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COMPARATIVE STUDY ON THE POLYPHENOL CONTENT FROM COMMONLY USED NATURAL PRODUCTS USING UV-VIS SPECTROSCOPY

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Abstract: *In this work we aimed to determine flavonoids, polyphenol and total phenolic acids in eight commercially available natural products: Tiliae flos, Chamomillae flos, Salviae folium, Crataegi flos, folium et fructus, Menthae folium, Hyperici herba, Millefolii herba, Cynarae folium. In oder to compare them, for each product there have been prepared three samples: alcoholic extract, infusion according to Romanian Pharmacopoeia 10th edition, infusion as indicated on the packaging. The results showed that the method of preparation has a major influence on obtaining the active principles needed for a therapeutic effect.*

Key words: *antioxidant agents, medicinal plants, spectrophotometry.*

1. Introduction

Polyphenolic compounds are the largest and most common group of secondary metabolites found in plants. Polyphenols usually found in plant materials can be divided in three groups: simple phenols, hydroxycinnamic acid derivatives and flavonoids [20].

Polyphenols have numerous biological effects, including antioxidant action by the scavenger activity of hydroxyl, peroxyl and superoxide radicals. Administration of flavonoids inhibits cardiovascular diseases and cancer risk [4], have anti-inflammatory, diuretic, anti-gout hepatoprotective and antiviral activity [17].

Polyphenolic compounds are widely distributed in plant species, especially in vegetables and fruits. There are, however, few data regarding the polyphenol content of medicinal plants [10], [11]. As a result, the present study focuses on the quantification of polyphenolic substances in Romanian medicinal plants.

In this work we aimed to determine flavonoids, polyphenol carboxylic acids and the total phenolic content from natural products frequently used in daily life as traditional remedies.

The studied plants are part of the families: *Tiliaceae* (*Tilia cordata* - linden), *Asteraceae* (*Chamomilla recutita* – chamomile, *Achillea millefolium* – yarrow, *Cynara scolymus* - artichoke), *Lamiaceae* (*Salvia officinalis* - sage, *Mentha piperita*

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– peppermint) *Rosaceae* (*Crataegus monogyna* – hawthorn), *Hypericaceae* (*Hypericum perforatum* – St. John's wort).

These plants are commonly used in Romanian households for different conditions, such as: digestive disorders (*Mentha piperita*), infections and inflammations (*Chamomilla recutita*), infections and inflammations of the oral cavity (*Salvia officinalis*), gastrointestinal disorders, wounds and inflammations, dysmenorrhea (*Achillea millefolium*), cardio-vascular diseases and anxiety (*Crataegus monogyna*), insomnia, common colds, cough, fever, flu (*Tilia cordata*), depression, wounds, burns, ulcers, biliary dyskinesia (*Hypericum perforatum*), hepato-biliary disorders (*Cynara scolymus*) [9].

In order to make a comparison of the actual level of the examined compounds obtained by alcoholic extraction, and the compounds obtained by the use of the usual preparations containing them, for each product three tests were performed: alcoholic extract, infusion according to Romanian Pharmacopoeia 10th edition (RP 10^{th} ed.), infusion as indicated on the packaging.

2. Material and methods

2.1. Samples

The analysed samples were purchased from the local markets and include the following items: *Tiliae flos, Chamomillae flos, Salviae folium, Crataegi flos, folium et fructus, Menthae folium, Hyperici herba, Millefolii herba, Cynarae folium*. All the samples are available in packs of 50 g and have the same year of production: 2013.

2.2. Sample preparation

For alcoholic extracts: 10 g of powdered plant material were extracted with 100 ml of an aqueous solution of ethanol 70% (v/v) at 40^0C for 1 hour. The extracts were filtered, the filter was washed with the same solvent and brought in 100 ml flasks.

For the aqueous extracts two methods were used: the first method prescribed by RP 10^{th} ed. [6]: 6 g of powdered plant material, except chamomile - 3 of powdered plant material is infused and left in contact for 30 minutes, after which the extracts were filtered and filled up to 100 g by rinsing with water. The second method of infusion was carried out in compliance with the instructions written on each pack of product.

2.3. Apparatus and reagents

The measurements were carried out using a V-530 Jasco UV-VIS spectrophotometer. All materials and reagents were of analytical grade.

2.4. Determination of total phenolic content

The total phenolic content in plant extracts was estimated by the Folin-Ciocalteu method described by Singleton [14], [15], [19]. 2 ml of the diluted plant extract was mixed with 10 ml of a 1:10 dilution of Folin-Ciocalteu reagent. After a period of time ranging from 30 seconds to 8 minutes, 8 ml of 7.5% Na_2CO_3 solution was added. The absorbance was determined after a reaction time of 2 h at 20^0 C, compared to a control blank (water plus reagent) with absorbance 0. The intensity of the blue color obtained was measured at a wavelength of 760 nm. The total concentration of phenolic compounds in the extract was calculated by comparison with a calibration curve prepared with gallic acid in the same way with concentrations between 0 and 500 μg/100 ml. The total phenolic content in the sample was expressed as gallic acid equivalents = amount (mg) of gallic acid found in a gram of dried plant material.

2.5. Determination of flavonoids

Flavonoids were determined by the spectrophotometric method officialised by RP 10^{th} ed., based on the reaction of aluminum chloride [6]. For 5 ml of sample there were added 5 ml of sodium acetate 100 g/l and 3 mL of aluminum chloride 25 g/l, and then it was brought up to a 25 ml

volumetric flask using ethanol. After 15 minutes the absorbance was determined at 470 nm using a liquid with a compensation liquid obtained similarly, but the reagents were replaced with 8 ml of water. Flavonoid concentration was calculated using a calibration curve prepared in parallel and in the same conditions as the samples obtained from a standard solution of rutoside.

2.6. Determination of polyphenol carboxylic acids

To determine the polyphenol carboxylic acids a method officialised by the European Pharmacopoeia 7th edition was used [5]. To 1 ml sample, there were added 2 ml of 0.5 M hydrochloric acid, 2 ml of a solution prepared by dissolving 10 g of sodium nitrite (R) and 10 g of sodium molybdate (R) in 100 ml water (R). Then 2 ml of 8.5% sodium hydroxide solution were added, and then brought to 10 ml using water (R). The absorbance was immediately measured at 525 nm, using a solution of 1 ml sample diluted with 1 ml water (R) as a compensation liquid. The results were expressed in chlorogenic acid.

3. Results and Discussions

Total phenolic content was expressed in mg gallic acid/g dry plant (Table 1), the amount of flavonoids was expressed in mg rutoside/g dry plant (Table 2) and the hydroxy cinnamic acids in mg chlorogenic acid/g dry plant (Table 3) in alcoholic and aqueous extracts.

Table 1

Total phenolic content expressed in mg/g dry plant in aqueous and alcoholic extracts

Sample	Total phenols		
	Alcoholic extract	Aqueous extract RP 10th ed.	Aqueous extract package
Tiliae flos	25.98	7.23	5.16
Chamomillae flos	19.71	2.75	2.70
Salviae folium	31.88	12.16	4.85
Crataegi flos, folium et fructus	35.40	9.11	5.68
Menthae folium	24.96	11.99	5.22
Hyperici herba	47.73	12.88	6.94
Millefolii herba	35.86	7.38	4.86
Cynarae folium	43.98	5.94	3.03

Table 2

Flavonoid content expressed in mg/g dry plant in aqueous and alcoholic extracts

Sample	Flavonoids		
	Alcoholic extract	Aqueous extract RP 10th ed.	Aqueous extract package
Tiliae flos	5.22	2.07	1.73
Chamomillae flos	4.25	1.95	1.62
Salviae folium	3.53	2.63	1.98
Crataegi flos, folium et fructus	3.26	2.2	2.08
Menthae folium	7.6	2.85	1.94
Hyperici herba	3.5	2.5	2.39
Millefolii herba	11.23	3.06	1.7
Cynarae folium	5.82	4.4	1.43

As can be seen from the previous tables, the amount of total polyphenols varied between 19.71 – 47.73 mg/g in alcoholic extracts, between 2.75 –12.88 mg/g in extracts obtained according to RP 10th ed. and between 2.70 – 6.94 mg/g in extracts

obtained according to package instructions.

Flavonoids varied between 3.26 - 11.23 mg/g in alcoholic extracts, between 1.95 – 4.4 mg/g in extracts obtained according to RP 10th ed. and between 1.43 – 2.39 mg/g in extracts obtained according to package instructions.

Hydroxy cinnamic acid content expressed in mg/g dry plant in aqueous and alcoholic extracts Table 3

Sample	Hydroxy cinnamic acids		
	Alcoholic extract	**Aqueous extract RP 10th ed.**	**Aqueous extract package**
Tiliae flos	17.82	5.15	3.01
Chamomillae flos	7.16	1.05	0.5
Salviae folium	27.56	8.28	2.13
Crataegi flos, folium et fructus	17.23	6.33	3.15
Menthae folium	14.86	8.60	3.06
Hyperici herba	36.1	9.05	3.75
Millefolii herba	13.22	3.54	1.54
Cynarae folium	8	0.9	0.8

Hydroxy cinnamic acid content was between 8 – 36.1 mg/g in alcoholic extracts, between 0.5 – 9.05 mg/g in extracts obtained according to RP 10th ed. and between 0.8 – 3.75 mg/g in extracts obtained according to package instructions.

The amounts of total polyphenols found in alcoholic extracts are comparable to those reported in the literature: *Chamomillae flos* –23.2 mg/g in 80% ethanol [1] or 36.79 mg/g in 50% ethanol [8]; *Tiliae flos* –14 mg/g in methanol extract [3], *Salviae folium* - 81.2 mg/g in 70% ethanol [12], *Crataegus monogyna* - between 28.3 and 114.3 mg/g in methanol extract [13], *Cynarae folium* - 50.5 mg/g in 75% ethanol extract [18], *Menthae folium* - 41.1 mg/g in 70% ethanol extract [2].

Regarding the total content of flavonoids, our results are similar to those reported by other authors, with differences related to the type of extract and standard substance used: *Salviae folium* - 2.56 mg/g in 70% ethanol extract [12]; *Menthae folium* - between 8.4 and 8.8 mg/g in 50% ethanol extract related to izocvercitrin [7]; *Chamomillae flos* – 9.48 mg/g in 50% ethanol extract related to luteolin-7-glucoside [8], *Millefolii herba* - between 13.7 and 39.7 mg/g related to apigenol [16].

The literature is poorer in data regarding the total content of hydroxy cinnamic acids in medicinal plants: *Menthae folium* - between 8 and 9.3 mg/g in 50% ethanol extract related to rosmarinic acid [2], [7]; *Chamomillae flos* -15.89 mg/g in 50% ethanol extract, expressed as chlorogenic acid [8]. The data reported are similar to the results of this study.

In the three types of analysed extracts, the highest amount of polyphenols was found in St. John's wort and the lowest in chamomile. Note that with respect to the total phenols, the extraction method strongly influences the concentration of the active ingredients, which were drastically reduced in aqueous extracts compared to the alcoholic ones and also the extract prepared according to package instructions compared with the infusion according to the Pharmacopoeia. Exceptions are the chamomile flowers whose aqueous extracts contain the same amount of polyphenolic compounds.

The total flavones seemed to be the least influenced by the method of extraction. Alcoholic extracts contain the highest amount of flavonoids, but aqueous extracts have comparable amounts of active substances.

4. Conclusions

For each plant, the values obtained from alcoholic extracts were higher than the values obtained from extracts prepared according to the RP 10^{th} ed. and as indicated on the package. Medicinal plants are important sources of antioxidant compounds, useful in preventing diseases mediated by free radicals, but the concentration of these compounds in preparations commonly consumed is quite low, which limits their effectiveness.

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References

1. Al Bahtiti N.H., 2012. Chemical Analysis and Biological Activity of Jordanian Chamomile Extracts. In: Advance J Food Sci Technol, 4(1): 22-25.
2. Aprotosoaie A.C., Raileanu E., 2013. The polyphenolic content of common Lamiaceae species available as herbal tea products in Romanian pharmacies. In: Rev. Med. Chir. Soc. Med. Nat., 117(1): 233-237.
3. Demiray S., Pintado M.E. et al., 2009. Evaluation of phenolic profiles and antioxidant activities of Turkish medicinal plants: Tilia argentea, Crataegi folium leaves and Polygonum bistorta roots. WASET, 30: 307-312.
4. Duthie G.G., Duthie S.J. et al., 2000. Plant polyphenols in cancer and heart disease: implications as nutritional antioxidants. In: Nutr. Res. Rev., 13: 79-106.
5. Fialova S., Tekeľová D. et al., 2008. The determination of phenolics compounds and antioxidant activity of mints and balms cultivated in Slovakia. In: Acta Fac. Pharm. Univ. Comen., 55: 96-102.
6. Fraisse D., Felgines C. et al., 2011. Caffeoyl Derivatives: Major Antioxidant Compounds of Some Wild Herbs of the Asteraceae Family. In: Food Nutr Sci, 2: 181-192.
7. Grigorescu E., Lazăr M.I. et al., 2001. Phytotherapeutic index. Imprint Lithography, Iasi.
8. Necula R., Aprotosoaie C. et al., 2011. Contributions to the study of the biochemical variability of the polyphenols existing in 8 Thymus pulegioides populations from northern Moldavia. In: Bulletin of the Transilvania University of Brasov, Vol. 4(53), Series VI, No. 2: 15-22.
9. Pogacnik L., Poklar U.N., 2011. Determination of antioxidants in medicinal herbs. Bulletin of the Transilvania University of Brasov, Vol. 4(53), Series VI, No. 2 : 97-102.
10. Ramu G., Krishna M.G., et al., 2012. Preliminary phytochemical and antioxidant study of hydroalcoholic extracts from selected genera of Indian Lamiaceae. In: Asian Pacific J Trop Biomed, p. 685-688.
11. Simirgiotis M.J., 2013. Antioxidant Capacity and HPLC-DAD-MS Profiling of Chilean Peumo (Cryptocarya alba) Fruits and Comparison with German Peumo (Crataegus monogyna) from Southern Chile. Molecules, 18: 2061-2080.

12. Singleton V.L., Orthofer R. et al., 1999. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. In: Methods Enzymol, 299:152-178.
13. Singleton V.L., Ross J.A., 1965. Colorimetry of total phenolics with phosphomolibdic-phosphotungstic acid reagents. In: Am J Enol Vitic, 37: 144-158.
14. Spinarova S., Petrikova K., 2003. Variability of the content and quality of some active substances within Achillea millefolium complex. In: Hort Sci, 30(1): 7-13.
15. Stănescu U., Hăncianu M. et al., 2004. Medicinal plants from A to Z; Monographs of drugs with therapeutical uses, Vol. 2 (in Romanian). Gr. T. Popa Publishing House, Iaşi.
16. Vamanu E., Vamanu A. et al., 2011. Antioxidant and Antimicrobial Activities of Ethanol Extracts of Cynara Scolymus (Cynarae folium, Asteraceae Family). In: Trop J Pharm Res, 10(6): 777-783.
17. Waterhouse A.L., 2001. Current Protocols in Food Analytical Chemistry. Determination of total phenolics. John Wiley & Sons, New York.
18. Whiting D.A., 2001. Natural phenolic compounds 1900-2000: a bird's eye view of a century's chemistry. In: Nat Prod Rep, 18: 583-606.
19. *** European Pharmacopoeia, 7th edition.
20. *** Romanian Pharmacopoeia 10th edition.