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EVALUATING ANTIOXIDANT CAPACITY AND BIOLOGICALLY ACTIVE CAPACITY FROM THYME

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Abstract
The study is to determine the content of flavonoids, polyphenolcarboxylic acids, total polyphenols and antioxidant capacity of dried thyme used in food. Investigations were performed on extracts obtained from hot and cold water solutions, methanol, ethanol and various mixtures of solvents. Data analysis shows that bioactive substances are well represented in the thyme, which may explain the remarkable antioxidant capacity observed and the interest of introducing this particular plant when creating foods with biological potential.
In recent years, antioxidant substances from plants are of interest to researchers, producers and consumers. Good sources of antioxidants are fresh fruit and vegetables, whole grains, due to the intake of vitamins, bioflavonoids, and components with antiradical potential. Many spices are the sources of phenolic compounds with an antioxidant capacity superior to that of fruit and cereals. They also have been recognized as having digestive stimulant action, carminative action, antimicrobial, anti-inflammatory, anti-mutagenic, anti-carcinogenic potential etc. Numerous studies have been published on the antioxidant capacity and the phenolic constituents of spices.
The purpose of the study is to assess the antioxidant capacity and phenolic compounds present in thyme using different extraction methods described in literature.
The choice of raw materials to obtain spices should be given special attention in its chemical compounds content which leads to a high antioxidant potential. Thus, it is possible to increase dietary intake of antioxidants with beneficial health effects.
Keywords: spices, antioxidant capacity, phenolic compounds

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1. INTRODUCTION

In last years, antioxidant substances from plants are of interest to researchers, producers and consumers. Good sources of antioxidants are fresh fruit and vegetables, whole grains, due to the intake of vitamins, bioflavonoids, and components with antiradical potential.
Many spices are the sources of phenolic compounds with an antioxidant capacity superior to that of fruit and cereals (Lu, M. et al, 2010). Concentrated in just a few grams of material, they may represent the simplest way to increase the content of phenolic compounds and antioxidant capacity of a daily diet, with potential health benefits (Suhaj, M., 2006).
Numerous studies have been published on the antioxidant capacity and the phenolic constituents of spices (Kaefer, C. & Milner, J., 2008). Among the factors leading to differences in composition and antioxidant activity of spices used for cooking, may be mentioned: the genetic factors, the degree of maturity of plants, cultivation techniques, post-harvest handling, storage conditions, methods applied for conditioning, processing techniques adopted etc. (Cai, Y., et al , 2004; Shan, B. et al 2005).
Thyme (*Thymus vulgaris*), one of the most popular plants, a 'bridge' between medicine, food and tradition, is an annual plant of the Lamiaceae family, with a taste/flavour of sweet-spicy, spicy-hot. It has various used, both culinary obtained for flavouring and seasoning (salads, sauces, fish dishes) and in medicine (being used as a remedy for many diseases: intestinal colic, intestinal bloating, acute and chronic headaches, stomach ulcer, urinary infections, anorexia, diarrhea, colitis fermentation).
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present in thyme using different extraction methods described in literature.

2. MATERIALS AND METHODS

For comparison purposes, qualitative and quantitative chemical study was performed on extracts from two samples of thyme: a sample consisting of thyme by market for seasoning (sample K), and a sample consisting of thyme processed for medicinal purposes (sample M).
Due to the fact that the way extraction is made decisively influences the results and gives a more complete perspective on the content of the analyzed compounds, extraction conditions were varied, working both at room temperature and reflux temperature, with several types of extraction solvents, met and recommended by the literature:
- water, cold (S_{1R}) and hot (S_{1C});
- methanol solution (50:50, v/v), cold (S_{2R}) and hot (S_{2C});
- with ethanol at 96°, cold (S_{3R}) and hot (S_{3C}); - with a mixture of methanol-water-acetic acid (90:9:1, v/v/v), cold (S4);
- with a mixture of methanol-acetone-water-formic acid (40:40:19, 9:0, 1, v/v/v/v), cold (S_5).
Thus, eight samples for each type of plant (spice – medicinal plant) were analyzed: five extracted hot and three extracted cold. They were obtained by leaching the plant material with an appropriate solvent. For this, each 2g of dried aerial part of plant were extracted with 20mL solvent (ratio of plant product/solvent of 1/10), hot (reflux temperature) and cold (room temperature). Extraction solutions were filtered and were analyzed in terms of total flavone content (expressed in rutoside) and total polyphenols (as gallic acid) and antiradical capacity.
For qualitative analysis of active principles from the obtained extracts the method of thin layer chromatography (TLC) was used. Reference solutions that were used are rutoside – chlorogenic acid – caffeic acid (E1) and rosmarinic acid (E2). The quantitative chemical study sought to quantify using spectrophotometry the biologically active compounds (flavonoids polyphenolcarboxylic acids, total polyphenols) of the analyzed samples, knowing that spicy aromatic plants contribute substantially to the shaping of antioxidant activity. A spectrophotometric method was used in visible and ultraviolet.
Total flavones content was expressed in g rutoside/100g dried plant. Content of polyphenolcarboxylic acids was expressed as g rozmarinic acid/100d dried plant product. Total polyphenol content was expressed as gallic acid equivalents (GAE). Determination of antiradical capacity was performed by measuring the ability of neutralizing radical 2,2-diphenyl-1-picrilhidrazil (DPPH) and transforming it in a reduced form by the plant extracts examined. Results were expressed as percentage inhibition of DPPH site.
To obtain these determination were used: spectrophotometers UV-VIS CARY 50, CECIL 2020, CINTRA 101, V-550 Jasco, applicator: CAMAG LINOMAT IV; G60F254 Merck silica gel HPTLC plates (100 x 100, 200 x 100).

3. RESULTS AND DISCUSSION

Cromatograms were viewed in four stages: viewing at 254 nm, before spraying with identification reagent. The purpose of this analysis was to identify the presence of polyphenolic antioxidant compound types; viewing at 366 nm before spraying with identification reagent; viewing at 366 nm after spraying with identification reagent, when the main types of flavonoids are highlighted in spots by position and size; view after spraying with DPPH reagent in order to identify any antiradical capacity.
Chemical qualitative study conducted by thin layer chromatography showed that, depending on the extraction solvent and extraction conditions (temperature, extraction technique), the obtained extractive solutions have a varied content of phenolic substances (flavonoids and polyphenols), responsible for the antioxidant action of the plants studied.
Comparing chromatoplates' appearance corresponding to K and M samples shows the

following: - content of flavonoid compounds in the K sample is lower than that in the M sample, although the amount and type of polyphenolcarboxylic acids are comparable; antioxidant activity is attributed mostly to polyphenolcarboxylic acids; extracts obtained with water, respectively alcohol 96°, by stirring at room temperature are significantly lower than those obtained using the same solvent extraction, but at reflux temperature; optimal extraction of active ingredients with antioxidant activity is provided by the mixture of methanol/water (50:50) at reflux temperature.

The results of spectrophotometric determinations are summarized in Table 1. As a first observation we concur that K sample's hot methanol extraction solution is the most complete. At the opposite pole stands cold ethanol extraction, the value being almost four times less than the maximum. The content of flavonoids in the extracts decreases in the order: methanol, hot> water, hot> methanol-acetone-water-formic acid > ethanol, hot> methanol, cold water-methanol-acetic acid > ethanol at cold water cooling. The M sample we can observe a different order for lower values.

If one compares the recorded values for flavonoids determination in tinctures (extracts from cold), it appears that they are much lower than extracts made at reflux temperature. This is evidence that flavonoids have increasingly better temperature extractability. Modest results were obtained for complex solvent extractions (S_4 and S_5). Comparing the two samples, the plant-spice has higher values for most types of extractions (seven out of eight).

Total polyphenol concentrations are consistent with the literature, with one exception: the ethanol extract, cold (Table 2). It can be noted the wide range of polyphenol content of thyme in the literature data (the difference between the maximum and minimum being 1,9 g GAE/100 s.u.), because the tests were conducted on different species of plants, some used as spices (mainly due to the contribution of volatile oil), others for medicinal purposes (due to complex bioactive), differing also in how the extraction is carried out.

Table 1. Quantitative determination of active biological substances in thyme extracts

Crt. No.	Sample	Flavonoids [g rutoside/ 100g s.u.]		Poliphenolcarbo -xylic acids [g rosmarinic acid/ 100 g s.u.]		Poliphenols [g GAE/ 100 g s.u.]	
		K	**M**	**K**	**M**	**K**	**M**
1.	S_{1R}	0,29	0,11	0,61	0,12	4,44	1,33
2.	S_{1C}	0,95	0,28	2,06	0,48	4,14	4,55
3.	S_{2R}	1,09	0,14	1,66	0,19	7,29	2,36
4.	S_{2C}	1,46	0,35	2,06	0,53	3,63	4,20
5.	S_{3R}	0,15	0,03	0,22	0,04	2,41	0,43
6.	S_{3C}	0,12	0,22	0,43	0,34	7,60	3,52
7.	S_4	0,60	0,10	1,71	0,11	6,43	1,63
8.	S_5	0,81	0,13	1,26	0,20	5,09	2,02

Table 2. Comparison of polyphenol content in the analyzed samples with data from literature

Sample	Polyphenol content of analyzed samples [g GAE/ 100 g s.u]		Literature data [g GAE/ 100 g s.u]	
	Minimum	Maximum	Minimum	Maximum
1	2,2	6,93	0,55	2,45
2	0,39	4,17		

The analysis showed that the sample obtained by cold extraction with methanol solution was most effective in removing the DPPH radicals (Table 3). The values obtained in the determination of antioxidant capacity are considerable for many extractions.

It can also be noted that high values of antioxidant capacity were obtained for complex solvent extracts, i.e. extracts from cold, although the values obtained for polyphenolic compounds have overwhelmingly modest values.

Table 3. Antioxidant activity of thyme extracts

Crt. No.	Solvent (sample)	% antioxidant activity against DPPH	
		K	M
1.	S_{1R}	75,86	48,56
2.	S_{1C}	88,69	85,35
3.	S_{2R}	90,83	83,07
4.	S_{2C}	91,22	88,43
5.	S_{3R}	53,38	24,09
6.	S_{3C}	92,98	83,52
7.	S_4	93,81	41,20
8.	S_5	89,84	57,74

4. CONCLUSION

Results of the study point out that aromatic plant have a considerable amount of polyphenolic compounds and a high antiradical activity, while the most effective method of extracting biologically active compounds was the extraction with 50% methanol solution, hot. The choice of raw materials to obtain spices should be given special attention in its chemical compounds content which leads to a high antioxidant potential.

The analyzed bioactive compounds are well represented in the thyme, but identifying them requires selection of optimal extraction conditions. In general, the principle of determination and analysis technique used influences the results obtained in determining antioxidant activity and compounds with antioxidant potential.

Thus, it is possible to increase dietary intake of antioxidants with beneficial health effects.

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