Foods 2021

MDPI

Proceedings

Plant Extracts as Potential Bioactive Food Additives †

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† Presented at the 2nd International Electronic Conference on Foods, 15–30 October 2021; Available online: https://foods2021.sciforum.net/.

Abstract: The bioactivity of infusions, decoctions and hydroethanolic extracts of six aromatic plants (basil, lemon balm, lavender, sage, spearmint, and tarragon) was evaluated in this work. The results highlighted several of these extracts with capability to prevent food spoilage (antimicrobial effects), promote health benefits (antioxidant and anti-inflammatory capacities) and, therefore, revealed the potential of natural plant extracts as food additives.

Keywords: antioxidants; antimicrobials; preservatives; anti-inflammatory

Citation: Silva, B.N.; Cadavez, V.; Caleja, C.; Pereira, E.; Calhelha, R.C.; Pinela, J.; Kostić, M.; Soković, M.; Teixeira, J.A.; Barros, L.; et al. Plant Extracts as Potential Bioactive Food Additives. **2021**, *68*, x. https://doi.org/10.3390/xxxxx

Academic Editor(s):

Received: date
Accepted: date
Published: date

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1. Introduction

Research has demonstrated that plant extracts have potential as food additives due to their numerous bioactive properties, which include antimicrobial and antioxidant capacities, acting in prevention or delay of food deterioration and avoiding product oxidation, respectively. Furthermore, they may offer health benefits to consumers, also due to their antioxidant abilities, as well as through anti-inflammatory properties.

In this context, *Ocimum basilicum* L. (basil), *Melissa officinalis* L. (lemon balm), *Lavandula stoechas* (lavender), *Salvia officinalis* L. (sage), *Mentha spicata* L. (spearmint), and *Artemisia dracunculus* L. (tarragon) were selected to produce extracts rich in bioactive molecules, with potential application in the food industry, since these plants have previously shown beneficial impacts on human health [1–5]. For that, three extraction methods were tested (infusion, decoction, and maceration) using nontoxic solvents, to assure the safety of the extracts for human consumption. Overall, this work aimed to evaluate the bioactivities of a variety of herbal extracts, and to assess their potential as food additives.

2. Materials and Methods

2.1. Plant Material and Extraction Procedures

Lavender, lemon balm, basil, tarragon, sage, and spearmint dry aerial parts were kindly provided by *Pragmático Aroma, Lda.* company ("Mais Ervas", Trás-os-Montes, Portugal), mechanically ground, and submitted to three extraction methods, namely infusion, decoction and maceration.

Infusions were performed by adding 2 g of plant material to 200 mL of boiling distilled water. Decoctions were performed by adding 2 g of plant material to 200 mL of

distilled water, heated, and boiled for 5 min. All aqueous mixtures were then immediately filtrated (7–10 μm), frozen, and lyophilized. Macerations were performed by adding 1 g of plant material to 30 mL of 80% ethanol (*v*/*v*) and stirring for 1 h at room temperature. The mixtures were filtrated (7–10 μm), 30 mL more of 80% ethanol (*v*/*v*) were added and the maceration was repeated for 1 h. Lastly, the ethanolic fraction was evaporated and the extracts were frozen and lyophilized. All extractions were performed in triplicate (n = 3).

2.2. Antimicrobial Activity

The extracts were screened against six bacterial strains: *E. coli* (ATCC 25922), *S. enterica* ser. Typhimurium (ATCC 13311), *E. cloacae* (ATCC 35030), *S. aureus* (ATCC 11632), *B. cereus* (clinical isolate), *L. monocytogenes* (NCTC 7973). Minimum inhibitory (MIC) and minimum bactericidal (MBC) concentrations were determined by a previously described serial microdilution method [6]. Sodium benzoate (E211) and potassium metabisulfite (E224) were screened as positive controls to confirm the sensitivity of the microorganisms to these widely used artificial preservatives. The results were expressed as MICs and MBCs, in mg/mL of the resuspended lyophilized extracts.

2.3. Antioxidant activity

The antioxidant activity was evaluated using a previously described in vitro assay based on the inhibition of the free radical-induced erythrocyte haemolysis (OxHLIA) [7,8]. The extracts capacity to inhibit the oxidative haemolysis was tested using sheep blood erythrocytes as ex vivo models. The extract concentration able to promote a Δt haemolysis delay of 60 min was calculated based on the half haemolysis time (Ht_{50} values) of the haemolytic curves of each extract concentration. Trolox was used as a positive control. The results are expressed as the extract concentration required to keep 50% of the erythrocyte population intact for 60 min (IC_{50}).

2.4. Anti-Inflammatory Activity

For the anti-inflammatory activity evaluation, a previously described assay was used [9]. For that, a mouse macrophage-like cell line RAW264.7 stimulated with lipopolysaccharides was used, and the extracts concentration tested ranged between 25–400 μg/mL. Nitric oxide (NO) production was studied with a Griess reagent system kit. Dexamethasone (50 μM) was used as a positive control. The results are expressed as the sample concentration (μg/mL) required to inhibit 50% of NO production (IC_{50}).

2.5. Statistical Analysis

Data are presented as mean ± standard deviation (SD) values. The statistical differences between mean values were obtained through one-way analysis of variance (ANOVA, $\alpha = 0.05$). Statistical analysis was conducted in R software (version 4.1.0, R Foundation for Statistical Computing, Vienna, Austria).

3. Results and Discussion

3.1. Antimicrobial Activity

The results show that all extracts exhibited antimicrobial activity against the tested pathogens (MIC ≤ 2 mg/mL). Among all extracts produced, sage infusion presented the lowest MIC and MBC values against *S. aureus* and *B. cereus* (MIC = 0.25 and MBC = 0.5 mg/mL in both cases), thus suggesting the greatest antimicrobial potential of this extract against these specific pathogens. Lemon balm decoction, on the other hand, presented the highest MIC and MBC values among all extracts produced, namely against *L. monocytogenes* (MIC = 2 and MBC = 4 mg/mL), which suggests a less effective antimicrobial action of this extract against this pathogen.

With some exceptions, the infusions, decoctions and hydroethanolic extracts produced revealed equal or lower MIC and MBC values (hence, equivalent or higher antimicrobial activities) than those of the commonly used artificial preservatives E211 and E224. Particularly, the results of E211 against *S. aureus* (MIC and MBC = 4 mg/mL); and those of E224 against *B. cereus* (MIC = 2 and MBC = 4 mg/mL) contrast noticeably with the lower results obtained from the plant extracts against such organisms. These are encouraging results for the possible replacement of common synthetic additives used by the industry with plant-based ingredients.

3.2. Antioxidant Activity

The results of the oxidative haemolysis assay (OxHLIA) are presented in Table 1. The results are expressed as IC_{50} values, meaning that higher values correspond to lower antioxidant potential. All extracts revealed antioxidant capacity against free radical-induced oxidative damage of biological membranes. Comparing the three extraction methods used, for each plant under analysis, hydroethanolic extracts revealed higher antioxidant potential (lower IC_{50} values), except those of sage and basil. In these two cases, decoction was the method leading to greater antioxidant power of the extracts.

Table 1. Antioxidant activity of the plant extracts (IC_{50} values, μg/mL).

Plant Sample	Infusion	Decoction	Hydroethanolic Extract
Tarragon	170 ± 2^{f}	92 ± 2^{e}	49 ± 2^{c}
Lemon balm	61 ± 1^{c}	27.0 ± 0.4^{b}	13.5 ± 0.4^{a}
Spearmint	84 ± 2^{d}	42.2 ± 0.6^{c}	12.5 ± 0.2^{a}
Lavender	49 ± 2^{b}	29 ± 1^{b}	15.4 ± 0.4^{a}
Sage	21.9 ± 0.8^{a}	8.9 ± 0.4^{a}	23.9 ± 0.9^{b}
Basil	97 ± 1^{e}	49 ± 1^{d}	89 ± 3^{d}

Trolox IC_{50} value: 21.8 ± 0.25 μg/mL. Values with different superscript letters in a column mean significant differences (ANOVA, $p < 0.05$).

From Table 1, it can be noted that the antioxidant power of each plant infusion was significantly different from all the other infusions ($p < 0.05$). Decoctions and hydroethanolic extracts also revealed differences in antioxidant activity depending on the used plant ($p < 0.05$), but not all of them were significant. According to the statistical analysis, sage infusion and sage decoction, and spearmint hydroethanolic extract showed the best antioxidant activities among all extracts.

3.3. Anti-Inflammatory Activity

The results of the anti-inflammatory activity assay are presented in Table 2.

Table 2. Anti-inflammatory activity of the plant extracts (IC_{50} values; μg/mL).

Plant Sample	Infusion	Decoction	Hydroethanolic Extract
Tarragon	$>400^{c}$	35 ± 0.5^{a}	44 ± 4^{b}
Lemon balm	$>400^{c}$	$>400^{d}$	$>400^{c}$
Spearmint	44.4 ± 0.7^{a}	44 ± 4^{b}	27 ± 2^{a}
Lavender	$>400^{c}$	$>400^{d}$	$> 400^{c}$
Sage	$>400^{c}$	$>400^{d}$	$> 400^{c}$
Basil	88.6 ± 0.5^{b}	64.5 ± 0.7^{c}	55 ± 5^{b}

Dexamethasone IC_{50} value: 6 ± 1 μg/mL. Values with different superscript letters in a column mean significant differences (ANOVA, $p < 0.05$).

Some extracts did not reveal anti-inflammatory action ($IC_{50} > 400$ μg/mL). Curiously, those that did present were not the ones with greatest antioxidant capacity (see Table 1),

as it could be expected. In fact, it has been reported in the literature that extracts with promising antioxidant activity would also possess anti-inflammatory potential, since antioxidants could reduce the inflammatory process that may be prompted by the overproduction of free radicals [9]. In this sense, the extracts of spearmint, basil, and tarragon stand out for their anti-inflammatory capability. It is also noticeable that spearmint and basil extracts show anti-inflammatory action regardless of the extraction method, unlike tarragon, which did not maintain its action when infusion was the extraction method used.

4. Conclusions

This study provides insight on the bioactivity of numerous herbal extracts. While only a few revealed anti-inflammatory potential, all infusions, decoctions, and hydroethanolic extracts showed encouraging outcomes in terms of antimicrobial and antioxidant capacities. In this sense, overall, this work emphasised the value of plant extracts as food natural ingredients to prevent spoilage (through antimicrobial action), deliver beneficial health effects (through antioxidant and anti-inflammatory action), and potentially replace artificial additives in the food industry.

Author Contributions: Conceptualization, V.C., J.A.T. and U.G.-B.; Data curation, B.N.S. E.P., C.C. and U.G.-B.; Formal analysis, B.N.S., and U.G.-B.; Funding acquisition, V.C., J.A.T. and U.G.-B.; Investigation, B.N.S., E.P., C.C., R.C.C., J.P., M.K., M.S. Methodology, E.P., C.C., M.K., M.S. and L.B.; Project administration, V.C., J.A.T. and U.G.-B.; Resources, L.B., J.A.T., V.C. and U.G.-B.; Software, B.N.S., and U.G.-B. Supervision, V.C., J.A.T., L.B. and U.G.-B.; Validation, B.N.S., E.P., C.C. and U.G.-B.; Visualization, B.N.S., E.P., C.C. and U.B.-B.; Writing—original draft, B.N.S.; Writing—review & editing, E.P., C.C. and U.G.-B. All authors have read and agreed to the published version of the manuscript.

Funding: The authors are grateful to the EU PRIMA program and the Portuguese Foundation for Science and Technology (FCT) for funding the ArtiSaneFood project (PRIMA/0001/2018) and for financial support through national funds FCT/MCTES to CIMO (UIDB/00690/2020). This study was supported by FCT under the scope of the strategic funding of UIDB/04469/2020 unit and BioTecNorte operation (NORTE-01-0145-FEDER-000004) funded by the European Regional Development Fund under the scope of Norte2020—Programa Operacional Regional do Norte. This work has been supported by the Ministry of Education, Science and Technological Development of Republic of Serbia (451-03-68/2020-14/200007).

Institutional Review Board Statement:

Informed Consent Statement:

Data Availability Statement: Summary data available upon request.

Acknowledgments: B. N. Silva wishes to acknowledge the financial support provided by FCT through the Ph.D. grant SFRH/BD/137801/2018. R. C. Calhelha, L. Barros, U. Gonzales-Barron and J. Pinela (CEECIND/01011/2018) acknowledge the national funding by FCT, P.I., through the Institutional Scientific Employment Program contract. The project Healthy-PETFOOD is acknowledged, for the contract of C. Caleja (Healthy-PETFOOD (POCI-01-0247-FEDER-047073)), as well as the Project Mobilizador Norte-01-0247-FEDER-024479: ValorNatural®, for the contract of E. Pereira.

Conflicts of Interest: The authors declare no conflicts of interest.

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