The comparison of *Ocimum basilicum* and *Levisticum officinale* extracts obtained using different extraction solvents and techniques

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In the present study, basil (*Ocimum basilicum* L.) and lovage (*Levisticum officinale* Koch.) extracts were obtained using maceration, ultrasound- and microwave-assisted extractions (UAE and MAE, respectively). Varying the different extraction solvents, including water, methanol, acetone, and ethyl acetate, the analyses of total polyphenol and flavonoid contents (TPC and TFC, respectively), as well as antioxidant properties (DPPH radical scavenging and cupric ion reducing activities) were carried out for all obtained plants' extracts. The total amount of extractive substances of the selected extracts was also measured. The highest TPC was achieved in water basil extract obtained using UAE, while the highest TFC was determined in the basil extracts prepared using maceration and MAE. The highest level of DPPH radical neutralization was observed for methanol and acetone extracts obtained by maceration (for both plants) as well as UAE and MAE (for lovage). However, the obtained results did not exhibit statistically significant correlation with the TPC and TFC. The highest cupric ion-reducing capacity was measured in methanol lovage extract prepared using UAE and in methanol basil and lovage extracts from MAE. Interestingly, a significantly higher amount of the extractive substances was measured in all methanolic lovage extracts compared to basil parallels. Thus, it can be concluded that the selection of the extraction medium and extraction technique depends on the used plant species, as well as on the future application and purpose (or role) of the prepared extracts.

Key words: basil; cupric ion reducing antioxidant potential; extraction; lovage; polyphenols.

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ABBREVIATIONS

CUPRAC - cupric ion reducing antioxidant potential
DPPH - 1,1-diphenyl-2-picryldrazil
GAE - gallic acid equivalent
MAE - microwave-assisted extraction
QE - quercetin equivalent
RSA - radical scavenging activity
TFC - total flavonoid content
TPC - total polyphenol content
UAE - ultrasound-assisted extraction

1. INTRODUCTION

Ocimum basilicum L. (sweet basil) is a perennial herbaceous plant that is widely cultivated, well-known and utilized in Southeast Asian cuisine (Snoussi et al., 2016). Its essential oil and extracts can be utilized as traditional medicine (Bora et al., 2011) and an additive of food products (Gülçin et al., 2007). Basil is traditionally used in anxiety, colds, fevers, migraines, diabetes, menstrual cramps, sinusitis, cardiovascular illnesses, nerve pain, insect bites, and headaches (Khan and Abourashed, 2011). It possesses anti-inflammatory, antioxidant, anti-thrombotic, antimicrobial, insecticidal, anticonvulsant, anti-hyperlipidemic, antiplatelet, immunomodulatory, cytotoxic, cardiotonic, and analgesic effects (Amrani et al., 2009; Khan and Abourashed, 2011). Terpenes, phenylpropanoids, alcohols, polyphenols, and aldehydes are the most significant constituents of both, oil and extracts (Ilić et al., 2018).

The perennial plant lovage, known as *Levisticum officinale*

Koch., is indigenous to Europe and is a member of the Apiaceae family (Ravindran et al., 2004). The majority of lovage cultivation centers are in central Europe, but some of the most important producers of lovage are Germany, Netherlands, and the USA (Ravindran et al., 2004). Traditional medicine has employed lovage, particularly for its carminative, digestive, diuretic, expectorant, antispasmodic, and diaphoretic properties (Sharma et al., 2021). The main components of lovage were monoterpene hydrocarbons, oxygenated monoterpenes, phthalides, terpenoids, and volatile acids (Khan and Abourashed, 2011).

As both researched herbs (basil and lovage) can be used for colds, stomach and neurological diseases, their synergistic effects in certain mass ratios and forms should be investigated in order to make them more potent (Amrani et al., 2009; Sharma et al., 2021).

Considering the selection of the optimal choice of extraction solvent, according to the literature, the use of ethyl acetate as a green extraction solvent is favorable compared to methanol and acetone (Tumbas Šaponjac et al., 2021). Even though methanol and acetone have a negative impact on the environment and living beings, their use in the industry was widespread due to their low cost and the simple technology of production (Joana Gil-Chávez et al., 2013). Pereira et al. (2011) indicate the number of advantages of ethyl acetate, including non-toxicity, biodegradability, and non-corrosivity. Moreover, it was found that ethyl acetate is better solvent for extraction of some phenolic compounds compared to methanol which can be attributed to a lower polarity and dielectric constant (Pereira et al., 2011). The main advantage of using methanol as an extraction solvent is its ability to extract the highest amount of polyphenolic and flavonoid compounds, due to the fact that it can easily infiltrate the plant tissue and thus enlargen the yield of bioactive components (Onyebuchi and Kavaz, 2020). Further, Downey and Hanlin (2016) report the use of acetone as an extraction medium for the extraction of condensed tannins. Water was one of the most used extraction solvent due to its low cost and eco-friendly status, but the limits are the border solubility of extracted organic compounds, as well as their polarity, the possibility to interact with other polyphenols, etc. (Jones and Kinghorn, 2006). Due to the wide application of ethanol as a solvent and its confirmed extraction efficiency for the extraction of polyphenols, the choice fell on the ethyl acetate which is an ester of ethanol and acetic acid.

It is difficult to develop a general protocol for the phenolic extraction from plant materials, thus the extraction process should be optimized for every plant source. Several studies confirmed the strong extraction efficiency of ethanol for bioactive substances from basil (Veronezi et al., 2014) and lovage (Kozłowska et al., 2021). Therefore, the purpuse of these investigations was the examination of the extraction posibilities of other types of polar or semipolar organic solvents which are the candidates to obtain green status and more extensive application.

The aim of the present study was the comparison of various basil and lovage extracts obtained using maceration, ultrasound- and microwave-assisted extractions (UAE and MAE, respectively), as well as four extraction mediums: water, methanol, acetone, and ethyl acetate. Prepared extracts were analyzed via total polyphenol content (TPC), total flavonoid content (TFC), and antioxidant potential (DPPH and CUPRAC assays). The total extractive substances have been determined for the selected extract as well.

2. MATERIALS AND METHODS

2.1. Plant material and reagents

The seeds of basil and lovage originated from the medicinal and aromatic plant collection of the Institute for Medicinal Plants Research "Dr. Josif Pančić", in Pančevo, Serbia. The leaves of basil and lovage used in this experiment came from pot-grown plants. Production of seedlings started by the end of February 2021, by sowing seeds in styrofoam containers filled with a sowing substrate. With the emergence and development of the first true leaves in middle of March 2021, seedlings were transplanted into plastic pots (∅ 13 cm) filled with 1 L of peat substrate. The plants have grown in pots for 70 days in the non-heated greenhouse in the production field of the Institute. Harvest of the plants took place at the beginning of the flowering phase, in the beginning of June 2021. The harvested aerial parts of plants were left to air-dry in a well-ventilated room for 15 days before being packaged in paper bags and stored until extraction at room temperature. The following reagents were used: deioinized water (Sigma-Aldrich Chemie GmbH, Germany), methanol (Zorka-Farm, Republic of Serbia), ethanol (Zorka-Farm, Republic of Serbia), acetone (Macron Fine Chemicals, Germany), ethyl acetate and sodium-carbonate (Fischer Chemicals, United Kingdom). Monosodium-phosphate monohydrate, disodium-phosphate dihydrate, and Folin-Ciocalteu reagent were bought from Merck, Germany. Gallic acid, quercetin, aluminum(III)-chloride, Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), potassium-hexacyanoferrate, sodium-hydroxyde, hydrochloric acid, and 1,1-diphenyl-2-picryldrazil (DPPH•) were all purchased from Sigma Aldrich, Germany. All reagents and standards were of practical grade, and they were used without any further purification.

2.2. Preparation of the extracts

The extraction process was carried out using three different techniques (maceration, UAE and MAE) and four solvents with different polarities (water, methanol, acetone, and ethyl acetate).

2.2.1. Maceration

Maceration was performed using a linear mechanical homogenizer (Roller mixer SRT6, Bibby Sterlin Ltd., Germany) at 25±5 °C for 24 h. The plant material of basil or lovage (1 g, diameter of particles was ∼0.5 mm) was extracted with 20 mL of the extraction solvent (water, methanol, acetone, or ethyl acetate). In the previous study (Jovanović et al., 2017a), the smaller particle size of plant material (0.3 mm) provided higher polyphenol yield, while the solid-to-solvent ratio was chosen as an optimal according to the study of the extraction of green basil (*Ocimum sanctum*) (Ghanta et al., 2022). The liquid was filtered using a conventional Buchner procedure of filtration (Buchner funnel and bottle, with quantitative filter paper, 0.2 μm). The permeate was collected and stored in a dark bottle at 4 °C until further analyses.

2.2.2. Ultrasound-assisted extraction

The amount of 1 g of the pulverized plant material was extracted using 20 mL of the same extraction mediums as in the case of maceration, for 10 min using an ultrasound bath at 60 °C (Sonorex, Bandelin, Germany). The raw extracts of basil and lovage were collected, and filtered using a Buchner method described in the previous sub-chapter. The extracts were stored in the dark place at 4 °C until further analyses.

2.2.3. Microwave-assisted extraction

The pulverized plant material (0.5 g) was extracted with 10 mL of an appropriate solvent (water, methanol, acetone, or ethyl

acetate), at 60 °C for 10 min using Microwave Synthesis Reactor (Monowave 300, Anton Paar, Germany). After microwave treatment, the content from the vessel was transferred into the centrifugation tube (45 mL) and centrifuged using an Eppendorf centrifuge (Eppendorf, 5430 R, Germany) in two cycles (10 min, 3000 rpm). The supernatant was collected and stored at 4 °C until further analyses. The main reason for the difference in the filtration step of MAE compared to the other two extraction techniques was the appearance of blockage of the filter medium. Namely, microwave-assisted extraction causes the degradation of plant particles with a simultaneous decrease the particle size due to its potency and extraction power. Thus, the newly formed particles incorporate into the structure of the filtration medium, due to which the solvent is disenabled to move through the pores.

2.3. Determination of total polyphenol and flavonoid contents

The total polyphenol content (TPC) of the extracts was determined using a modified Folin-Ciocalteu assay (Zuhair et al., 2013). Shortly, 0.02 mL of properly diluted extract, 0.1 mL of Folin-Ciocalteu water reagent (1:1, v/v), and 1.5 mL of deionized water were transferred into a 2 mL flask and mixed uniformly. After 5 min, 0.3 mL of sodium-carbonate (20 %, w/v) was added, followed by the addition of deionized water up to 2 mL. After 120 min of standing in the dark, the absorbance was measured at 765 nm using 1800 UV/Vis spectrophotometer (Shimadzu, Japan). Gallic acid was used as an analytical standard for the construction of the calibration curve (30-500 μg/mL), and the results of TPC were expressed as micrograms of gallic acid equivalents per milliliter of the extract (μg GAE/mL). All analyses were performed in triplicate.

The total flavonoid content (TFC) of the extracts was determined using the method of Zuhair et al. (2013) with some modifications. Shortly, 0.25 mL of extract was mixed with 0.075 mL of sodium-nitrite (5 %, w/v) and 1.25 mL of deionized water. After the addition of the mentioned reagents, the mixture was left in dark for 5 min. After that, 0.15 mL of aluminum(III)-chloride (10 %, w/v) and 0.5 mL of sodium-hydroxide (1 mol/l) were added to the mixture. Finally, the mixture was filled up with deionized water to 2 mL. The absorbance of all samples was measured at 425 nm, while the results were expressed as micrograms of quercetin equivalents per milliliter of the extract (μg QE/mL). Quercetin was used for the construction of a standard curve (1-30 μg/mL).

2.4. Determination of antioxidant capacity

2.4.1. DPPH assay

The antioxidant capacity of basil and lovage extracts was analyzed in terms of the ability of the sample to donate the hydrogen atom, using the stable DPPH• radical (Jovanović et al., 2021a). All analytes were prepared as follows: 0.2 mL of plant extract was mixed with 2.8 mL of the DPPH• radical solution (DPPH$^{\bullet}$(s) dissolved in methanol). The mixture was left for 30 min in the dark to intensify the reaction of radical neutralization. After that time, the absorbance was measured at 517 nm. The sample of control was prepared by mixing 0.2 mL of solvent used in the extraction and 2.8 mL of DPPH$^{\bullet}$ radical solution. The radical scavenging activity (RSA, %) was calculated as follows:

$$\text{RSA (\%)} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100\,,$$

where $A_{control}$ was the absorbance of DPPH working solution and the extraction solvent, while A_{sample} was the absorbance of DPPH working solution and the extract.

2.4.2. CUPRAC assay

The cupric ion-reducing antioxidant capacity was determined according to the experimental procedure defined by (Özyürek et al., 2011). The solution of cupric(II) ion (1×10^{-2} mol/dm^3) was prepared by dissolving 0.0853 g of the cupper(II)-chloride dihydrate(s) into 250 mL of deionized water. Ammonium-acetate buffer solution (1 mol/dm^3, neutral pH) was prepared by dissolving 19.27 g of ammonium acetate(s) in 250 mL of deionized water. The fresh solution of neocuproine was prepared by dissolving 0.078 g of neocuproine(s) in 50 mL of methanol concentration of 7.5×10-3 mol/dm^3. Each reaction mixture consists of 0.8 mL of the examined extract in the appropriate solvent (1 mL cupper(II)-chloride dihydrate solution, 1.2 mL of buffer solution of ammonium acetate, and 1 mL of neocuproine solution in methanol). After incubation for 30 min in the dark place (25±5 °C), the absorbance was recorded at 450 nm. All of these conditions were implemented for L-ascorbic acid solution in ethanol, which was used as a standard. The L-ascorbic acid solution (5×10^{-3} mol/dm^3) was prepared by dissolving 6.26×10^{-3} g of L-ascorbic acid(s) in ethanol and diluted in five different concentrations. The results of CUPRAC assay were expressed as miligrams of L-ascorbic acid equivalents amounts of reduced Cu(II) per milliliter of raw extract.

2.5. Determination of the total extractive substances

The total extractive substances were measured for selected basil and lovage extracts and this parameter was calculated as follows:

$$\text{Total extractive substances (\%)} = \frac{Weight\ of\ dry\ extract}{Weight\ of\ raw\ extract} \times 100\,,$$

All samples of raw extracts were evaporated under vacuum conditions for 45 min (40 °C, 400 mbar) using the Buchi vacuum rotavapor (R-114, Büchi, Germany).

2.6. Statistical analysis

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

3. RESULTS AND DISCUSSION

Basil and lovage extracts, obtained by using maceration, UAE, and MAE, using water, methanol, acetone, or ethyl acetate, as an extraction solvent, were characterized in terms of TPC, TFC, DPPH radical scavenging potential, and cupric ion reducing antioxidant capacity (Table 1 and Figure 1, respectively). The total extractive substances of the selected extracts were also determined, and the results are presented in Table 2.

3.1. Chemical characterization of the extracts

Polyphenol and flavonoid contents of the obtained basil and lovage extracts are presented in Table 1 and Supplements 1 and 2. The highest TPC in water samples was achieved in basil extract obtained in an ultrasound bath, followed by basil extracts obtained using maceration and microwave reactor, and lovage extract from UAE (Table 1, Supplement 1). The highest value of total polyphenols in the methanol sample was determined in the basil extract from maceration, whereas all other methanol extracts have shown statistically significantly lower TPC. In the case of acetone as an extraction medium, basil extract from MAE had statistically significantly higher polyphenol concentration, while basil extract obtained by maceration has shown the highest TPC among all ethyl acetate extracts.

Table 1. Total polyphenol and flavonoid contents of basil (*Ocimum basilicum* L.) and lovage (*Levisticum officinale* Koch.) extracts obtained by different extraction techniques.

Species	Solvent	Extraction type	Total phenolics content [μg/mL]	Total flavonoid content [μg/mL]
Basil	water	M	30.64±1.15 b	5.31±0.58 d
		UAE	35.46±1.14 a	7.16±0.74 c
		MAE	30.81±2.05 b	14.67±1.17 a
	methanol	M	31.76±2.08 a	15.67±1.11 a
		UAE	28.35±1.07 b	4.70±0.41 d
		MAE	28.02±1.45 b	12.20±1.08 b
	acetone	M	8.23±0.58 e	5.64±0.31 a
		UAE	14.33±1.18 b	3.38±0.18 c
		MAE	20.77±1.00 a	2.68±0.41 d
	ethyl-acetate	M	22.21±1.55 a	3.14±0.11 c
		UAE	14.76±1.05 b	3.10±0.19 c
		MAE	15.02±1.13 b	8.16±0.22 b
Lovage	water	M	11.58±0.98 d	4.49±0.37 d
		UAE	33.01±1.00 b	4.97±0.15 d
		MAE	27.41±0.32 c	11.86±1.00 b
	methanol	M	22.38±1.17 c	7.89±1.15 c
		UAE	26.60±1.41 b	4.20±0.17 d
		MAE	18.68±0.94 d	8.35±0.85 c
	acetone	M	5.21±0.07 e	4.40±0.13 b
		UAE	9.25±0.17 d	4.30±0.22 b
		MAE	11.96±0.77 c	2.70±0.44 d
	ethyl-acetate	M	8.18±1.48 c	2.82±0.35 c
		UAE	5.37±0.14 d	1.39±0.41 d
		MAE	14.16±1.15 b	9.54±0.71 a

[a] Abbreviations M, UAE and MAE, stand for maceration, ultrasound and microwave assisted extractions, respectively.
[b] Values with the same letter (a-f) in each group of the extraction solvent showed no statistically significant differences ($p<0.05$; n=3; analysis of variance, Duncan's *post-hoc* test).

Literature data also showed that extraction technique significantly affected TPC; namely, water and alcoholic wild thyme extracts had significantly higher polyphenol concentrations after UAE compared to the extracts prepared at room and high temperature (Jovanović et al., 2017b). Additionally, Poorhashemi et al. (2019) have reported that UAE was better than maceration for extraction of polyphenols and antioxidant activity of the extracts of *Myristica fragrans*. According to Jovanović et al. (2017b), the advantage of the UAE compared to the conventional extraction techniques was the similar content of polyphenols obtained using a lower solvent consumption and a shorter extraction time. However, in the case of basil and lovage extracts, it cannot be noticed a trend, and there was no only one extraction technique that gives the highest polyphenol yield for all extraction solvents. Therefore, it can be concluded that every plant source and extraction solvent requires the examination of the appropriate extraction methods which will give the highest extraction efficiency in terms of polyphenol yield. In all basil and lovage extracts, water and methanol have given the samples with statistically significantly higher polyphenol concentrations compared to acetone and ethyl acetate samples. Jovanović et al. (2017a) have reported that the highest TPC was determined in *Thymus serpyllum* water extracts compared to the extracts obtained using a pure organic solvent. Usually, methanol was widely used to extract antioxidants from different plant materials. According to Jayasinghe et al. (2003), the TPC of methanol basil extracts was in a wide range and depends on the moisture content of the herb (fresh or dry), particle size, and used solid-to-solvent ratio. Considering all of the presented results, it can be concluded that water and methanol as solvents with the highest polarity provide the highest extraction efficiency (expressed as polyphenol concentration) compared to the low polarity solvents, such are ethyl acetate and acetone. Some literature reports indicate that methanol provides the highest extraction efficiency in comparison to other polar solvents. Namely, in the work of Jayasinghe et al. (2003), a methanol extract of basil shows 6.12 % of extraction efficiency, while acetone and ethyl acetate show smaller values, about 3.32 %, and 3.12 %, respectively. The high polarity of methanol can promote the solubility of phenolic compounds and thus boost the extraction efficiency of these compounds. Also, the differences in polyphenol concentration may be due to the use of different parts of the herbal material in the process of extraction, as well as the implemented technique (maceration, UAE, and MAE). The study of Anokwuru et al. (2011) showed that methanol can more effectively extract flavonoids from plant materials than acetone and ethyl acetate. Also, the concentration of flavonoids usually varies between various plant species, while the anatomical part of the processed plant can significantly influence the chemical composition of the extract. Moreover, flavonoids predominantly can be present in the external parts of plants, especially in the generative plant organs (Anokwuru et al., 2011). Further, it is very important to emphasize that choice of the extraction technique, solvent, dielectric properties, and polarity strongly influence flavonoid extraction effi-

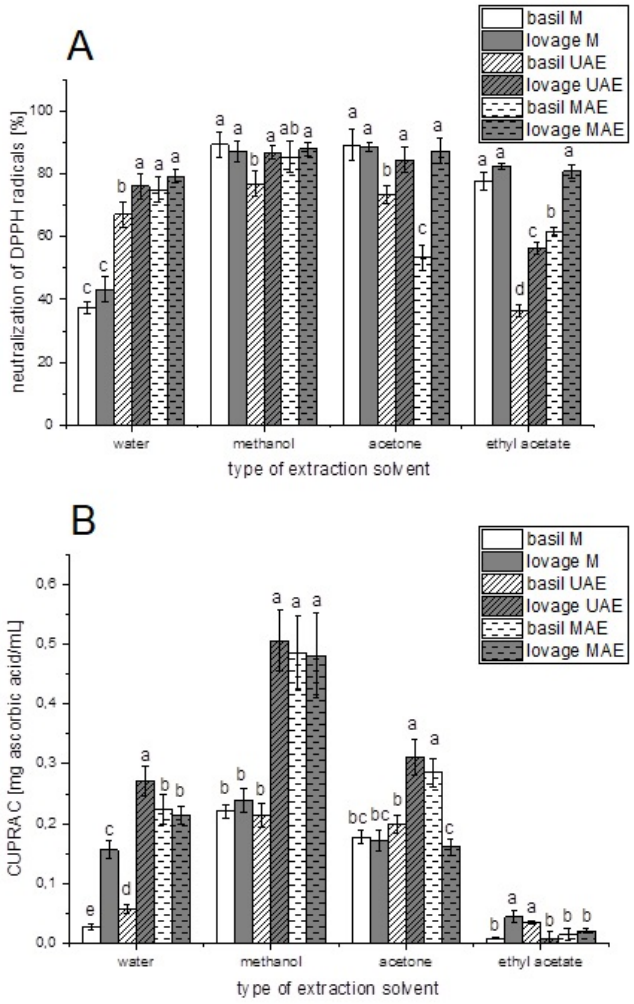

Fig. 1. DPPH radical scavenging activity (A) and cupric ion reducing antioxidant potential (B) of basil (*Ocimum basilicum* L.) and lovage (*Levisticum officinale* Koch.) extracts obtained by maceration (M), ultrasound- and microwave-assisted extractions (UAE and MAE, respectively); values with the same letter (a-e) in each group of the extraction solvent showed no statistically significant differences ($p<0.05$; n=3; analysis of variance, Duncan's *post-hoc* test).

ciency (Chávez-González et al., 2020). The extraction efficiency depends on the type of flavonoid molecule to be recovered as well (Chávez-González et al., 2020). Namely, semipolar solvents like ethyl acetate, or apolar solvents like chloroform, diethyl ether, and methylene chloride are commonly used for the extraction of the apolar derivates of flavonoids such as myricetin, quercetin, and isorhamnetin. Contrary, highly polar solvents like methanol and its aqueous mixture effectively extract flavonoid glycosides and aglycones (naringenin and neohesperidin) due to their polar nature (Chávez-González et al., 2020). There is no standard analytical procedure for the maximal exploitation of polyphenol substances from a medicinal plant.

Interestingly, flavonoid concentration did not correlate with polyphenol content in the majority of the extracts (Table 1). In water samples, the highest TFC was achieved in basil extract from MAE, followed by lovage extract from MAE, whereas in methanol extracts, the highest TFC was achieved using basil as a plant source and maceration, followed by basil extract from MAE. In acetone extracts, the highest flavonoid concentration was determined in basil extract obtained using maceration, followed by lovage extracts obtained by maceration and UAE. Regarding the results of ethyl acetate extracts, TFC was the highest in lovage extract from MAE, followed by basil extract from the same extraction technique. In all examined samples, water and methanol extract possessed statistically significantly higher flavonoid yield in comparison to acetone and ethyl acetate extracts, as in the case of TPC. The results from the research of Pereira et al. (2009) suggest that water was more potent to extract polyphenols and flavonoids of *Melissa officinalis* than an ethanol. Moreover, Jacotet-Navarro et al. (2015) compared a few different extraction methods for the recovery of rosmarinic acid, flavonoids, and glycosylated derivatives. That report suggests that when extraction was performed using the sonication method with methanol as a solvent, the recovery of the previously mentioned polyphenol classes was high. Further, microwave extraction affects the egress of the polar flavonoid molecules in the polar extraction media by simultaneously increasing the internal pressure of the solid and generating high extraction efficiency (Jovanović et al., 2017b). Moreover, Liazid et al. (2007) carried out a study that investigated the stability of different flavonoids by varying the temperature during the microwave-assisted extraction process using methanol as a solvent. That study provides proof that flavonoid molecules are stable at temperatures above 100 °C (kaempferol), while some fractions like catechin, epicatechin, and myricetin are unstable, and degrade quickly.

3.2. Antioxidant potential of the extracts

The antioxidant potential of *O. basilicum* L. and *L. officinale* extracts was determined using DPPH radical scavenging and CUPRAC assays; the results are presented in Figure 1 and Supplements 3 and 4.

Regarding the results of DPPH radical scavenging activity of all water extracts, it was the highest in basil and lovage extracts from MAE, and lovage extract from UAE (Figure 1A). There was no statistically significant difference between methanol extracts, except in the case of basil extract from UAE, which had the lowest activity, but its scavenging activity did not statistically differ from basil extract from MAE. The highest antioxidant potential in acetone extracts was determined in basil and lovage extracts from maceration and lovage extracts from UAE and MAE. Ethyl acetate basil and lovage extracts from maceration, and lovage extract from MAE have shown statistically significantly higher antioxidant capacity in comparison to other ethyl acetate extracts. Water basil and lovage extracts from maceration and ethyl acetate basil extract prepared using UAE have exerted the lowest DPPH radical scavenging potential. According to the presented results of the DPPH neutralization capacity of basil and lovage extracts, it can be noticed that solvent type and extraction procedure had a statistically significant influence on the mentioned variable. The obtained results are in agreement with the literature data, where different extraction mediums (water and ethanol) and different extraction techniques (maceration, extraction at high temperature, and extraction by using an ultrasound probe) significantly affected the DPPH radical scavenging activity of wild thyme extracts (Jovanović et al., 2021b).

Further, comparing the correlation between DPPH antioxidant capacity and TPC or TFC, it can be concluded that all extracts with the highest polyphenol and flavonoid yields did not possess the highest radical scavenging activity. In specific, DPPH radical scavenging activity achieved the maximal level for several extracts: methanol and acetone basil and lovage extracts obtained by maceration (for both plants) and UAE and MAE (for lovage). Additionally, methanol basil extract from maceration possessed the highest TPC and TFC as well (Table 1). As discussed above, the highest content of polyphenols was measured in basil and lovage aqueous extracts obtained by UAE (Table 1). However, this TPC was not correlated with

the DPPH$^{\bullet}$ neutralization power of the extracts. A potential reason for this inconsistency can be in the presence of the other large organic molecules, which can be adsorbed at 517 nm causing the disturbances. Kozłowska et al. (2021) have found a significant correlation between antioxidant capacity and the content of phenolic acids (rosmarinic, chlorogenic, caffeic, ferulic, and neochlorogenic acids) in ethanol extract of lovage. Nevertheless, the reduction of DPPH radicals may be due to the reducing property of flavonoids (Hirano et al., 2001). Namely, there are non-measured flavonoids that cannot react with $AlCl_3$ because they do not contain the necessary chelating functional groups for Al^{3+} binding. Additionally, Jovanović et al. (2021b) have also reported that the DPPH radical scavenging capacity of alcoholic wild thyme extracts was higher in comparison to aqueous extracts. Furthermore, the same study has shown that the extracts obtained using an ultrasound probe possessed statistically significantly higher antioxidant activity in comparison to maceration. Therefore, the combination of the type of plant material, extraction medium, and extraction technique can significantly influence antioxidant capacity and it should be investigated and optimized for every plant source. Due to the enough examined chemistry and oxidation-reduction potential of the Folin reagent, there is proof that some types of organic molecules like proteins, reduced sugars, and organic acids can reduce Folin reagent (Everette et al., 2010) without possibility to react with DPPH radicals and neutralize them. In accordance with that, in some cases when experiments were based on colorimetric measurements, the higher concentrations of phenolic compounds can be overrated. The results of our study in which methanol and acetone provide effective neutralization of DPPH radical compared to water extract match with the results from the study of Stanković (2011) which examined the DPPH activity of the methanolic and acetone extract of *Marrubium peregrinum* L. The higher solubility of some flavonoid derivates like flavonones and phenylpropanoids in methanol and acetone than in water influences the better biological activities of methanol and acetone plant extracts (Jovin et al., 2008). However, the research of Warsi and Sholichah (2017) showed that DPPH antioxidant capacity of the ethyl acetate extract of the basil leaves was more potent than the ethanol parallel. According to Pérez-Jiménez et al. (2008), the antioxidant activity of plants is related to the cumulative synergistic action of various antioxidant compounds, including polyphenols, carotenoids, terpenoids, vitamin C, vitamin E, Maillard compounds, and minerals in traces.

As can be seen in Figure 1B, cupric ion-reducing antioxidant potential was the highest in lovage extract from UAE (among water samples), basil and lovage extracts from MAE, and lovage extract from UAE (among methanol samples), basil extract from MAE and lovage extract from UAE (among acetone samples), and basil extract from UAE and lovage extract obtained using maceration (among ethyl acetate samples). The samples of the lovage-UAE, lovage-MAE, and basil-MAE showed the highest potential for the reduction of cupric ions when methanol was used as a solvent. Other samples significantly lower reduced the cupric ion, and the antioxidant activity of some of them cannot be evaluated as effective in this type of system. For instance, the ethyl acetate extracts of basil and lovage do not generate enough reduction potential, so the results for CUPRAC assays are consequently lower. Also, the results from the study of Zengin et al. (2019) match with the findings from this study. Namely, ethyl acetate as a semipolar solvent will extract semipolar organic compounds better, than water and methanol, which will extract dominantly polar organic compounds more efficiently, and a small amount of semipolar or unipolar organic compounds. Also, basil aqueous extract possessed stronger antioxidant activity when compared to ethyl acetate, which can be ascribed to the solubility of some specific fractions of flavonoids (Jayasinghe et al., 2003). Finally, the methanolic extract of lovage, with a significantly lower TPC, showed a higher reduction capacity in comparison to the water, which had a higher content of polyphenols. This unusualness was exclusively bounded to the implementation of UAE and MAE techniques, while maceration did not provide the extracts with significant reduction capacity. Moreover, Anand et al. (2019) reported that the methanolic extract of basil generates higher antioxidant activity in comparison to the aqueous extract when the measurements were performed using conventional, *in vitro* radical scavenging, ion chelating, and reducing potential. As reported, the difference in chemical compositions of the two extracts was the main reason. According to the results from the CUPRAC test and polyphenol/flavonoid content, it was not shown the correlation between the TPC or TFC and reduction power, as in the case of the results from the DPPH assay. In this case, the combination of the extraction solvent and the implemented technique of the extraction was crucial for the antioxidant potential. As discussed above, the highest TPC was in basil and lovage aqueous extracts obtained by UAE, whereas the highest TFC was in the water and methanol basil extracts prepared by maceration and MAE, respectively (Table 1). Only high TFC of methanol basil extract from MAE was correlated with high cupric ion-reducing the power of the extract. These investigations show that TPC and TFC did not correlate strictly with the antioxidant activity because some other forms of organic molecules (plant pigments, free organic acids, tannins, sugars, proteins) can intensify the reactions with free radicals and neutralize them (Özyürek et al., 2011). Also, the reaction between natural antioxidants and radical forms can cause a decrease in the absorbance value in the CUPRAC assay, and these changes can be influenced by catalytic factors of oxidation like light, pH, oxygen, and polarity of the solvent. Moreover, the cupric ions can be reduced by some different types of antioxidants, like polyphenols, flavonoides, thiols, D-ascorbic acid, mannitol, glucose, and similar (Özyürek et al., 2011). All methanol extracts of basil and lovage have shown significant antioxidant potential in the DPPH assay, while in the CUPRAC test, only methanol basil extract prepred using MAE and basil and lovage extracts prepared using UAE and MAE have exerted significant antioxidant capacity. However, in water extracts, the samples which showed high DPPH radical scavenging potential also exerted a high ion-reducing capacity. All acetone extracts (except the basil sample from MAE) had high DPPH antioxidant capacity, whereas only acetone extracts from UAE (lovage) and MAE (basil) showed high ion-reducing capacity. In the DPPH assay, ethyl acetate basil and lovage extracts prepared using maceration and MAE (only for lovage) have exerted significant antioxidant activity, while all ethyl acetate extracts were a poor source of antioxidant compounds responsible for cupric ion-reducing antioxidant capacity.

3.3. Total extractive substances of the selected extracts

Total extractive substances were determined in the methanol extracts of both plant sources (basil and lovage) obtained from all used extraction techniques, and the results are presented in Table 2. Although some literature data indicates a direct correlation between the content of polyphenol molecules and the antioxidant capacity of plant extract, sometimes the mentioned correlations are not consistent due to the fluctuation of polyphenolic constituents (Rajakaruna et al., 2022). In support of these results, choosing the methanolic extract for the determination of total extractive substances can be explained

by the fact that the distribution of phenolic constituents in all of the examined extracts was different.

Table 2. Total extractive substances of the selected *Ocimum basilicum* L. and *Levisticum officinale* Koch. methanolic extracts.

Species	Extraction technique	Total extractive substances [%]
Basil	Maceration	9.22±0.18 b
	Ultrasound-assisted extraction	9.00±0.24 b
	Microwave-assisted extraction	8.92±0.30 b
Lovage	Maceration	12.96±0.59 a
	Ultrasound-assisted extraction	13.00±0.71 a
	Microwave-assisted extraction	14.06±0.80 a

[a] Values with the same letter in each group of the extraction technique showed no statistically significant differences ($p<0.05$; n=3; analysis of variance, Duncan's *post-hoc* test).

As can be seen from Table 2, there was no statistically significant difference between total extractive substances from different extraction procedures, but lovage extracts have shown statistically significantly higher extraction yield in comparison to basil. These results contribute to showing that the total amount of extractive substances in the methanolic extract of basil and lovage depends on different factors, primarily on the process parameters in the implemented technique of extraction. The higher amount of total extractive substances of lovage extract did not correlate with the total amount of phenolic derivates and this occupation can be corroborated by the fact that other ballast matters (simple and complex carbohydrates, lipids, and proteins) were presented in the extracts (Jovanović et al., 2017b; Leal et al., 2008). Leal et al. (2008) showed that organic acids (quinic, tartaric, and malic acids) and rhamnose can influence yield (total extractive substances) as well. Additionally, there is an assumption that some non-volatile and non-polar organic compounds like waxes, phytosterols, and fatty oils can contribute to a higher amount of total extractive substances. Teofilović (2016) declared that the relative amount of extracted substances in the extraction period can be the orientation of the criterion of the amount of destroyed matters with the assumption that all of the extracted substances are uniformly distributed through the total volume of the plant material. The amount of total extractive substances can be influenced by the gradient of concentrations between free solvent and internal liquid (bonded solvent with the plant particles). In that way, the particle size of homogenized plant material, porosity, microcapillaries, and the contact between solvent and plant particles determine the efficiency of extraction, as well as the amount of total extractive substances (Teofilović, 2016). Certainly, the concentration and polarity of solvent from one side, and the choice of the appropriate ratio between the plant material and extraction agent for another, can directly influence the amount of total extractive substances.

CONCLUSION

In this research, four different extraction solvents (with gradual polarity decrease, water, methanol, acetone, and ethyl acetate) and three extraction methods (maceration, UAE, and MAE) were used to obtain liquid basil and lovage extracts, which were then evaluated via polyphenol and flavonoid contents, as well as *in vitro* antioxidant activity (DPPH and CUPRAC assays). The highest polyphenol content was achieved in water basil extract obtained in UAE, whereas the highest flavonoid concentration was determined in water and methanol basil extracts prepared using maceration and MAE, respectively. DPPH radical neutralization did not correlate with the TPC and TFC, and achieved the maximal level for several extracts: methanol and acetone extracts obtained by maceration (for both plants) and UAE and MAE (for lovage). The highest cupric ion-reducing capacity was measured in methanol lovage extract prepared using UAE and in basil and lovage extracts from MAE. All methanolic lovage extracts possessed a significantly higher amount of the extractive substances in comparison to basil parallels. The selection of the extraction medium and extraction technique depends on the used plant species, as well as on the future application of the prepared extracts. Further experiments should be based on the encapsulation of selected basil and lovage extracts, and their implementation in various food, pharmaceutical, and cosmetic formulations.

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