution 80% showed a 83% scavenger activity against free radicals [15]; the extract with methanol solution 50% showed a 80% scavenger activity against free radicals [16]; the ethanolic extract – 99% [17].

4. Conclusions

The value of concentration in compounds with antioxidant potential (flavonoids, polyphenolcarboxilic acids, total polyphenols) in the extracts of the same vegetal material, obtained with different solvents and at different temperatures varies very much, so the solvents and the working conditions influence significantly the obtained results. Thus:

1. The flavonoids and the polyphenolcarboxilic acids are extracted better with water, at high temperature, but the efficiency is close to the extraction with methanol solution, at reflux temperature and with complex mixture S_5.
2. The polyphenols are extracted very well with methanolic solution, at high temperature, but also with the mixture of solvents methanol – acetone – water – formic acid (S_5).
3. Water and methanol have the capacity of extracting all the three groups of analysed compounds both from the analysed condiment and the medicinal plant.
4. Ethanol reaches valuable extractions at high temperature (hot), especially in the case of the condiment and it is less efficient at low temperature.
5. The mixtures of solvents extract selectively the target compounds, varying a lot from a sample to another. The mixture S_5 was more efficient, especially in the case of medicinal plant.
6. The extraction temperature influences positively the substances extractability when it is higher. A valuable extract from the antioxidant capacity point of view may be obtained at room temperature, although the polyphenolic compounds vary a lot, in relation to the studied sample. The

efficiency of the solvent and the extraction temperature are confirmed by the values obtained for the total extract (the total dry substance).

Comparing the two samples (K and M), one can observe higher values of antioxidant compounds for the medicinal plant in comparison with the condiment.

Oregano – both the condiment, and the medicinal plant – have a high antiradical capacity, depending to a lesser extent on the extraction solvent, but more on the temperature.

5. References

[1] ZHANG, L., RAVIPATI, A. S., KOYYALAMUDI, S. R., JEONG, S., REDDY, N., SMITH, P. T., BARTLETT, J., SHANMUGAM, K., MÜNCH, D., WU, M., Antioxidant and Anti-inflammatory Activities of Selected Medicinal Plants Containing Phenolic and Flavonoid compounds, J Agric Food Chem, 2011, vol. 59, p. 12361–12367;

[2] FRANZ, CH., BAUER, R., CARLE, R., TEDESCO, D., TUBARO, A., ZITTERL-EGLSEER, K., Study on the assessment of plants/herbs, plant/herb extracts and their naturally or synthetically produced components as "additives" for use in animal production, available at http://www.efsa.europa.eu /en/supporting/pub /070828.htm, 2009;

[3] GOZE, I., ALIM, A., TEPE, A. S., SOKMEN, M., SEVGI, K., TEPE, B., Screening of the antioxidant activity of essential oil and various extracts of *Origanum rotundifolium Boiss*. From Turkey, Journal of Med. Plants Research, 2009, vol. 3, p. 246-254;

[4] CAILLET, S., YU, H., LESSARD, S., LAMOUREUX, G., AJDUKOVIC, D., LACROIX, M., Fenton reaction applied for screening natural antioxidants, Food Chemistry, 2007, vol. 100, p. 542–545;

[5] DUKE, JIM, Phytochemical and Ethnobotanical Databases, USDA, ARS, National Genetic Resources Program, 2013, available at http://www.ars-grin.gov/duke/;

[6] JIMENEZ-ALVAREZ, D., GIUFFRIDA, F., GOLAY, P. A., COTTING, C., LARDEAU. A., KEELY, B. J., Antioxidant Activity of Oregano, Parsley, and Olive Mill Wastewaters in Bulk Oils and Oil-in-Water Emulsions Enriched in Fish Oil, J. Agric. Food Chem., 2008**,** vol. 56 (16), p. 151–7159;

[7] GÜLÇIN, I, Antioxidant activity of food constituents: an overview, Arch Toxicol, 2012, vol. 86, p. 345–391;

[8] MATERSKA, M., 2010, Evaluation of the lipophilicity and stability of phenolic compounds in herbal extracts, Acta Sci. Pol., Technol. Aliment, vol. 9, p. 61-69;

[9] GRAYER, R. J., VIEIRA, R. F., PRICE, A. M., KITE, G. C., SIMON, J. E., PATON, A. J., Characterization of cultivars within species of Ocimum by exudate flavonoid profiles, Biochemical Systematics and Ecology, 2004, vol. 32, p. 901-913;

[10] HAMINIUK, C., MACIEL, G., PLATA-OVIEDO, M., PERALTA, R., Phenolic compounds in fruits – an overview, International Journal of Food Science & Technology, 2012, vol. 47, p. 2023–2044;

[11] KRUMA, Z., M. ANDJELKOVIC, R. VERHE, V. KREICBERGS, Phenolic compounds în basil, oregano and thyme, Baltic Conference on Food Science and Technology, FOODBALT-2008, 3, Jelgava (Latvia), available at http://llufb.llu.lv/conference/foodbalt/2008/Foodbalt-Proceedings-2008-99-103.pdf;

[12] WU, X., BEECHER, G. R., HOLDEN, J. M., HAYTOWITZ, D. B., GEBHARDT, S. E., PRIOR, R. L. L., Lipophilic and hydrophilic antioxidant capacities of common foods în the United States, J. Agric. Food Chem., 2004, vol. 52, p. 4026-4037;

[13] NINFALI, P., MEA, G., GIORGINI, S., ROCCHI, M., BACCHIOCCA, M., Antioxidant capacity of vegetables, spices and dressings relevant to nutrition, British Journal of Nutrition, 2005, vol. 93, p. 257–266;

[14] SHAN, B., CAI, Y. Z., SUN, M., CORKE, H., Antioxidant Capacity of 26 Spice Extracts and Characterization of Their Phenolic Constituents , J. Agric. Food Chem., 2005, vol. 53 (20), pp 7749–7759;

[15] CAPECKA, E., MARECZEK, A. LEJA, M., Antioxidant activity of fresh and dry herbs of some Lamiaceae species, Food Chemistry, 2005, vol. 93, p. 223-226;

[16] CERVATO, G., CARABELLI, M., GERVASIO, S., CITTERA, A., CAZZOLA, R., AND CESTARO, B., Antioxidant properties of oregano (Origanum vulgare) leaf extracts, Journal of Food Biochemistry, 2000, vol. 24, p. 453-465;

[17] EXARCHOU, V., NENADIS, N., TSIMIDOU, M., GEROTHANASSIS, I. P., TROGANIS, A. & BOSKOU, D, Antioxidant activities and phenolic composition of extracts from Greek oregano Greek sage, and summer savory, Journal of Agricultural and Food Chemistry, 2002, vol. 50, p. 5294–5299.