biology and life sciences forum

MDPI

Proceeding Paper

Plant Extracts as Potential Bioactive Food Additives †

Beatriz Nunes Silva [1,2,3,4], Vasco Cadavez [1,2], Cristina Caleja [1,2], Eliana Pereira [1,2], Ricardo C. Calhelha [1,2], José Pinela [1,2], Marina Kostić [5], Marina Soković [5], José António Teixeira [3,4], Lillian Barros [1,2] and Ursula Gonzales-Barron [1,2,*]

1 Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal; beatrizsilva@ceb.uminho.pt (B.N.S.); vcadavez@ipb.pt (V.C.); ccaleja@ipb.pt (C.C.); eliana@ipb.pt (E.P.); calhelha@ipb.pt (R.C.C.); jpinela@ipb.pt (J.P.); lillian@ipb.pt (L.B.)
2 Laboratório Para a Sustentabilidade e Tecnologia em Regiões de Montanha, Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal
3 CEB—Centre of Biological Engineering, University of Minho, 4710-057 Braga, Portugal; jateixeira@deb.uminho.pt
4 LABBELS—Associate Laboratory, 4710-057 Braga, Portugal
5 Institute for Biological Research "Siniša Stanković"—National Institute of Republic of Serbia, University of Belgrade, Bulevar Despota Stefana 142, 11000 Belgrade, Serbia; marina.kostic@ibiss.bg.ac.rs (M.K.); mris@ibiss.bg.ac.rs (M.S.)
* Correspondence: ubarron@ipb.pt; Tel.: +35-12-7330-3325
† Presented at the 2nd International Electronic Conference on Foods—"Future Foods and Food Technologies for a Sustainable World", 15–30 October 2021; Available online: https://foods2021.sciforum.net/.

Abstract: Plant extracts have been proposed as antimicrobial agents and health-promoters to be included in a variety of food products. In this sense, this work aimed to evaluate the bioactivities of infusions, decoctions and hydroethanolic extracts of six aromatic plants, namely, basil, lemon balm, lavender, sage, spearmint, and tarragon. The novelty of this study is related to the recent trend to replace chemical additives with more natural, plant-based ones, to meet consumers' demands. The results highlighted the antimicrobial, antioxidant, and anti-inflammatory effects of several of these extracts, thus emphasising their capability to prevent food spoilage and promote health benefits. In this sense, our research revealed the potential of some plant extracts as potential food additives.

Keywords: antioxidants; antimicrobials; preservatives; anti-inflammatory

check for updates

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. *Biol. Life Sci. Forum* **2021**, *6*, 116. https://doi.org/10.3390/Foods2021-11010

Academic Editor: Antonio Cilla

Published: 14 October 2021

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 [1]. Furthermore, they may offer health benefits to consumers, also due to their antioxidant abilities, as well as through anti-inflammatory properties [1]. Considering the increasing demand by consumers for foods with health-promoting effects beyond basic nutrition, over the past years, the food industry has been making efforts to switch from synthetic additives to others that are plant-based.

In this sense, *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) have shown beneficial impacts on human health, as revealed by centuries of traditional medicine use and other researchers [2–6], and they are readily available in Portugal.

For this reason, the objective of this work was to evaluate the biological activities of extracts from these plants obtained through different conventional, yet "green", extraction methodologies (infusion, decoction, and maceration) using nontoxic solvents (water and 80% ethanol (*v*/*v*)), which is important to assure the safety of the extracts for human consumption [7]. More specifically, the antibacterial, antioxidant, and anti-inflammatory

activities of the extracts were evaluated. In this context, the research hypothesis is that basil, lemon balm, lavender, sage, spearmint, and tarragon could be used to produce extracts rich in bioactive molecules, with potential application in the food industry.

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), and *L. monocytogenes* (NCTC 7973). Bacteria strains were subcultured twice by streaking on blood agar and incubated at 37 °C for 48 h and then 24 h to ensure that bacterial cells were in the exponential growth phase. Following incubation in agar, single colonies from the second plate were inoculated into individual tubes containing sterile water and the bacterial suspensions were adjusted to a concentration of approximately 1.5×10^8 CFU/mL (0.5 McFarland). Minimum inhibitory (MIC) and minimum bactericidal (MBC) concentrations were determined by a previously described broth microdilution method, with some modifications [8]. The lowest concentration that completely inhibited bacterial growth in the microdilution well was regarded as the minimal inhibitory concentration (MIC). The minimal bactericidal concentrations (MBC) were also determined by subcultivation of 10 μL of the microplate wells into blood agar plates. The lowest concentration that showed no growth after this subculturing was regarded as the MBC.

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 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) [9].

An erythrocyte solution (2.8%, *v*/*v*; 200 μL) prepared in phosphate-buffered saline (PBS, pH 7.4) was mixed with 400 μL of either: (i) extract solution (13–800 μg/mL in PBS), (ii) PBS solution (control), (iii) distilled water (for complete haemolysis), or (iv) the positive control Trolox (7.81–250 μg/mL in PBS). After pre-incubation at 37 °C for 10 min with shaking, 200 μL of 2,2′-azobis(2-methylpropionamidine) dihydrochloride (AAPH, 160 mM in PBS; from Sigma-Aldrich) were added and the optical density was measured at 690 nm every 10 min in a microplate reader (Bio-Tek Instruments, ELX800) until complete haemolysis [7]. The percentage of the erythrocyte population that remained intact (*P*) was calculated by Equation (1), where S_t and S_0 correspond to the optical density of the sample at t and 0 min, respectively, and CH_0 is the optical density of the complete haemolysis at 0 min.

$$P\% = 100\left(\frac{S_t - CH_0}{S_0 - CH_0}\right) \quad (1)$$

Results were expressed as delayed time of haemolysis (Δt) using Equation (2), where Ht_{50} is the 50% haemolytic time (min) graphically obtained from the haemolysis curve of each sample concentration.

$$\Delta t \text{ (min)} = Ht_{50} \text{ (sample)} - Ht_{50} \text{ (control)} \quad (2)$$

Finally, the Δt values were correlated to the different sample concentrations and, from the obtained correlation, the concentration able to promote a Δt haemolysis delay was calculated. The results were expressed as IC_{50} values (μg/mL) at Δt 60 min, i.e., the sample concentration required to keep 50% of the erythrocyte population intact for 60 min.

2.4. Anti-Inflammatory Activity

For the anti-inflammatory activity evaluation, a previously described assay was used [10]. Cells from the mouse macrophage-like cell line RAW264.7 were seeded in 96-well plates and their attachment to the plate allowed overnight. Afterwards, cells were treated with different concentrations of the extracts (25–400 μg/mL) for 1 h, and then stimulated with LPS (1 μg/mL) for 18 h. Through this procedure it was possible to observe the occurrence of induced changes in nitric oxide (NO) basal levels, which was studied with a Griess Reagent System kit. Nitrite level produced was determined by measuring optical density at 515 nm, in a microplate reader, and then compared with the standard calibration curve. Dexamethasone (50 μM) was used as 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) [11].

3. Results and Discussion

3.1. Antimicrobial Activity

The MIC is defined as the lowest concentration of an antimicrobial agent that completely inhibits growth of the organism in the microdilution wells as detected by the unaided eye [8]. The results of this study 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 at Δt 60 min, which indicates the sample concentration required to keep 50% of the erythrocyte population intact for 60 min. This means that higher IC_{50} values correspond to lower antioxidant potential. All extracts revealed antioxidant capacity against free radical-induced oxidative damage of biological membranes. Com-

paring 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

Nitric oxide (NO) is a signalling molecule that plays a key role in the pathogenesis of inflammation, as it gives an anti-inflammatory effect under normal physiological conditions but induces inflammation due to overproduction in abnormal situations [12].

The results of the anti-inflammatory activity assay are presented in Table 2. The results are expressed as the sample concentration required to inhibit 50% of NO production, thus, higher IC_{50} values reflect lower anti-inflammatory potential.

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 [10]. 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. and 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.G.-B.; Writing—original draft, B.N.S.; Writing—review and 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: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Summary data available upon request.

Acknowledgments: B.N.S. wishes to acknowledge the financial support provided by FCT through the Ph.D. grant SFRH/BD/137801/2018. R.C.C., L.B., U.G.-B. and J.P. (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 conflict of interest.

References

1. Vinceković, M.; Viskić, M.; Jurić, S.; Giacometti, J.; Kovačević, D.B.; Putnik, P.; Donsì, F.; Barba, F.J.; Jambrak, A.R. Innovative technologies for encapsulation of Mediterranean plants extracts. *Trends Food Sci. Technol.* **2017**, *69*, 1–12. [CrossRef]
2. Behbahani, B.A.; Shahidi, F.; Yazdi, F.T.; Mortazavi, S.A.; Mohebbi, M. Antioxidant activity and antimicrobial effect of tarragon (*Artemisia dracunculus*) extract and chemical composition of its essential oil. *J. Food Meas. Charact.* **2017**, *11*, 847–863. [CrossRef]
3. Carocho, M.; Barreira, J.C.M.; Bento, A.; Fernández-Ruiz, V.; Morales, P.; Ferreira, I.C.F.R. Chestnut and lemon balm based ingredients as natural preserving agents of the nutritional profile in matured "Serra da Estrela" cheese. *Food Chem.* **2019**, *204*, 185–193. [CrossRef] [PubMed]
4. Sharifi-Rad, M.; Ozcelİk, B.; Altın, G.; Daşkaya-Dİkmen, C.; Martorell, M.; Ramírez-Alarcón, K.; Alarcón-Zapata, P.; Morais-Braga, M.F.B.; Carneiro, J.N.P.; Leal, A.L.A.B.; et al. *Salvia* spp. plants-from farm to food applications and phytopharmacotherapy. *Trends Food Sci. Technol.* **2018**, *80*, 242–263. [CrossRef]
5. Antolak, H.; Kregiel, D. Food Preservatives from Plants. In *Food Additives*; Karunaratne, D.N., Pamunuwa, G., Eds.; IntechOpen: London, UK, 2017; pp. 45–85.
6. Ez zoubi, Y.; Bousta, D.; Farah, A. A Phytopharmacological review of a Mediterranean plant: *Lavandula stoechas* L. *Clin. Phytoscience* **2020**, *6*, 9. [CrossRef]
7. Granato, D.; Santos, J.S.; Salem, R.D.; Mortazavian, A.M.; Rocha, R.S.; Cruz, A.G. Effects of herbal extracts on quality traits of yogurts, cheeses, fermented milks, and ice creams: A technological perspective. *Curr. Opin. Food Sci.* **2018**, *19*, 1–7. [CrossRef]
8. Soković, M.; Glamočlija, J.; Marin, P.D.; Brkić, D.; van Griensven, L.J.L.D. Antibacterial Effects of the Essential Oils of Commonly Consumed Medicinal Herbs Using an In Vitro Model. *Molecules* **2010**, *15*, 7532–7546. [CrossRef] [PubMed]

9. Silva de Sá, I.; Peron, A.P.; Leimann, F.V.; Bressan, G.N.; Krum , B.N.; Fachinetto, R.; Pinela, J.; Calhelha, R.C.; Barreiro, M.F.; Ferreira , I.C.F.R.; et al. In vitro and in vivo evaluation of enzymatic and antioxidant activity, cytotoxicity and genotoxicity of curcumin-loaded solid dispersions. *Food Chem. Toxicol.* **2019**, *125*, 29–37. [CrossRef] [PubMed]
10. Jabeur, I.; Tobaldini, F.; Martins, N.; Barros, L.; Martins, I.; Calhelha, R.C.; Henriques, M.; Silva , S.; Achour, L.; Santos-Buelga, C.; et al. Bioactive properties and functional constituents of *Hypericum androsaemum* L.: A focus on the phenolic profile. *Food Res. Int.* **2016**, *89*, 422–431. [CrossRef] [PubMed]
11. R Core Team. *R: A Language and Environment for Statistical Computing*; R Foundation for Statistical Computing: Vienna, Austria, 2021; Available online: https://www.R-project.org/ (accessed on 5 June 2021).
12. Sharma, J.N.; Al-Omran, A.; Parvathy, S.S. Role of nitric oxide in inflammatory diseases. *Inflammopharmacology* **2007**, *15*, 252–259. [CrossRef] [PubMed]