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COMPARISON OF LIQUID AND LYOPHILIZED SERPYLLI HERBA WASTE EXTRACTS PREPARED AT DIFFERENT pH VALUES

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Abstract

Polyphenol recovery and physico-chemical properties of the extracts depend on the type of solvents and their pH values. Additionally, due to the presence of different biologically active compounds in plant waste, plant extracts obtained from industrial by-processing can find potential applications in various products. In the present study, Serpylli herba waste extracts were prepared using maceration (60 min), a solid-to-solvent ratio of 1:30, the particle size of plant waste 0.3 mm, and two types of the extraction medium: 50% ethanol (pH 6) and 50% ethanol with glacial acetic acid (pH 2.5). The lyophilization process was chosen as the next step (-75°C, 0.011 mbar, for 24 h). Comparison of liquid and lyophilized extracts prepared at different pH values was done *via* analyzing total polyphenol content (TPC, Folin-Ciocalteu method), total flavonoid content (TFC, colorimetric assay), antioxidant capacity (ABTS and DPPH assays), zeta potential, and conductivity (photon correlation spectroscopy). TPC of liquid extracts prepared at pH 2.5 and pH 6 amounted to 1.38 and 1.23 mg GAE/mL, respectively, while lyophilized parallels had 271.7 and 188.8 mg GAE/g, respectively. The same trend is noticed in the case of TFC: 0.368 and 0.334 mg CE/mL for liquid extracts obtained at pH 2.5 and pH 6, respectively, and 102.5 and 99.7 mg CE/mL for lyophilized parallels. ABTS radical scavenging activity of the liquid extracts at pH 2.5 and pH 6 was 0.767 and 0.750 mmol TE/L, respectively and for the lyophilized parallels was 0.136 and 0.111 mmol TE/g. IC_{50} (concentration for neutralization of 50% of DPPH free radicals) was 1.12 and 1.75 mg/mL for the liquid extracts prepared at pH 2.5 and pH 6, respectively and 0.331 and 0.391 mg/mL for the lyophilized parallels. The zeta potential of the liquid extracts at pH 2.5 and pH 6 was 1.74 and -2.56 mV, respectively, whereas the zeta potential of lyophilized parallels was -3.55 and -18.7 mV. Conductivity of the liquid extracts was 0.864

(pH 2.5) and 0.423 mS/cm (pH 6), whereas for the lyophilized extracts it was 0.199 (pH 2.5) and 0.452 mS/cm (pH 6). The presented results provide the information on physico-chemical properties of Serpylli herba waste liquid and lyophilized extracts that can add value and improve the quality of the existing food, functional food, pharmaceutical and cosmetic products, as well as for drinking water and wastewater treatment.

Keywords: lyophilization, polyphenols, Serpylli herba, waste, zeta potential

1. Introduction

The Lamiaceae family consists primarily of herbs and shrubs, with approximately 200 genera and 3200 species, and is distinguished by aromatic herbage, quadrangular stems, and verticillate inflorescences. *Thymus serpyllum* L. (creeping thyme, wild thyme, or mother of thyme) is thyme species from the Lamiaceae family, which grows in almost all the countries bordering the Mediterranean and is extensively cultivated in Central Europe, Asia, and the United States (1). According to the literature (2, 3), Serpylli herba contains a wide range of active compounds, such as essential oil (0.4-2.3%), flavonoids (luteolin-7-O-glucuronide, luteolin-7-O-glucoside, luteolin-7-O-rutinoside, apigenin, etc.), and phenolic acids (rosmarinic, syringic, vanilic, chlorogenic, p-coumaric, and caffeic acids). The all mentioned compounds could be responsible for Serpylli herba antioxidant, antimicrobial, anthelmintic, carminative, expectorant, analgesic, stimulant, diaphoretic, antispasmodic, diuretic, and anti-inflammatory properties (1, 4-6).

Plant waste, such as tea dust, banana peel, lemon skin grape, olive, apple, and carrot pomace, contains various biologically active components that can find potential applications in food, pharmaceutical, and cosmetic industries (6-9). There is the possibility to use dust particles for the isolation of active components that can be used as natural additives in food products and ingredients in pharmaceutical and cosmetic preparations (10). Tea waste of Serpylli herba contains essential oil, polyphenols, monoterpenes, polysaccharides, and proteins (5, 11, 12).

The pH of the extraction solvent is an important factor that influences the solubility of the target components. Herbal sources contain various polyphenol compounds that have different solubility at different pH values (13). The ethanolic plant extracts can be used in different industry sectors, due to their activity against different hazards and human pathogens, incorporation into different carriers, and the presence of various biologically active compounds (6, 14-16). Nevertheless, bioactive components in the liquid extracts are susceptible to chemical and physical degradation. Therefore, one of the most used drying techniques is lyophilization which uses freezing and low pressure to obtain a dried formulation. Lyophilized samples are stable over long periods due to the prevention of hydrolytic and oxidative reactions toward the bioactive and non-active substances during storage (10, 17).

Chemical characterization of ethanol and lyophilized extracts of Serpylli herba waste included determination of total polyphenol and flavonoid contents (TPC and TFC, respectively) and radical scavenging activity (ABTS and DPPH assays), whereas analyzed physical properties were conductivity and zeta potential.

2. Experimental

2.1. Plant material and reagents

Dried and grinded Serpylli herba (particle size 0.3 mm, herbal dust or waste) was from the Institute for Medicinal Plants Research ”Dr Josif Pančić”, Pančevo, Serbia. The following reagents of the analytical purity grade were used: ethanol and sodium carbonate (Fisher Scientific, UK), Folin-Ciocalteu reagent, gallic acid and glacial acetic acid (Merck, Germany), sodium nitrite (Alkaloid, Macedonia), potassium persulfate (Centrohem, Serbia), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) - ABTS, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid - Trolox, and 2,2-diphenyl-1-picrylhydrazyl - DPPH (Sigma-Aldrich, USA).

2.2. Preparation and lyophilization of the extracts

Serpylli herba waste extracts were prepared using maceration (60 min) in the shaker (KS 4000i control, IKA, Germany), a solid-to-solvent ratio of 1:30, the particle size of plant waste 0.3 mm, and two types of extraction medium: 50% ethanol (pH 6) and 50% ethanol with glacial acetic acid (pH 2.5).

Before the lyophilization, ethanol was removed from the extracts at 50 mbar and 50°C for 30 min, using Heizbad Hei-VAP rotary evaporator (Heidolph, Germany). Subsequently, the samples were lyophilized (-75˚C, 0.011 mbar, for 24 h) in Beta 2-8 LD plus lyophilizator (Christ, Germany).

2.3. Determination of total polyphenol and flavonoids content

Total polyphenol content (TPC) was determined spectrophotometrically using the modified Folin-Ciocalteu method (12), while total flavonoid content (TFC) was evaluated by a colorimetric assay described by Barros et al. (18). TPC was expressed as milligram of galic acid equivalent per milliliter of liquid extract or per gram of lyophilized extract (mg GAE/mL or mg GAE/g, respectively). TFC was expressed as milligram of catechin equivalent per milliliter of liquid extract or per gram of lyophilized extract (mg CE/mL or mg CE/g, respectively).

2.4. Determination of ABTS and DPPH radical scavenging activity

The ABTS and DPPH assays are based on the reduction of free radicals in alcohol solution by polyphenols (12). ABTS radical scavenging capacity was expressed as mmol Trolox equivalents per milliliter of liquid extract or per gram of lyophilized extract (mmol TE/mL or mmol TE/g, respectively). DPPH radical scavenging capacity was expressed as the concentration of the extract required to scavenge 50% of free radicals, IC50 (mg/mL). All absorbance readings were performed on UV spectrophotometer, UV-1800 (Shimadzu, Japan).

2.5. Determination of zeta potential and conductivity

The Zeta potential (mV) and conductivity (mS/cm) of the obtained extracts were determined by photon correlation spectroscopy (PCS) in Zetasizer Nano Series, Nano ZS (Malvern Instruments Ltd., UK). Each sample was measured three times at room temperature. Lyophilized samples were diluted before the analysis.

2.6. Statistical analysis

The analysis of variance (one-way ANOVA) followed by Duncan's post hoc test (STATISTICA 7.0) was used for the statistical analysis and the differences were considered statistically significant at $p<0.05$, $n=3$.

3. Results and Discussion

Comparison of liquid and lyophilized extracts prepared at different pH values (2.5 and 6) was performed *via* analyzing TPC, TFC, ABTS and DPPH antioxidant capacity, zeta potential, and conductivity.

3.1. Total polyphenol and flavonoid contents of the extracts

With the aim to investigate the influence of different pH values of the extraction medium on chemical composition of ethanol Serpylli herba extracts, the analyses of TPC and TFC were performed. The results are presented in Table 1.

Table 1. Total polyphenol content (TPC), total flavonoid content (TFC), ABTS and DPPH antioxidant capacity of Serpylli herba extracts (liquid and lyophilized) prepared using 50% ethanol (pH 6) and 50% ethanol with glacial acetic acid (pH 2.5).

Sample	TPC [mg GAE/mL or mg GAE/g]	TFC [mg CE/mL or mg CE/g]	ABTS [mmol TE/mL or mmol TE/g]	IC_{50} DPPH [mg/mL]
liquid pH 2.5	1.38±0.01^{a*}	0.368±0.004^{b}	0.767±0.027^{a}	1.12±0.04^{a}
liquid pH 6	1.23±0.06^{b}	0.332±0.002^{a}	0.775±0.035^{a}	1.75±0.04^{b}
lyophilized pH 2.5	271.1±17.1^{a}	102.5±1.1^{a}	0.136±0.001^{b}	0.331±0.015^{b}
lyophilized pH 6	188.8±14.2^{b}	99.7±0.9^{b}	0.111±0.004^{a}	0.391±0.023^{a}

*Values with different letters (a-b) in each extract group showed statistically significant differences ($p<0.05$; $n=3$; analysis of variance, Duncan's *post-hoc* test); GAE, gallic acid equivalent; CE, catechin equivalent; TE, Trolox equivalent; IC_{50}, the concentration of the extract required to neutralize 50% of DPPH radicals.

As can be seen from Table 1, the influence of the extraction medium pH value on the TPC and TFC of the extracts was statistically significant. Namely, the TPC of the liquid extract prepared at pH 2.5 was statistically higher (1.38±0.01 mg GAE/mL) compared to the extract prepared at pH 6 (1.23±0.06 mg GAE/mL). Additionally, the lyophilized samples had TPC of 271.7±17.1 mg GAE/g (pH 2.5) and 188.8±14.2 mg GAE/g (pH 6). The same trend is noticed in the case of TFC, 0.368±0.004 and 0.334±0.002 mg CE/mL for liquid extracts obtained at pH 2.5 and pH 6, respectively, and 102.5±1.1 and 99.7±0.9 mg CE/mL for lyophilized parallels. The obtained results of TPC and TFC are in agreement with the literature data, where the concentration of polyphenols in grape extracts increased with the decrease of pH from alkaline to acidic values (19). According to Friedman and Jurgens (20), the release of polyphenols from grapes was significantly higher in extremely acidic surroundings compared to pH 6.

3.2. Antioxidant potential of the extracts

The impact of different pH values of three extraction mediums on the antioxidant capacity of ethanol Serpylli herba extracts was also examined using two antioxidant tests (ABTS and DPPH methods) and the results are presented in Table 1.

The effect of the extraction medium pH value on ABTS and DPPH radical scavenging capacity of the extracts was statistically significant, as in the case of TPC and TFC (Table 1). ABTS radical scavenging potential of the liquid extracts at pH 2.5 and pH 6 was 0.767 and 0.750 mmol TE/L, respectively. The antioxidant potential for the lyophilized parallels was 0.136 and 0.111 mmol TE/g.

In DPPH assay, IC50 was significantly lower, i.e. higher antioxidant potential, at pH 2.5 (1.12±0.04 mg/mL) in comparison to the liquid extract prepared at pH 6 (1.75±0.04 mg/mL) and 0.331±0.015 and 0.391±0.023 mg/mL for the lyophilized parallels.

The obtained results are in agreement with the literature data, where several studies have shown a positive correlation between polyphenol concentration and antioxidant capacity (21-23). Namely, polyphenols are the most important components that possess the antioxidant capacity and play an important role, as reducing agents, free radical scavengers, potential complexes of prooxidant metals, and quenchers of singlet oxygen, in protection against disorders caused by oxidant damage (21). Dastmalchi et al. (22) have reported that the fraction of the extracts from species of the Lamiaceae family, which has the highest flavonoid content, also possesses the highest antioxidant activities. According to Miraj et al. (21), the radical scavenging activities of plant extracts have arisen from the content of some representatives of polyphenol compounds, including quercetin, quercitrin, rutin, gallic, rosmarinic, and caffeic acids.

3.4. Zeta potential and conductivity of the extracts

The Zeta potential and conductivity of Serpylli herba extracts are presented in Figure 1. Zeta potential (absolute value), as a measurement of the system stability, was the highest for the lyophilized extract prepared at pH 6 (-18.7±0.7 mV), followed by lyophilized extract prepared at pH 2.5 (-3.55±0.15 mV). The zeta potential of the liquid extracts prepared at pH 2.5 and pH 6 was 1.74±0.16 and -2.56±0.21 mV, respectively (Figure 1). Determination of the extract zeta potential is important from the aspect of its future application, including potential encapsulation and use in drinking water treatment. The Zeta potential of plant extracts depends on extraction conditions and thus of the extracted compounds, and absolute values vary from 2 mV to 15 mV (24).

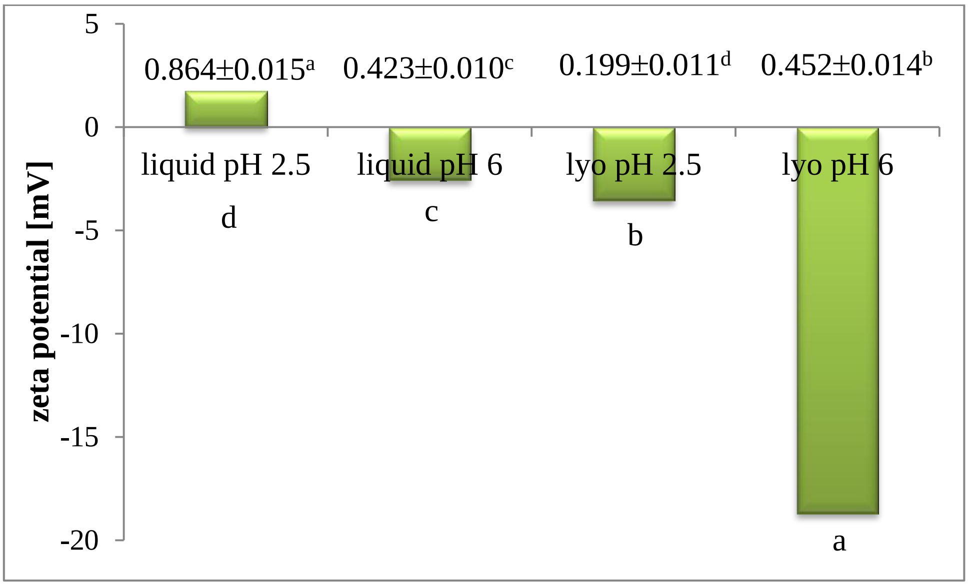

Figure 1. Zeta potential (bars) and conductivity (numbers above bars, mS/cm) of Serpylli herba extracts (liquid and lyophilized) prepared using 50% ethanol (pH 6) and 50% ethanol with glacial acetic acid (pH 2.5).

Suliman et al. (25) and Jurinjak Tušek et al. (26) have reported that the conductivity of the extracts can be used as a predictor of their antioxidant capacity and the extracts that possessed a higher antioxidant potential showed a higher value of conductivity as well. The conductivity of the liquid extracts was 0.864±0.015 and 0.423±0.010 mS/cm (for pH 2.5 and 6), whereas for the lyophilized parallels it was 0.199±0.011 and 0.452±0.014 mS/cm (Figure 1, numbers above the bars). However, the conductivity is influenced by the presence of extraneous ions, therefore analysis of the extract antioxidant activity using antioxidant assays is necessary.

4. Conclusion

In the presented research, the comparison of liquid and lyophilized Serpylli herba waste extracts prepared at different pH values was done via determination of TPC, TFC, antioxidant capacity, zeta potential, and conductivity. The liquid and lyophilized extracts prepared at pH 2.5 had statistically higher TPC and TFC in comparison to parallels prepared at pH 6. ABTS and DPPH radical scavenging potential were in a positive correlation to the polyphenol and flavonoid concentrations in the extracts. Zeta potential was the highest for the lyophilized extract prepared at pH 6, while conductivity was the highest for the liquid extract prepared at pH 6 which was not correlated to the antioxidant capacity of the extract. Thus, the pH value of the extraction medium, as well as extract form should be chosen depending on the future application of the extract.

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6. References

1. Khan, I., Abourashed E. Leung's encyclopedia of common natural ingredients used in food, drugs, and cosmetics. John Wiley & Sons, Inc. **2010.**
2. Monographs, Serpylli herba, Wild Thyme European Scientific Cooperative on Phytotherapy (ESCOP). Notaries House, United Kingdom. **2014.**
3. PDR for Herbal Medicines, Third ed. Thomson PDR, New Jersey. **2004.**
4. Hussain, A. I., Anwar, F., Chatha, S. A. S., Latif, S., Sherazi, S. T. H., Ahmad, A., Worthington, J., Sarker S.D. Chemical composition and bioactivity studies of essential oils from two *Thymus* species from the Pakistani flora. LWT – *Food Sci. Technol.*, **2013**, *50*, 185-192.
5. Jarić, S., Mitrović, M., Pavlović. P. Review of ethnobotanical, phytochemical, and pharmacological study of *Thymus serpyllum* L. *eCAM*, **2015**, *2015*, 1-10.
6. Jovanović, A., Vajić, J., Mijin, D., Zdunić, G., Šavikin, K., Branković, S., Kitić, D., Bugarski, B. Polyphenol extraction in microwave reactor using by-product of *Thymus serpyllum* L. and biological potential of the extract. *JARMAP*, **2022**, *31(10)*, 100417.
7. Brown da Rocha, C., Noreña, C. Microwave-assisted extraction and ultrasound-assisted extraction of bioactive compounds from grape pomace. *Int. J. Food Eng.,* **2020**, *16(1–2)*, 20190191.
8. Nastasi, J. R., Kontogiorgos, V., Daygon, V. D., Fitzgerald M. Pectin-based films and coatings with plant extracts as natural preservatives: A systematic review. *Trends Food Sci. Technol.*, **2022**, *120*, 193-211.

9. Panić, M., Radić Stojković, M., Kraljić, K., Škevin, D., Radojčić Redovniković, I., Gaurina Srček, V., Radošević K. Ready-to-use green polyphenolic extracts from food by-products. *Food Chem*., **2019**, *283*, 628-636.
10. Munin, A., Edwards-Lévy, F. Encapsulation of natural polyphenolic compounds: A review. *Pharmaceutics,* **2011**, *3*, 793–829.
11. Čančarević, A., Bugarski, B., Šavikin, K. & Zdunić, G. Biological activity and ethnomedicinal use of *Thymus vulgaris* and *Thymus serpyllum*. Lekovite Sirovine, **2013**, *33*, 3–17.
12. Jovanović, A., Djordjević, V., Petrović, P., Pljevljakušić, D., Zdunić, G., Šavikin K., Bugarski B. The influence of different extraction conditions on polyphenol content, antioxidant and antimicrobial activities of wild thyme. *JARMAP*, **2021**, *25*, 100328.
13. Zam, W., Bashour, G., Abdelwahed W., Khayata W. Effective extraction of polyphenols and proanthocyanidins from Pomegranate's peel. *Int J Pharm Pharm Sci.*, **2012**, *4*, 675-682.
14. Albu, S., Joyce, E., Paniwnyk, L., Lorimer, J.P., Mason, T.J. Potential for the use of ultrasound in the extraction of antioxidants from *Rosmarinus officinalis* for the food and pharmaceutical industry. *Ultrason. Sonochem.*, **2004**, *11*, 261-265.
15. Armendáriz-Barragán, B., Zafar, N., Badri, W., Galindo-Rodríguez, S. A., Kabbaj, D., Fessi, H., Elaissari, A. Plant extracts: from encapsulation to application. *Expert Opin. Drug Deliv.*, **2016**, *13*, 1165-1175.
16. Tavares, W. R., Barreto, M. D. C., Seca, A. M. Aqueous and ethanolic plant extracts as bio-insecticides-Establishing a bridge between raw scientific data and practical reality. *Plants*, **2021**, *10*, 920-950.
17. Fang, Z., Bhandari, B. Encapsulation of polyphenols—A review. *Trends Food Sci. Technol*., **2010**, *21*, 510–523.
18. Barros, L., Baptista, P., Ferreira, I. Effect of *Lactarius piperatus* fruiting body maturity stage on antioxidant activity measured by several biochemical assays. *FCT*, **2007**, *45*, 1731-1737.
19. Librán, C., Mayor, L., Garcia-Castello, E., Vidal-Brotons, D. Polyphenol extraction from grape wastes: Solvent and pH effect. *Agric. Sci*., **2013**, *4*(9B), 56-62.
20. Friedman, M., Jurgens, H.S. Effect of pH on the stability of plant phenolic compounds. *J. Agric. Food Chem.*, **2000**, *48*, 2101–2110.
21. Miraj, S., Kopae, M. R., Kiano, S. *Melissa officinalis* L: A review study with an antioxidant prospective. *eCAM*, **2016**, *22(3)*, 385-394.

22. Dastmalchi, K., Dorman, H. D., Oinonen, P. P., Darwis, Y., Laakso, I., Hitunen, R. Chemical composition and *in vitro* antioxidative activity of a lemon balm (*Melissa officinalis* L.) extract. LWT, *Food Sci. Technol.*, **2008**, *41*, 391-400.
23. Triantaphyllou, K., Blekas, G., Boskou, D. Antioxidative properties of water extracts obtained from herbs of the species Lamiaceae. *Int J Food Sci Nutr.*, **2001**, *52*, 313-317.
24. Skaf, D., Punzi, V., Rolle, J., Cullen, E. Impact of *Moringa oleifera* extraction conditions on zeta potential and coagulation effectiveness. *J. Environ. Chem. Eng.*, **2021**, *9(1)*, 104687.
25. Suliman, R. S., Asmani, F., Suliman, M. S., Hussain, M., Khan, J., Kaleemullah, M., Othman, N. B., Tofigh, A., Yusuf, E. A new approach for predicting antioxidant property of herbal extracts. *Int. J. Pharmacogn. Phytochem.*, **2015**, *7(1)*, 166-174.
26. Jurinjak Tušek, A., Benković, M., Valinger, D., Jurina, T., Belščak-Cvitanović, A., Gajdoš Kljusurić, J. Optimizing bioactive compounds extraction from different medicinal plants and prediction through nonlinear and linear models. *Ind Crops Prod.*, **2018**, *126*, 449-458.