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Extraction, Chemical Characterization, and Antioxidant Activity of Bioactive Plant Extracts †

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Abstract: Natural extracts have been proposed as preservatives to increase the safety of various food products. In this work, the phytochemical and antioxidant profiles of French lavender (Lavandula stoechas), lemon balm, basil, tarragon, salvia, and spearmint extracts were studied. The results show that hydroethanolic extracts may be more effective as biopreservatives if moderate temperatures are used in the extraction process, as they revealed higher phenolic content. More specifically, the results also show that lemon balm extracts present a great potential to be used as biopreservatives, due to their high-level phenolic and flavonoid contents and antioxidant activity.

Keywords: phenolics; flavonoids; solid–liquid; Soxhlet; aqueous extraction; ethanolic extraction

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1. Introduction

Plant extracts have been proposed as alternative biocides and antioxidants to be incorporated in foods or their packaging [1–3], as research has focused on obtaining extracts of increased biological interest from natural sources. In this work, basil, lemon balm, French lavender, salvia, spearmint, and tarragon were selected as they are readily available in Portugal, and there is scientific evidence of their antimicrobial and antioxidant properties [1,4–6]. Solid-liquid and Soxhlet extractions were selected as these are often used by the food industry to extract valuable bioactive compounds because they usually promote good extraction results and are easy to implement [7]. In this sense, this work aims to study the phytochemical and antioxidant profile of such plant extracts, as obtained by distinct extraction methods and solvents, and to assess their potential to be used as food preservatives.

2. Materials and Methods

2.1. Plant Material and Extraction Procedures

French lavender, lemon balm, basil, tarragon, salvia, and spearmint dry aerial parts were provided by Pragmático Aroma Lda. ("Mais Ervas", Trás-os-Montes, Portugal), and then mechanically ground. The extractions were performed using 70% (*v*/*v*) ethanol (Et70) and distilled water as solvents in a shaking water bath (at 150 rpm) at 60 °C for 90 min (solid–liquid extraction); and using a Soxhlet apparatus (at approximately 90 or 120 °C for Et70 and distilled water, respectively, for 7 cycles). Both methods used a sample/solvent ratio of 1:20. After filtration (7–10 μm filter paper), the extracts were stored in a refrigerator at 4 °C until use.

2.2. Total Phenolic and Flavonoid Content Determinations

The total phenolic content (TPC) was determined using the Folin–Ciocalteu assay [8]. A calibration curve (R^2 = 0.994) was prepared using a standard solution of gallic acid, and the final values were expressed as milligrams of gallic acid equivalents (GAE) per gram of dry plant material (mg GAE/g dry plant). The total flavonoid content (TFC) was determined by aluminium chloride colorimetric method [9]. A calibration curve (R^2 = 0.999) was prepared using (+)-Catechin, and the results were expressed as milligrams of catechin equivalents (CE) per gram of dry plant material (mg CE/g dry plant).

2.3. Antioxidant Activity Determination

The antioxidant activity was measured using the free radical scavenging (DPPH), the radical cation decolorization (ABTS), and the ferric reducing antioxidant power (FRAP) methods to evaluate distinct mechanisms of action of the extracts. The free radical scavenging (DPPH) and the radical cation decolorization (ABTS) assays were conducted as described by Ballesteros et al. [10], with some modifications. Calibration curves (R^2 = 0.996–0.998) were prepared with a standard solution of Trolox and a corresponding control was used for each solvent. The radical scavenging activity for the DPPH and ABTS methods (% inhibition) was calculated as Equation (1).

$$\%\ \text{Inhibiton} = \frac{A_c - A_s}{A_c} \times 100 \quad (1)$$

where A_s is the sample absorbance and A_c the control sample absorbance. The results were expressed as micrograms of Trolox equivalent (TE) per gram of dry plant material (μg TE/g dry plant).

Ferric reducing antioxidant power (FRAP) assay was performed as described by Meneses et al. [11]. A calibration curve was prepared using an aqueous solution of ferrous sulphate (R^2 = 0.977). FRAP values are expressed as micromoles of ferrous equivalent per g of dry plant material (μmol Fe^{2+}/g dry plant).

2.4. Identification and Quantification of Individual Phenolic Compounds

The samples were analyzed by a Shimadzu Nexera X2 UPLC chromatograph equipped with a Diode Array Detector (DAD) (Shimadzu, SPD-M20A). The separation was performed on a reversed-phase Acquity UPLC BEH C18 column (2.1 mm × 100 mm, 1.7 μm particle size; from Waters) and a precolumn of the same material at 40 °C. The flow rate of the solvents (0.1% formic acid and acetonitrile) was 0.4 mL/min. A comparison between the UV spectra (at different wavelengths) and the retention times of each standard was used to identify and quantify the phenolic compounds. All analyses were made in triplicate.

2.5. Statistical Analysis

All experiments were performed in triplicate and data is presented as mean ± standard deviation (SD) values. The significance of variables was evaluated by analysis of variance (α = 0.05) whereas principal component analysis (PCA) was used on phenolic compounds to discriminate between extracts. Statistical analysis was conducted in R software (version 3.6.2, R Foundation for Statistical Computing, Vienna, Austria).

3. Results and Discussion

3.1. Total Phenolic Content and Total Flavonoid Content

The TPC of the extracts produced are depicted in the two top plots of Figure 1. In the case of solid-liquid extraction, TPC of salvia and spearmint extracts are solvent-dependent ($p < 0.05$), unlike the remaining plant extracts. For the Soxhlet extractions, basil, lemon balm, French lavender, and spearmint TPC appear to be affected by the solvent used ($p < 0.05$). Evaluating the influence of extraction technique when the solvent is water, only the

extracts of salvia and French lavender seem to show differences in TPC ($p < 0.05$), with Soxhlet extracts presenting higher values than solid-liquid ones. When ethanol 70% is used as solvent, extracts of basil, lemon balm, salvia, and spearmint present differences ($p < 0.05$) in TPC between extraction methods, with a tendency for higher values in the solid-liquid extracts.

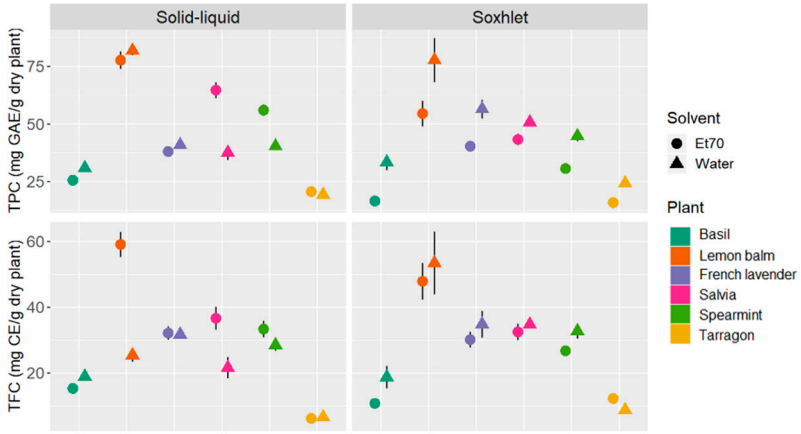

Figure 1. Total phenolic content, TPC (**top plots**), and total flavonoid content, TFC (**bottom plots**), of the studied plant extracts as influenced by solvent and extraction method.

The TFC of all extracts produced are shown in the two bottom plots of Figure 1. Statistical analysis revealed that solvent type has an impact ($p < 0.05$) on the TFC of lemon balm and salvia extracts by solid-liquid method; and of basil, lemon balm, spearmint, and tarragon in Soxhlet extracts. Lemon balm and salvia aqueous extracts presented distinct TFC depending on the extraction method ($p < 0.05$), whereas the remaining plants did not show any differences. As for the hydroethanolic extracts, extraction method influenced the TFC of lemon balm, spearmint, and tarragon extracts ($p < 0.05$).

Unlike other extracts, the aqueous solid-liquid extract of lemon balm presented high discrepancy between TFC and TPC values, which is explained by the liquid chromatography results: lemon balm hydroethanolic extract presented 17.88 ± 1.04 mg/g dry plant of naringin; against 2.33 ± 0.11 mg/g dry plant of the same flavonoid in the aqueous extract. This suggests that for lemon balm, solid-liquid method using water as solvent may not be sufficient to obtain some flavonoids.

These results are explained by the fact that alcoholic solvents are more effective in extracting phenolic compounds than water, as they improve their solubility from the raw material to the solvent medium [12–14], and that lower temperatures are more adequate to extract such compounds as they avoid thermal degradation and oxidation [13]. Hence, it is expected that ethanol 70% paired with Soxhlet method leads to lower TPC and TFC, since phenolic compounds are likely to be extracted early in the process and then exposed to a higher temperature for a longer time. Oppositely, higher TPC and TFC are obtained when ethanol 70% is used at lower temperatures, when using the solid-liquid extraction for example. Therefore, it is possible to assume that, for Soxhlet extractions, water may be the most appropriate solvent, whereas for solid-liquid extractions, a mixture of water/ethanol is more adequate.

3.2. Antioxidant Activity

The results of the antioxidant activity assays are shown in Table 1. In this work, regardless of the extraction method or solvent, lemon balm extracts presented the highest antioxidant activities (measured by FRAP, DPPH and ABTS), whereas tarragon extracts showed the lowest activities. However, when hydroethanolic solid-liquid extraction was applied, only lemon balm deviated significantly ($p < 0.05$) from the other plants. This result was expected as it agrees with the TPC results.

Comparing the results for the solid-liquid method, all assays showed differences ($p < 0.05$) between the aqueous and hydroethanolic extracts for all plants, except for basil and tarragon. For the Soxhlet method, FRAP and DPPH assays presented differences between solvents for basil and French lavender extracts; while ABTS assay revealed such differences for basil, French lavender, and spearmint.

Water extracts revealed differences ($p < 0.05$) between methods for lemon balm, French lavender, and salvia in FRAP and ABTS assays, but only for French lavender in DPPH assay. Hydroethanolic extracts showed distinct results between methods for French lavender and spearmint in FRAP assay, and for French lavender, spearmint, and basil in DPPH assay; while ABTS assay revealed that only tarragon extracts are not significantly different depending on the method used.

These results suggest that the antioxidant activity of extracts, except those of tarragon, are generally dependent on the extraction method and/or solvent applied, as supported by the TPC results, and previously justified in Section 3.1.

Table 1. Antioxidant activity of extracts obtained from the studied plants measured by FRAP, DPPH and ABTS methods (mean ± standard deviation).

		Extraction Method/Solvent			
	Plant	**Solid-Liquid**		**Soxhlet**	
		Water	**Et70**	**Water**	**Et70**
FRAP (μmol Fe^{2+}/g dry plant)	Basil	400.5 ± 9.76	342.5 ± 11.7	508.6 ± 19.7	256.5 ± 1.52
	Lemon balm	692.8 ± 97.7	1166 ± 34.3	1182 ± 126	1094 ± 22.9
	French lavender	478.1 ± 112	682.4 ± 39.8	818.0 ± 65.5	511.2 ± 80.6
	Salvia	561.9 ± 14.7	846.4 ± 44.7	791.3 ± 35.0	857.2 ± 66.7
	Spearmint	621.1 ± 65.6	808.1 ± 57.2	776.9 ± 8.57	688.6 ± 36.3
	Tarragon	143.2 ± 2.72	202.5 ± 6.45	232.2 ± 3.71	204.4 ± 19.1
DPPH (μmol TE/g dry plant)	Basil	157.4 ± 10.6	152.8 ± 10.0	193.0 ± 1.20	85.38 ± 0.97
	Lemon balm	302.9 ± 29.0	365.3 ± 0.98	362.8 ± 19.5	359.8 ± 2.36
	French lavender	183.2 ± 54.8	282.8 ± 11.0	292.6 ± 37.5	212.8 ± 41.6
	Salvia	210.5 ± 9.78	309.9 ± 7.84	265.7 ± 0.50	280.8 ± 23.8
	Spearmint	219.6 ± 28.0	312.0 ± 21.3	268.1 ± 0.50	231.5 ± 12.8
	Tarragon	57.84 ± 1.54	42.95 ± 14.1	94.90 ± 1.50	62.95 ± 1.07
ABTS (μmol TE/g dry plant)	Basil	197.0 ± 7.90	216.5 ± 6.84	227.3 ± 3.48	122.6 ± 0.98
	Lemon balm	410.0 ± 75.3	596.2 ± 15.4	532.8 ± 31.1	492.1 ± 8.12
	French lavender	261.9 ± 63.3	381.4 ± 15.4	389.1 ± 51.9	276.9 ± 52.9
	Salvia	264.8 ± 3.16	436.8 ± 11.4	382.7 ± 4.72	356.9 ± 27.6
	Spearmint	323.9 ± 30.9	434.5 ± 31.0	379.2 ± 6.21	289.2 ± 10.1
	Tarragon	86.96 ± 2.24	114.2 ± 3.71	130.4 ± 4.47	102.1 ± 19.0

3.3. Identification and Quantification of Individual Phenolic Compounds

To assess the extracts' chemical profiles, tentative identification/quantification of phenolic compounds was performed by Ultra Performance Liquid Chromatography (UPLC). Of the fifteen compounds identified, rosmarinic, ferulic, and ellagic acids, naringin, hesperidin, resveratrol, and quercetin were found in all the extracts.

PCA was conducted to visualize the influence of phenolic compounds on the differentiation of the extracts (Figure 2). The first two principal components, PC1 and PC2, accounted for 29% and 18% of the total variance, respectively. PCA was unable to fully distinguish between the solvents (not shown) but showed good discrimination between the methods, suggesting a greater difference in phenolic compounds for extracts obtained from different methods than from different solvents. The results also indicate that Soxhlet extracts generally contain higher concentrations of rosmarinic acid, kaempferol, hesperidin, and ellagic acid, whereas solid–liquid extracts present greater amounts of vanillic,

syringic, cinnamic, and *o*-coumaric acids, resveratrol, and quercetin, for example. Overall, the hydroethanolic extracts presented higher values of rosmarinic acid, resveratrol, and hesperidin, as these phenols are poorly soluble in water [14].

Vanillic and syringic acids were found in small concentrations (0.20–0.34 and 0.19–0.21 mg/g dry plant, respectively) in a few solid–liquid extracts (aqueous basil and salvia; and hydroethanolic tarragon and basil), but in none of the Soxhlet samples. Chlorogenic acid was found in the aqueous tarragon, mint, and lemon balm Soxhlet extracts (0.18–0.55 mg/g dry plant), and in the hydroethanolic lemon balm, basil, and French lavender solid–liquid extracts (0.64–1.42 mg/g dry plant).

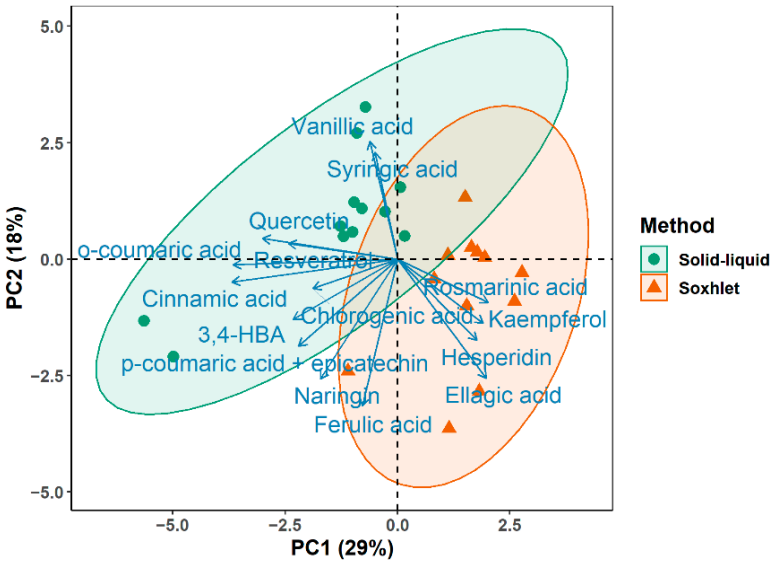

Figure 2. Principle component analysis (PCA) on the studied plant extracts.

Cinnamic acid was found in all the solid–liquid extracts, with the hydroethanolic extracts revealing higher concentrations (0.74–9.70 mg/g dry plant) than the aqueous ones (0.50–3.00 mg/g dry plant). Likewise, *o*-coumarin was found in all the solid–liquid extracts (0.41–2.22 mg/g dry plant), but only in the tarragon Soxhlet samples (0.05–1.25 mg/g dry plant). *p*-coumaric acid and epicatechin were identified in some samples of every method/solvent combination, but more often in the hydroethanolic solid–liquid extracts. Kaempferol was also retrieved in all method/solvent combinations except for the aqueous solid–liquid extracts, and more often for the hydroethanolic Soxhlet samples.

These results are valuable, as they help to select the best method/solvent combination depending on the desired phenolic compound to be retrieved from the extract.

4. Conclusions

These outcomes provide insight on the phytochemical profile and antioxidant activity of plant extracts, and on the effect of extraction methods and solvents on such characteristics. Solid-liquid hydroethanolic extracts showed potential as biopreservatives, due to their higher phenolic content. Overall, lemon balm extracts presented the highest TPC, TFC and antioxidant activities among all plants, thus suggesting the potential of this natural resource to be incorporated in foods as a preservative.

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U.G.-B.; Writing-original draft, B.N.S.; Writing—review and editing, J.A.T., P.F.-S., V.C. and U.G.-B. All authors have read and agreed to the published version of the manuscript.

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