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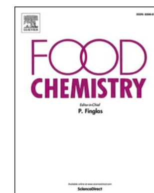

Research paper

Chemical profiles and bioactivities of polyphenolic extracts of *Lavandula stoechas* L., *Artemisia dracunculus* L. and *Ocimum basilicum* L.

Beatriz Nunes Silva [a,b,c], Vasco Cadavez [a,b], Cristina Caleja [a,b], Eliana Pereira [a,b], Ricardo C. Calhelha [a,b], Adriana K. Molina [a,b], Tiane Finimundy [a,b], Marina Kostić [e], Marina Soković [e], José António Teixeira [c,d], Lillian Barros [a,b], Ursula Gonzales-Barron [a,b,*]

[a] *Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal*
[b] *Laboratório Associado para a Sustentabilidade e Tecnologia em Regiões de Montanha (SusTEC), Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal*
[c] *CEB - Centre of Biological Engineering, University of Minho, Campus de Gualtar, 4710–057 Braga, Portugal*
[d] *LABBELS – Associate Laboratory, 4710-057 Braga, Portugal*
[e] *Institute for Biological Research "Siniša Stanković"- National Institute of Republic of Serbia, University of Belgrade, Bulevar Despota Stefana 142, 11000 Belgrade, Serbia*

A R T I C L E I N F O

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A B S T R A C T

This study assessed the chemical profiles and bioactivities of the infusions, decoctions and hydroethanolic extracts of tarragon, basil and French lavender. The extracts were chemically characterised (HPLC-DAD-ESI/MS) and their bioactivities were evaluated in vitro. All extracts revealed antimicrobial, antifungal and antioxidant properties. French lavender extracts showed higher total phenolic content, regardless of the extraction method used, and antioxidant and antitumour capacities, but no anti-inflammatory action. All basil and two of the tarragon extracts revealed anti-inflammatory power. Thus, tarragon, basil and French lavender extracts may be considered for inclusion in foods, as preservatives or functional ingredients. Nonetheless, further studies must be conducted to evaluate the pharmacokinetic parameters of the bioactive compounds.

1. Introduction

For centuries, plants have been relied upon as traditional medicines to support and promote human health. Nowadays, they continue to be used to treat multiple conditions and complaints. In the case of French lavender (*Lavandula stoechas* L., Lamiaceae), basil (*Ocimum basilicum* L., Lamiaceae) and tarragon (*Artemisia dracunculus* L., Asteraceae), the first has been used for its anti-inflammatory, antispasmodic, sedative and carminative properties, and to treat rheumatic diseases and nephrotic syndromes (Ez Zoubi, Bousta, & Farah, 2020; Domingues, Delgado, Gonçalves, Zuzarte, & Duarte, 2023); basil has been used for the treatment of headaches, cough, constipation, skin warts, parasites and renal malfunctions, and its reported properties include antimicrobial, anticonvulsant, antioxidant and anticarcinogenic effects (Kamelnia, Mohebbati, Kamelnia, El-Seedi, & Boskabady, 2023; Othman et al., 2021); tarragon, in turn, possesses anti-inflammatory, antipyretic, antiseptic, eupeptic, laxative, carminative, stomachic, antispasmodic, antiparasitic, antimicrobial, vermifuge and emmenagogue effects, and some of its popular uses include the treatment of skin conditions and gastritis, for example (Ekiert et al., 2021; Hassanzadeh, Tayarani-Najaran, Nasery, & Emami, 2016).

Over recent years, the sustainability movement has encouraged the use of natural resources, such as medicinal plants, that are often available and easily accessible but undervalued, and consumers have shown increased concern to take on healthier lifestyles, which include consumption of foods with health-boosting effects past basic nutrition. Additionally, the increasing number of research focusing on medicinal plants and spices has shed a light on them as good sources of phytochemicals, with interesting biological capacities and therapeutic effects (de la Rosa, Martínez-Ruíz, Domínguez-Avila, & Alvarez-Parrilla, 2018).

* Corresponding author at: Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal

E-mail addresses: beatriznsilvaa@gmail.com (B.N. Silva), vcadavez@ipb.pt (V. Cadavez), ccaleja@ipb.pt (C. Caleja), eliana@ipb.pt (E. Pereira), calhelha@ipb.pt (R.C. Calhelha), amolina@ipb.pt (A.K. Molina), tiane@ipb.pt (T. Finimundy), marina.kostic@ibiss.bg.ac.rs (M. Kostić), mris@ibiss.bg.ac.rs (M. Soković), jateixeira@deb.uminho.pt (J.A. Teixeira), lillian@ipb.pt (L. Barros), ubarron@ipb.pt (U. Gonzales-Barron).

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Among these, the antimicrobial, antioxidant, anti-inflammatory and antiproliferative activities of medicinal plants are of particular importance.

Several authors have reported that the misuse and overconsumption of chemical preservatives from food products may induce numerous health issues, including gut microbiota dysbiosis (Silva, Teixeira, Cadavez, & Gonzales-Barron, 2023). The negative impact of such additives on consumers' health has prompted the search for novel antibacterial and antifungal agents, such as those originated from plant sources, which have shown relevant antimicrobial capabilities (Oulahal & Degraeve, 2022). Additionally, as stated by Licá, Soares, Mesquita, and Malik (2018), natural products obtained from plants are known reliable antioxidant resources, and reports have shown that they cause fewer side effects when compared to synthetic drugs. Similarly, the current anti-inflammatory synthetic drugs are known to cause several side-effects, and for that reason, there has been a continuous search for novel anti-inflammatory medicines, in particular, those that are plant-derived (Licá et al., 2018). In regard to the antiproliferative capacity, literature has demonstrated the value of plant-derived compounds in cancer inhibition and treatment, with their potential as inhibitors of various stages of tumour having been reported several times (Nawaz et al., 2022).

In this context, the food industry is now aiming to include natural extracts into products as a strategy to limit the use of synthetic additives and to produce functional foods, even though this is not always straightforward. In vitro results are not always transferable to food matrices, natural extracts can be unstable and have a negative impact on the organoleptic characteristics of foods due to their high concentration in terpenoids and phenolic compounds (Dinani & van der Goot, 2022; Martínez-Zamora, Peñalver, Ros, & Nieto, 2021), interactions between the extracts and the food components may occur, resulting in degradation and loss of bioactive compounds, among other challenges and concerns such as the economic costs, legislation and practical effectiveness of introducing plant extracts as preservatives in the food industry (Silva et al., 2023).

While these aspects must be considered, the interest in plant extracts with bioactive molecules as food additives has grown considerably in the past decades, and considering the results of a recent meta-analysis study which highlighted the potential of several plant essential oils as antimicrobial agents (Silva, Cadavez, Teixeira, & Gonzales-Barron, 2021), the objective of this study was to contribute with the analysis of the phenolic composition and biological properties of extracts obtained using non-toxic solvents and from easily accessible Mediterranean plants, namely tarragon, basil and French lavender. In particular, the antimicrobial, antifungal, antioxidant, anti-inflammatory, and antiproliferative activities of the extracts were evaluated.

2. Materials and methods

2.1. Plant material and extracts preparation

The dry aerial parts of tarragon, French lavender and basil were obtained from *Pragmático Aroma, Lda.* (Bragança, Portugal) company and mechanically ground.

Infusions were prepared by adding 2 g of each plant material to 200 mL of boiling distilled water and leaving the mixture at room temperature for 5 min. For the decoctions, 2 g of plant material was added to 200 mL of distilled water and boiled for 5 min. Both aqueous mixtures were then filtered (7–10 μm), frozen and lyophilised (FreeZone 4.5, Labconco, Kansas City, MO, USA). Hydroethanolic extracts (HE) were also produced through dynamic macerations, where 1 g of plant material was added to 30 mL of ethanol at 80% (*v*/*v*) and stirred for 1 h at room temperature. The supernatants were filtered (7–10 μm), additional 30 mL of ethanol 80% (*v*/*v*) were mixed with the extraction residues, and the maceration was repeated for 1 h. The ethanolic fraction was then evaporated (Büchi R-210, Flawil, Switzerland) and the extracts frozen and lyophilised. Extractions were performed in triplicate ($n = 3$).

2.2. HPLC-DAD-ESI/MS analysis of phenolic compounds

Phenolic compounds were investigated using a previously validated method (Restivo, Degano, Ribechini, & Colombini, 2014). First, the lyophilised extracts were redissolved in ethanol 20% (*v*/*v*) up to a final concentration of 10 mg/mL and filtered (0.22 μm). Then, individual phenolic compounds were analysed by Dionex Ultimate 3000 UPLC (Thermo Scientific, San Jose, CA, USA) coupled with a diode-array detector (at 280, 330, and 370 nm). MS detection was performed in an API 3200 Qtrap (Applied Biosystems, Darmstadt, Germany) equipped with an ESI source and a triple quadrupole-ion trap mass analyzer. Nitrogen served as the curtain (20 psi) and collision gas (medium). The ion spray voltage was set at − 4500 V in the negative mode. The MS detector was programmed for recording in two consecutive modes: Enhanced MS (EMS) and enhanced product ion (EPI) analysis. EMS was employed to show full scan spectra, so as to obtain an overview of all of the ions in the sample. Settings used were: declustering potential (DP) − 40 V, entrance potential (EP) − 7 V, and collision energy (CE) − 20 V. EPI mode was performed in order to obtain the fragmentation pattern of the parent ion (s) in the previous scan using the following parameters: DP − 40 V, EP − 10 V, CE − 25 V, and collision energy spread (CES) 0 V. Spectra were recorded in negative ion mode between m/z 100 and 1800 m/z.

Chromatographic separation was performed using a Waters Spherisorb S3 ODS-2 C18 column (4.6 mm × 150 mm, 3 μm, Waters, Milford, MA, USA) at 35 °C. The solvents used were water/formic acid 0.1% (A) and acetonitrile (B), with a flow rate of 0.5 mL/min. The elution gradient for solvent B was the following: 10–15% eluent B up to 5 min, 15–20% B up to 5 min, 20–25% B 10 min, 25–35% B 10 min, 35–50% B 10 min and column re-equilibration for 10 min. Phenolic compounds were identified by comparing their retention time, UV-VIS and mass spectra with those of corresponding standard solutions, when available. Otherwise, peaks were tentatively identified comparing the obtained information with available data from the literature (Clifford, Johnston, Knight, & Kuhnert, 2003; Clifford, Knight, & Kuhnert, 2005; Lee, Kim, Liu, Oh, & Lee, 2005). Quantification was performed using calibration curves prepared with appropriate standards (between 100 and 2.5 mg/L). Limits of detection and quantification were determined, and, in all cases, the coefficient of linear correlation was $R^2 > 0.99$ (supplementary materials, Table S1). The acquisition and data processing were carried out with the Xcalibur® data system (Thermo Scientific, San Jose, CA, USA). The results are expressed in mg per g of dry extract (mg/g). All analyses were made in triplicate ($n = 3$).

2.3. Biological evaluation

2.3.1. Antibacterial and antifungal activity

Escherichia coli (ATCC 25922), *Salmonella enterica* ser. typhimurium (ATCC 13311), *Enterobacter cloacae* (clinical isolate), *Staphylococcus aureus* (ATCC 11632), *Bacillus cereus* (food isolate), *Listeria monocytogenes* (NCTC 7973), *Aspergillus fumigatus* (human isolate), *Aspergillus niger* (ATCC 6275), *Aspergillus versicolor* (ATCC 11730), *Penicillium funiculosum* (ATCC 36839), *Penicillium verrucosum* var. *cyclopium* (food isolate) and *Trichoderma viride* (IAM 5061) were used.

The minimum inhibitory concentrations (MIC) were evaluated by a broth microdilution method as described by Kostić et al. (2017) and determined as the lowest concentrations without visible growth in the microplate wells. The minimum bactericidal and fungicidal concentrations (MBC and MFC) were also determined. The MBC was defined as the smallest concentration with no visible growth after serial sub-cultivation of 10 μL into microdilution plates with 100 μL of tryptic soy broth per well and incubation for 24 h at 37 °C; whereas the MFC was defined as the lowest concentration with no visible growth after serial sub-cultivation of 2 μL of the content of the wells and incubation at 28 °C for 72 h.

Sodium benzoate (E211) and potassium metabisulfite (E224) were used as positive controls. The results were expressed as mg/mL of the resuspended lyophilised extracts.

2.3.2. Antioxidant activity

The cell-based essays of formation of thiobarbituric acid reactive substances (TBARS) and inhibition of oxidative haemolysis (OxHLIA) were performed using previously described methodologies (Silva de Sá et al., 2019; Takebayashi, Chen, & Tai, 2010). The lyophilised extracts were initially redissolved in distilled water (for TBARS) or phosphate-buffered saline (PBS, pH 7.4) (for OxHLIA) to different concentrations and Trolox was used as a positive control in both essays.

TBARS: porcine brain cell homogenates were used, and lipid peroxidation inhibition was evaluated by measuring the colour intensity (532 nm) of the malondialdehyde-thiobarbituric acid complexes formed in the system. The results were expressed as the extract concentration (μg/mL) required to inhibit 50% of the TBARS formation (IC_{50}).

OxHLIA: an erythrocyte solution (2.8%, *v/v*; 200 μL) prepared in PBS was added to 400 μL of: i) extract solution (13–800 μg/mL in PBS), ii) PBS solution (negative control), iii) distilled water (for complete haemolysis), or iv) Trolox (7.81–250 μg/mL). After a pre-incubation (37 °C, 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, Winooski, VT, USA) until complete haemolysis. The results were expressed as IC_{50} values (μg/mL) for a Δt of 60 min and 120 min, which indicate the sample concentration required to protect 50% of the red blood cells from the haemolytic action of AAPH for 60 and 120 min, respectively.

2.3.3. Inhibition of nitric oxide production

The inhibition of nitric oxide production was evaluated as described by Jabeur et al. (2016). After culture, the cell line RAW264.7 was seeded in 96-well plates at 150.000 cells/well and their attachment to the plate allowed overnight. Subsequently, cells were treated with different concentrations of the extracts (6.25–400 μg/mL) for 1 h, followed by stimulation with lipopolysaccharides (1 μg/mL) for 18 h. This procedure allowed observation of the occurrence of induced changes in nitric oxide basal levels. For that, the tested sample and lipopolysaccharides were dissolved in supplemented DMEM, and determination of nitric oxide content was performed using a Griess Reagent System kit (Promega, Madison, WI, USA). Nitrite level produced was determined by optical density measurement at 540 nm, in a microplate reader (Bio-Tek Instruments, ELx800, Winooski, VT, USA), and comparison with the standard calibration curve. Dexamethasone (Sigma, St. Louis, Missouri, EUA; 50 μM) was used as positive control. The results are expressed as the sample concentration (μg/mL) required to inhibit 50% of nitric oxide production (IC_{50}).

2.3.4. Antiproliferative activity

The lyophilised extracts were redissolved in water and successively diluted to obtain various concentrations. The sulforhodamine B essay was used (Guimarães et al., 2013). Extracts were incubated with the tested cell lines (190 μL, 10,000 cells/mL), and final concentrations ranged between 6.25 and 400 μg/mL. Six human tumour cell lines were tested: AGS (gastric adenocarcinoma), CaCo-2 (colorectal adenocarcinoma), HeLa (cervical carcinoma), MCF-7 (breast adenocarcinoma), NCI-H460 (non-small cell lung cancer) and non-tumour hFOB (human foetal osteoblasts). Ellipticine (Sigma, St. Louis, Missouri, EUA) was used as a positive control. The results were expressed as the extract concentration required to inhibit 50% of the cell growth (GI_{50}).

2.4. Statistical analysis

Data were presented as mean ± standard deviation (SD) values. The statistical differences of the means were obtained through one-way analysis of variance ($\alpha = 0.05$). The pheatmap function from the pheatmap package was used to produce clustered heatmaps (Kolde, 2018). Statistical analysis was conducted in R software.

3. Results and discussion

3.1. Phytochemical composition

The phenolic composition of tarragon, basil and French lavender extracts is reported in Tables S2, S3 and S4 of the supplementary material, respectively. The phenolic compounds and their concentrations are also displayed in the heatmaps of Figs. 1, 2 and 3, respectively.

In the heatmaps, the greater the height at which any two objects are joined, the smaller the similarity. In this sense, the left dendrogram organises compounds detected in similar concentrations across different extraction methods; and the upper dendrogram informs about comparable total phenolic content (TPC) across the three extracts produced, for each plant.

In tarragon extracts, twenty phenolic compounds were identified. Fig. 1 and Table S2 suggest that tarragon infusion and HE have a more similar profile and TPC (25.05 and 32.33 mg/g extract, respectively), compared to the decoction (42.27 mg/g extract). In basil extracts, seventeen compounds were classified, and in this case, the decoction and HE appear to be more closely related with each other than each of them with basil infusion (Fig. 2). This was evident by the much higher TPC of the infusion (43.41 mg/g extract) in comparison to the decoction and HE (15.31 and 18.26 mg/g extract), as shown in Table S3. In French lavender extracts, eighteen compounds were identified, and the difference in TPC between methods was less evident than for basil, but still observable. In this case, the infusion and decoction revealed higher and more similar concentration of compounds (41.99 and 43.45 mg/g extract), compared to the HE (37.06 mg/g extract), as indicated by the upper dendrogram of Fig. 3. Overall, the infusions of basil and French lavender stood out for their higher TPC (43.41 and 43.45 mg/g extract), whereas basil decoction revealed the lowest TPC (15.31 mg/g extract).

Comparing the total amount of phenolic acids with the total flavonoids, the former were superior in quantity and diversity in all extracts produced (Tables S2, S3 and S4). In the case of tarragon extracts, four flavonoids were identified, compared to sixteen phenolic acids, and depending on the extract type, total flavonoids concentration ranged between 17.4% and 26.7% of the TPC (Table S2). In both basil and French lavender extracts, only two flavonoids were detected, and the remaining compounds identified were phenolic acids. In basil extracts, flavonoid content ranged from 8.37% to 10.1% of the TPC, whereas in those of French lavender, flavonoid content was between 26.6% and 32.9% of all phenolic compounds content.

Basil and French lavender extracts contained six phenolic acids in common, namely, rosmarinic acid, salvianolic acid B, caftaric acid, caffeic acid, caffeic acid hexoside and 3-*p*-coumarouylquinic acid. Rosmarinic acid was the major compound in these extracts, with concentrations between 5.57 and 17.54 mg/g extract (Fig. 2 and Table S3) in the case of basil, and between 13.59 and 16.91 mg/g extract (Fig. 3 and Table S4) in the case of French lavender. It is evident that the extraction type influenced the recovery of this compound more in the case of basil than French lavender, as suggested by the wider range of concentrations observed the first case. The biological properties of rosmarinic acid that have been reported include anticarcinogenic, antioxidant, antiviral, antibacterial, anti-aging, antidiabetic, cardio-, hepato- and nephroprotective, antidepressant, antiallergic and anti-inflammatory activities (Nadeem et al., 2019).

Tarragon extracts did not show any compounds in common with those detected in the other plant extracts. In this case, quercetin-3-*O*-rutinoside, 5-*O*-caffeoylquinic acid and 1,3,5-*O*-tricaffeoylquinic acid were the most abundant compounds. While the two caffeoylquinic acids were detected in comparable concentrations for the three extraction methods, quercetin-3-*O*-rutinoside content was very dependent on the type of extraction. In terms of potential benefits with therapeutic applications, caffeoylquinic acids have been linked to anti-inflammatory, antioxidant, antibacterial, cancer-related, antiviral, anti-Alzheimer, and neuroprotective activities, and showed potential to improve

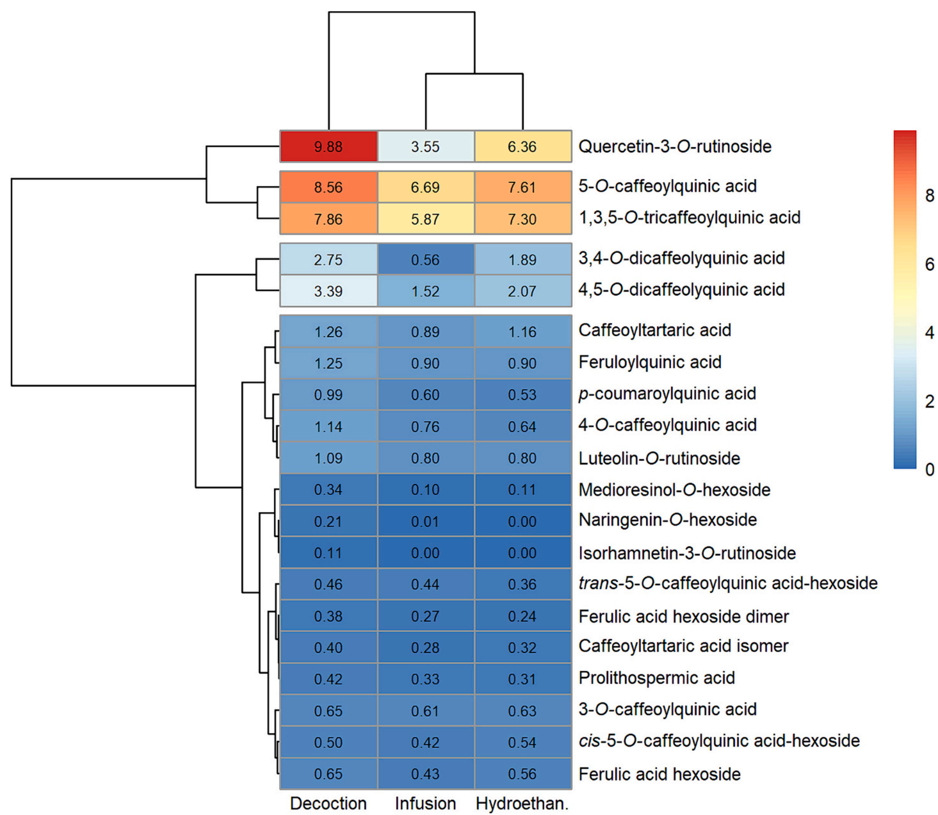

Fig. 1. Clustered heatmap of phenolic compounds identified in tarragon extracts (units: mg/g).

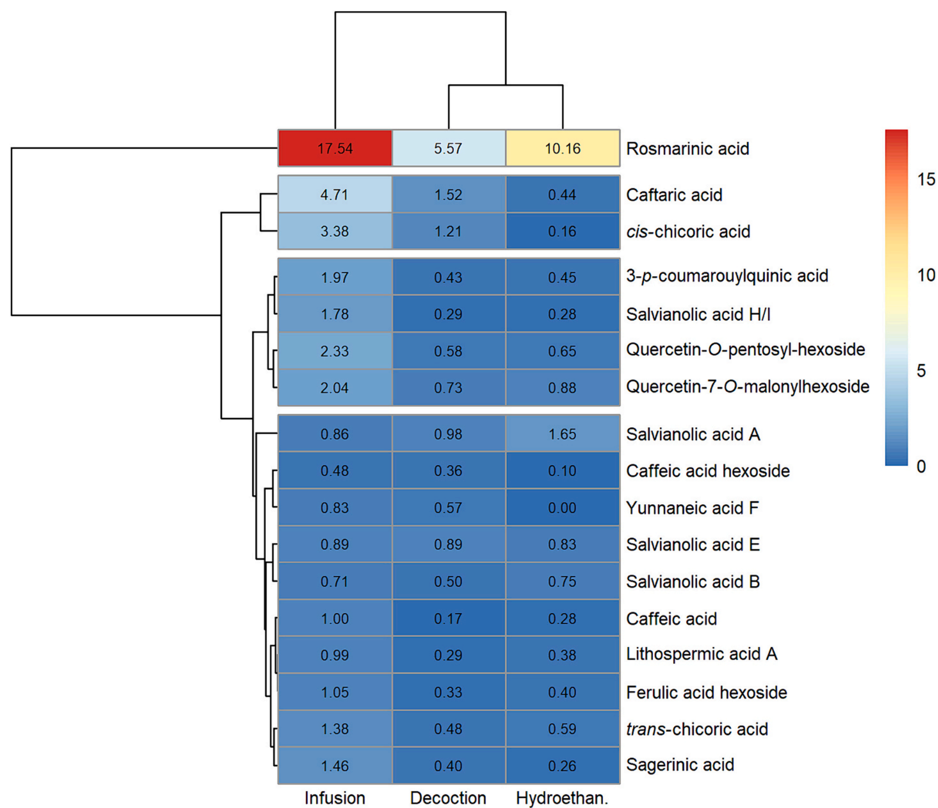

Fig. 2. Clustered heatmap of phenolic compounds identified in basil extracts (units: mg/g).

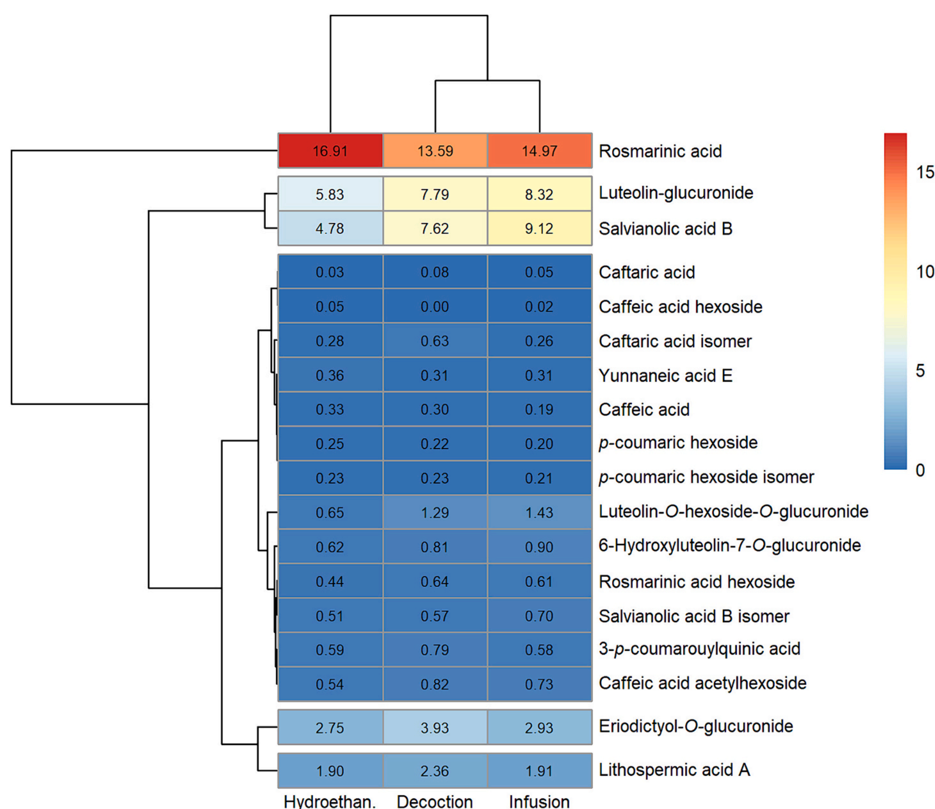

Fig. 3. Clustered heatmap of phenolic compounds identified in French lavender extracts (units: mg/g). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

cognitive decline and lifestyle-related diseases, such as diabetes (Magaña, Kamimura, Soumyanath, Stevens, & Maier, 2021). In its turn, quercetin-3-*O*-rutinoside was reported to have anti-inflammatory, antidiabetic, antioxidant, anticarcinogenic, cytoprotective, antiplatelet, antithrombic, vasoprotective, and cardioprotective activities (Fitzpatrick & Woldemariam, 2017; Raoof & Mohamed, 2018).

The presence of quercetin-3-*O*-rutinoside, hydroxycinnamates (particularly di- and tricaffeoylquinic acids and ferulic acids) and/or other compounds of the same class as those detected in our tarragon extracts was also reported by Lin and Harnly (2012), Miron, Herrero, and Ibáñez (2013), Ribeiro et al. (2016) and Majdan et al. (2020).

In the case of basil extracts, Kwee and Niemeyer (2011) identified rosmarinic and chicoric acids as the dominant phenolic acids, and caftaric and caffeic acids at lower concentrations; Hossain, Rai, Brunton, Martin-Diana, and Barry-Ryan (2010) detected caffeic and rosmarinic acids, as well; and Spréa et al. (2022) and Carocho et al. (2016) also reported the presence of the same phenolic acids, plus sagerinic, salvianolic and yunnaneic acids. These results mostly agree with those of our work.

The phenolic compounds detected by Ceylan, Usta, Usta, Maltas, and Yildiz (2015) and Contreras, Algieri, Rodriguez-Nogales, Gálvez, and Segura-Carretero (2018) in French lavender extracts included caffeic acid, *p*-coumaric acid, rosmarinic acid, ferulic acid, eriodictyol, quercetin, luteolin-7-*O*-glucuronide, apigenin-7-*O*-glucoside and apigenin-7-*O*-glucuronide. The outcomes of these studies and of the chromatographic analysis of *Lavandula stoechas* L. extracts by Palacio et al. (2020) are generally in agreement with our results.

3.2. Antibacterial and antifungal activity

The MIC, MBC and MFC determined for each extract are available in Tables S5 and S6 of the supplementary material, respectively.

All extracts revealed antimicrobial activity against *E. coli*, *S. Typhimurium*, *E. cloacae*, *S. aureus*, *B. cereus* and L. *monocytogenes*. However, the pathogens showed varying susceptibility to different extracts, depending on the extraction method and the plant used, as suggested by the MIC values of 0.5 or 1 mg/mL; and MBC of 1 or 2 mg/mL. Only *B. cereus* was equally affected by the nine extracts, as the MIC and MBC values were the same in all cases (MIC = 0.5 mg/mL; MBC = 1 mg/mL).

All fungi species were susceptible to all the extracts (MIC $\leq$0.5 mg/mL; MFC $\leq$ 1 mg/mL), but especially *T. viride* in the presence of the HE (MIC $\leq$0.125 mg/mL; MFC $\leq$ 0.25 mg/mL). With some exceptions, infusions and decoctions showed uniform activity (MIC = 0.25 and MFC = 0.5 mg/mL) for all tested fungi.

Most infusions, decoctions and HE showed equivalent or superior bactericidal and fungicidal activities when compared to those of E211 and E224. Moreover, the concentration of synthetic additives needed to inhibit each type of bacteria varies widely (from 0.5 to 4 mg/mL, in the case of E211), thus indicating species selectivity. Plant extracts, on the other hand, appeared to inhibit all bacteria at a reduced range of concentrations (0.5 to 1 mg/mL).

Ueda et al. (2021) determined MIC of hydroethanolic basil extracts of 2 mg/mL for *S. aureus*, *L. monocytogenes*, *E. coli*, *S. typhimurium* and *E. cloacae*, 1 mg/mL for *B. cereus*, 0.25 mg/mL for *A. fumigatus, P. funiculosum* and *P. verrucosum*, and 0.5 mg/mL for *A. niger*, *A. versicolor* and *T. viride*. Ribeiro et al. (2016) tested hydroethanolic (80% (*v*/*v*)) tarragon extracts and reported MIC values of 0.12 mg/mL for *S. typhimurium*, 0.08 mg/mL for *S. aureus*, *L. monocytogenes* and *A. niger*, 0.06 mg/mL for *E. coli* and *A. versicolor*, 0.04 mg/mL for *E. cloacae*, *A. fumigatus*, *T. viride*, *P. funiculosum* and *P. verrucosum*, and 0.02 mg/mL for *B. cereus*. For HE obtained with ethanol 96% (*v*/*v*), Behbahani, Shahidi, Yazdi, Mortazavi, and Mohebbi (2017) described higher MIC values: 8 mg/mL for *E. coli*, 4 mg/mL for *B. cereus*, and 2 mg/mL for *S. aureus*, *C. albicans* and *A. fumigatus*. Regarding ethanolic extracts of

French lavender, Canli, Yetgin, Benek, Bozyel, and Altuner (2019) reported MIC values of 0.0359 mg/mL for *C. albicans*, *L. monocytogenes*, *S. enteritidis* and *S. typhimurium*, and 0.01795 mg/mL for two *S. aureus* strains.

3.3. Antioxidant activity

The antioxidant capacity of the extract measured by the TBARS and OxHLIA essays are shown in Table 1.

The results show that all extracts have antioxidant activity, but in different degrees. From both essays, for each extraction method, different plants yield different results ($p < 0.05$). In the TBARS essay, French lavender infusion and decoction, as well as tarragon HE presented the highest antioxidant capacities. In the OxHLIA essay, the three French lavender extracts presented the greatest protective capacity, with the HE standing out for its lowest IC_{50} values, even better than Trolox.

In OxHLIA, the antioxidant behaviour was monitored over time, at two Δt, as some antioxidants may react more quickly and become depleted in the system, while others may offer prolonged antioxidant protection over time. With two exceptions (tarragon and French lavender HE), the concentration necessary to protect 50% of the red blood cells from the haemolytic action of AAPH for 120 min was less than double the concentration necessary for this protection for 60 min. This means that most extracts had anti-haemolytic activity for longer exposure times, whereas the HE of tarragon and French lavender were not as efficient for 120 min compared to 60 min.

The antioxidant activity of tarragon, French lavender and basil extracts has been determined previously; however, comparison of results is not straightforward, as different extraction methods, solvents and antioxidant essays have been employed (Behbahani et al., 2017; Karabagias, Karabagias, & Riganakos, 2019; Miron et al., 2013; Ribeiro et al., 2016; Ueda et al., 2021).

3.4. Inhibition of nitric oxide production

The ability of the extracts to inhibit nitric oxide production is presented in Table 2.

None of the French Lavender extracts revealed inhibition of nitric oxide action at the tested concentrations ($IC_{50} > 400$ μg/mL). Only those of basil and tarragon proved this capability, with basil extracts showing inhibition of nitric oxide action regardless of the extraction method, unlike tarragon, which did not maintain its capacity when the infusion method was used. Tarragon decoction showed the highest inhibition of nitric oxide capacity, considering its IC_{50} of 34.6 ± 0.53 μg/mL, followed by tarragon HE, with $IC_{50} = 44.1 \pm 3.96$ μg/mL.

In line with our results, Takeuchi et al. (2020) also observed the anti-inflammatory effects of basil extracts in vitro, and Eidi, Oryan, Zaringhalam, and Rad (2015) reported on the anti-inflammatory capacity of ethanolic tarragon extracts in adult mice. To our knowledge, studies on the anti-inflammatory potential of French lavender extracts (not essential oils) are scarce. Only two studies were identified: one by Algieri et al. (2016) reporting on the anti-inflammatory effects of the hydroalcoholic French lavender both in vitro and in vivo; and one by Ez Zoubi, Bousta, Lachkar, and Farah (2014), which evaluated the in-vivo anti-inflammatory effect.

Table 2
Inhibition of nitric oxide activity of plant extracts (IC_{50} values; μg/mL) measured by nitric oxide production inhibitory capacity (mean ± SD, $n = 4$).

Plant sample	Infusion	Decoction	Hydroethanolic extract
Tarragon	> 400 ^{b}	34.6 ± 0.53 ^{a}	44.1 ± 3.96 ^{a}
French lavender	> 400 ^{b}	> 400 ^{c}	> 400 ^{b}
Basil	88.6 ± 0.47 ^{a}	64.5 ± 0.68 ^{b}	54.7 ± 5.37 ^{a}

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

3.5. Antiproliferative activity

Table 3 details the antiproliferative activity of extracts. Tarragon infusion was active against HeLa and MCF-7 cells; but only against MCF-7 cells in the case of tarragon HE; French lavender decoction was active against AGS, CaCo-2 and HeLa cells, but also against MCF-7 in the case of its infusion. French lavender and basil HE revealed inhibitory potential against all tumour cell lines.

All infusions and decoctions were non-toxic against hFOB ($GI_{50} > 400$ μg/mL). However, toxicity for hFOB cells was detected in all HE, compromising the use of such extracts as food additives.

From literature, the basil HE produced by Ueda et al. (2021) did not show hepatotoxicity in PLP2 cells (non-tumour) at the maximum tested concentration of 400 μg/mL. Carocho et al. (2016) screened basil decoctions, which revealed antiproliferative activity against HeLa cell line ($GI_{50} = 254 \pm 5$ μg/mL), but no effect on MCF-7 and NCI-H460 lines, and no hepatotoxicity for PLP2 non-tumour cells ($GI_{50} > 400$ μg/mL). Ribeiro et al. (2016) determined the cytotoxic properties of hydroethanolic tarragon extracts and observed their ability to inhibit the growth of MCF-7 ($GI_{50} = 272 \pm 22$ μg/mL) but not of NCI-H460 ($GI_{50} > 400$ μg/mL), in concordance with our study. However, the authors reported inhibitory effects in HeLa cell line ($GI_{50} = 245 \pm 14$ μg/mL), which we did not observe. Furthermore, their extracts did not show any effect on non-tumour cells (PLP2), contrary to ours. As for French lavender extracts, Siddiqui et al. (2019) and Nunes et al. (2017) evaluated their antiproliferative activity using the microculture tetrazolium essay (MTT). Siddiqui et al. (2019) prepared an ethanolic fraction from a methanolic extract and reported a major reduction in the survival percentage of tumour cells HEP G2. Similarly, the results obtained by Nunes

Table 1
Antioxidant activity of plant extracts (IC_{50} values, μg/mL) measured by the TBARS essay (mean ± SD, $n = 9$) and by the OxHLIA essay (mean ± SD, $n = 3$).

Essay		Plant sample	Infusion	Decoction	Hydroethanolic extract
TBARS		Tarragon	392 ± 16.8 ^{c}	549 ± 23.4 ^{c}	177 ± 4.16 ^{a}
		French lavender	182 ± 3.67 ^{a}	186 ± 4.67 ^{a}	239 ± 6.99 ^{c}
		Basil	210 ± 2.98 ^{b}	213 ± 7.47 ^{b}	206 ± 4.53 ^{b}
OxHLIA	$\Delta t = 60$ min	Tarragon	170 ± 2.24 ^{c}	91.8 ± 1.61 ^{c}	48.5 ± 1.64 ^{b}
		French lavender	48.8 ± 1.64 ^{a}	28.6 ± 1.01 ^{a}	15.4 ± 0.44 ^{a}
		Basil	97.2 ± 1.40 ^{b}	49.2 ± 1.21 ^{b}	89.4 ± 2.89 ^{c}
	$\Delta t = 120$ min	Tarragon	262 ± 3.75 ^{c}	141 ± 2.34 ^{c}	117 ± 2.92 ^{b}
		French lavender	94.6 ± 1.44 ^{a}	45.3 ± 1.00 ^{a}	32.5 ± 0.53 ^{a}
		Basil	151 ± 2.05 ^{b}	93.4 ± 2.54 ^{b}	160 ± 4.43 ^{c}

TBARS essay: Trolox IC_{50} value = 5.4 ± 0.3 μg/mL. OxHLIA essay: Trolox IC_{50} value = 21.8 ± 0.25 μg/mL ($\Delta t = 60$ min) and 43.5 ± 1.00 μg/mL ($\Delta t = 120$ min). For each essay, values with different superscript letters in a column mean significant differences (ANOVA, $p < 0.05$).

Table 3
Antiproliferative activity of plant extracts (GI_{50} values; µg/mL) measured by the sulforhodamine B essay (mean ± SD, $n = 2$).

Extraction	Plant sample	AGS [1]	CaCo-2 [2]	HeLa [3]	MCF-7 [4]	NCI-H460 [5]	hFOB [6]
Infusion	Tarragon	> 400 [b]	> 400 [b]	323 ± 7.48 [a]	117 ± 2.01 [a]	> 400	> 400
	French lavender	223 ± 5.39 [a]	259 ± 0.03 [a]	255 ± 36.2 [a]	213 ± 2.34 [b]	> 400	> 400
	Basil	> 400 [b]	> 400 [b]	> 400 [b]	> 400 [c]	> 400	> 400
Decoction	Tarragon	> 400 [b]	> 400 [b]	> 400 [b]	> 400	> 400	> 400
	French lavender	177 ± 7.26 [a]	315 ± 0.14 [a]	342 ± 5.07 [a]	> 400	> 400	> 400
	Basil	> 400 [b]	> 400 [b]	> 400 [b]	> 400	> 400	> 400
Hydroethanolic extract	Tarragon	> 400 [c]	> 400 [c]	> 400 [c]	237 ± 2.23 [b]	> 400 [c]	290 ± 3.05 [c]
	French lavender	234 ± 5.40 [b]	294 ± 0.35 [b]	310 ± 4.60 [b]	190 ± 1.21 [a]	306 ± 4.51 [a]	257 ± 2.06 [a]
	Basil	113 ± 9.55 [a]	264 ± 1.53 [a]	257 ± 9.78 [a]	186 ± 0.04 [a]	366 ± 4.01 [b]	275 ± 0.60 [b]

Ellipticine GI_{50} values: 1.23 ± 0.03 µg/mL (AGS), 1.21 ± 0.02 µg/mL (CaCo-2), 1.91 ± 0.12 µg/mL (HeLa), 1.02 ± 0.02 µg/mL (MCF-7), 1.01 ± 0.01 µg/mL (NCI-H460) and 1.21 ± 0.08 µg/mL (hFOB). Values with different superscript letters in a column mean significant differences (ANOVA, $p < 0.05$).

et al. (2017) indicate antiproliferative action on HEP G2 cells, but also some impact was measured in fibroblasts, which suggests toxicity against non-tumour cells. Tayarani-Najaran et al. (2021) also investigated the antiproliferative effects of French lavender methanol extract, and the results indicated that pre-treatment of the PC12 cells with the extract could significantly decrease 6-OHDA cytotoxicity and cell apoptosis, thus suggesting an important neuroprotective and anti-apoptotic activity.

3.6. Final remarks

The present study reports on several relevant biological activities of extracts obtained from tarragon, French lavender and basil, using distinct extraction methods and solvents. Even though it may not be a limitation per se, it is important to keep in mind that the results of this and other similar studies are indissociable from and specific for the extraction method, solvent, temperature and extraction time that were tested, as research has shown the significant impact of these variables on compound selectivity (particularly, on the polyphenolic content), and, consequently, on their biological activities (Ferreira-Santos et al., 2024; Silva et al., 2021). What is more, the raw material itself may originate extracts of different compositions and biological capacities, since, for example, the part of the plant that is used (roots, leaves, stems, etc.) and its geographic origin (city, country) are likely to influence the chemical profile of the plant and, therefore, of the extract. Considering this, it is important to guarantee that the methodology and all the conditions used are well described, so that results are reproducible and comparison between results originating from different studies using distinct methodologies are possible.

While the in vitro results of our study shed a light on the potential of these natural agents as antimicrobial, health-promoting additives, further in situ studies are needed to validate their action when they are introduced into food matrices, considering that most plant phenolics have a lower activity in food than in vitro (Oulahal & Degraeve, 2022). Studies have shown that interactions occur between the food matrix and the bioactive compounds of the natural extracts, and that the corresponding biological capacities are differently affected by the distinct components: for example, high protein or high lipid content has been reported to reduce the antimicrobial efficacy of plant extracts (Weiss, Loeffler, & Terjung, 2015). Other matrix characteristics, such as the water activity, pH, and even microflora composition, for example, can also have a role in the biological activities of plant extracts in foods. Furthermore, the environmental conditions to which the food product is exposed can also affect the bioactivities of the plant extracts, as light and high temperatures, for example, can degrade phenolic compounds (Ferreyra, Bottini, & Fontana, 2023). In this sense, when a natural extract is intended to be added to a specific food product, in situ tests must be carried out in that particular matrix to ensure that the biocapacities of the additive are not lost. Often, higher quantities of the extract will be needed in situ to provide the same biological activities as those observed in vitro, which can pose a problem in terms of organoleptic characteristics and, consequently, consumer acceptance.

If the plant extract maintains its efficiency in the food matrix, regulatory approval will still be needed for its implementation as a food additive, and this requires data on the chemical composition of the plant-derived ingredient, information on maximum levels for microorganisms and possible contaminants, as well as exposure assessment and toxicological studies (EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS), 2012; EFSA Scientific Committee, 2009).

Lastly, the economic feasibility of implementing these natural extracts in foods must also be evaluated, as the food industry will not consider replacing synthetic compounds with natural alternatives if the latter are more expensive and not cost-effective.

4. Conclusions

The extracts produced using tarragon, basil and French lavender revealed antimicrobial, antifungal and antioxidant properties. No trend was observed as far as the extraction method (infusion, decoction or hydroethanolic maceration) that would provide the best bioactivities in each essay. Instead, higher differences were observed according to the plant tested. To this, French lavender extracts stood out for having overall high TPC, across the methods tested, whereas the phenolic content of the other plant extracts was more influenced by the extraction procedure used. Most French lavender extracts showed the highest oxidation inhibitory capabilities and were able to damage tumour cells; however, they showed no inhibition of nitric oxide production, and, in the case of the HE, toxicity against healthy cells.

Considering these results, these extracts appear as potential natural additives for the preservation of foods and the elaboration of functional foods. Nonetheless, additional studies should be conducted to evaluate the stability and sensory appreciation of extracts when incorporated into foods, the bioavailability and bioaccessibility of the bioactive compounds of the extracts, and the interaction between the extract and the food matrix.

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CRediT authorship contribution statement

Beatriz Nunes Silva: Data curation, Formal analysis, Investigation, Software, Validation, Visualization, Writing – original draft. **Vasco Cadavez:** Conceptualization, Formal analysis, Funding acquisition, Project administration, Resources, Software, Supervision, Validation. **Cristina Caleja:** Data curation, Investigation, Methodology, Validation, Visualization, Writing – review & editing. **Eliana Pereira:** Data curation, Investigation, Methodology, Validation, Visualization, Writing – review & editing. **Ricardo C. Calhelha:** Investigation. **Adriana K. Molina:** Investigation. **Tiane Finimundy:** Investigation. **Marina Soković:** Investigation, Methodology. **José António Teixeira:** Resources, Supervision. **Lillian Barros:** Methodology, Resources, Supervision. **Ursula Gonzales-Barron:** Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – review & editing, Conceptualization, Data curation, Formal analysis, Funding acquisition.

Declaration of competing interest

None.

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2024.139308.

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