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Comparative study on the phenolic composition and *in vitro* bioactivity of medicinal and aromatic plants from the Lamiaceae family

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ABSTRACT

Medicinal and aromatic plants (MAP) have been described as a source of phenolic compounds with potential as antioxidant, antiproliferative and antimicrobial agents. MAP from the Lamiaceae family (*Origanum vulgare* L., *Thymus vulgaris* L., *Ocimum basilicum* L., *Salvia officinalis* L., *Melissa officinalis* L., and *Matricaria chamomilla* L.) were selected to perform a phytochemical and biological screening for their further exploitation as natural bioactive ingredients. The total content of phenolic compounds varied from 184.02 mg/g extract in *M. officinalis* to 17.97 mg/g extract in *M. chamomilla.* Caffeic and rosmarinic acids were the main phenolic acids found in the respective hydroalcoholic extracts. The extracts showed a promising antioxidant activity *in vitro*, being related the phenolic compositions of the extracts, furthermore, all extracts being able to combat lipid peroxidation in TBARS assays with an IC_{50} under 26 μg/mL, moreover all the plant extract has prevented the oxidative haemolysis in OxHLIA assays at concentrations below 67 μg/mL in a Δt 60 min and under 118 μg/mL for a Δt 120 min. Regarding to the bactericidal and fungicidal action the plant extracts were able to inhibit growth against bacteria associated with food hazards, such as *Salmonella typhimurium* (MIC < 1) and *Listeria monocytogenes* (MIC < 1), regarding to fungicidal activity it can be highlighted the MIC values under to 0.25 for *Aspergillus versicolor* and *Trichoderma viride*. Overall, the selected Lamiaceae plants stood out as a source of active phytochemicals that can be used by different industries, such as food and cosmetics.

1. Introduction

By definition, "a medicinal plant is any plant that contains substances that can be used for therapeutic purposes or which are precursors for the synthesis of useful drugs" (Sofowora et al., 2013). Throughout history, plants have been used as foods and medicinal agents, due to their therapeutic and nutritional properties. With the development of modern science, it has been shown that the phytotherapeutic effects of plants are related to biologically active compounds formed through secondary metabolites (Kralova and Jampilek 2021). Phenolic compounds are considered one of the main groups of secondary metabolites in plants (Wang et al., 2020). Their bioactive potential is mainly due to their action against free radicals by an antioxidant, redox and metal chelation capacity, acting as reducing agents, hydrogen donors or singlet oxygen quenchers (Carocho & Ferreira, 2013). Therefore, several health beneficial effects are attributed to these compounds, such as antioxidant, anti-inflammatory, antibacterial, cytotoxic actions, among others (Ferreira et al., 2017). Moreover, in recent years, there has been a global trend toward using natural substances from plants as source of antioxidants and functional ingredients, being exploited by the food industry in order to suit the consumer market (Dziki et al., 2014). In addition, their biological activities have also been explored in other industry sectors, such as in the cosmetic, packaging and textile industries (Albuquerque et al., 2021). Consequently, a great interest has been expressed in medicinal and aromatic plants (MAP), as they are considered an underexplored reservoir of valuable substances, and their research is ongoing work (Christaki et al., 2019).

Among different families, Lamiaceae stands out for including plants

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commonly used worldwide, mainly in culinary as aroma and/or flavour enhancers. Besides being used as food additives, the Lamiaceae family is a source of phytochemical compounds that have beneficial effects on the consumers health (Kozłowska et al., 2015), such as antioxidants (Ydyrys et al., 2021), antibacterial (Fidan et al., 2019) and cytotoxic (Ghalkhani et al., 2021) properties, associated to the quantity and quality of phenolic compounds present in their extracts.

For this study, six MAP from the Lamiaceae family (namely *Origanum vulgare* L., *Thymus vulgaris* L., *Ocimum basilicum* L., *Salvia officinalis* L., *Melissa officinalis* L., and *Matricaria chamomilla* L.) were selected conform a previous triage in respect of the bioactive potential to perform possibility of including extracts from MAEs belonging to the Lamiaceae family used on a daily basis, enhancing the use of these natural bioactive ingredients rather than synthetic antioxidants or antimicrobial agents in food and/or cosmetic products can be explored, meeting the trend in the reduction of artificial products by these industries, adding values to new products.

2. Materials and methods

2.1. Samples preparation

The selected Lamiaceae plants were obtained dried from a producer specialised in MAP (Cantinho das Aromáticas, Porto, Portugal) and transported to the laboratory at room temperature. To obtain the hydroethanolic extract 2 g of the dry plant were extracted with 50 mL of ethanol:water (80:20, *v/v*) under stirring, at room temperature for 1 h. The mixture was filtered through a Whatman paper filter No. 4, and the residue was re-extracted by repeating the procedure. After gathering the two filtrates, ethanol was eliminated under vacuum at 40 °C by using a rotary evaporator (Büchi R-210, Flawil, Switzerland). The obtained solution was frozen and further lyophilized (Freezone 4.5, Labconco, Kansas City, MO, USA). Water was treated in a Milli-Q water purification system (TGI Pure Water Systems, Greenville, SC, USA). Ethanol (98.8 %) was of analytical grade from Fisher Scientific (Lisbon, Portugal).

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

The phenolic compounds were determined according to Spréa, Fernandes, Calhelha, et al. (2020). The samples were dissolved in an aqueous solution of ethanol (20 % *v/v*) up to a final concentration of 10 mg/mL and filtered through disposable 0.22 μm filters. The MAP extracts were analysed using a Dionex Ultimate 3000 ultra-performance liquid chromatographic equipment (Dionex Ultimate 3000 UPLC and Linear Ion Trap LQT XL, Thermo Scientific, San Jose, CA, USA) coupled with a diode array detector and an electrospray ionisation mass spectrometry detector (HPLC-DAD-ESI/MS). Double online detection was carried out in the DAD using 280 nm and 370 nm as the preferred wavelengths and in a mass spectrometer (MS) connected to the HPLC system via the DAD cell outlet. Data acquisition and processing were achieved with the Xcalibur® data system (Thermo Scientific, San Jose, CA, USA). Spectra were recorded in negative ion mode between *m/z* 100 and 1500. The phenolic compounds were identified by comparing their retention time, UV–vis and mass spectra with those obtained from standard compounds, when available, and with data from scientific literature. Standards (*p*-coumaric acid, naringenin, quercetin-3-*O*-glucoside, rosmarinic acid, apigenin-7-*O*-glucoside, protocatechuic acid, caffeic acid, catechin, chlorogenic acid and taxifolin from Sigma-Aldrich) were also used to obtain the calibration curves required for quantifying the identified compounds in each analysis. The results were expressed in mg per g of lyophilized extract. For each formulation, the analyses were carried out in triplicate. Acetonitrile (99.9 %), *n*-hexane (95 %) and ethyl acetate (99.8 %) were of HPLC grade and ethanol (98.8 %) was of analytical grade from Fisher Scientific (Lisbon, Portugal).

2.3. Bioactivity evaluation

2.3.1. Antioxidant activity

The cell-based assays of inhibition of oxidative haemolysis (OxHLIA) and formation of thiobarbituric acid reactive substances (TBARS) *in vitro* were performed to evaluate the antioxidant activity of the plant extracts. The extracts were initially diluted in water to different concentrations and Trolox was used as a positive control.

OxHLIA assay. A red blood cell solution (2.8 %, *v/v*; 200 μL) prepared in phosphate-buffered saline (PBS, pH 7.4) was mixed with 400 μL of either: extract solution (6.25–700 μg/mL in PBS), PBS (negative control), distilled water (baseline), or the 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) was added, and the optical density was measured at 690 nm every ~ 10 min in a microplate reader (Bio-Tek Instruments, ELX800) until complete haemolysis. The results were expressed as IC_{50} values (μg/mL) for Δt of 60 and 120 min (Spréa, Fernandes, Finimundy, et al., 2020), which translate the extract concentration required to protect 50 % of the red cells from the haemolytic action of AAPH for 60 and 120 min.

TBARS assay. The extracts (0.039–2.5 mg/mL) were tested for their ability to prevent the ferrous sulphate-induced lipid peroxidation using porcine brain cell homogenates by monitoring the colour strength (at 532 nm) provided by malondialdehyde-thiobarbituric acid (MDA-TBA) complexes (Spréa, Fernandes, Calhelha, et al., 2020). The results were expressed as IC_{50} (μg/mL), which translate the extract concentration providing 50 % of antioxidant activity).

2.3.2. Cytotoxic potential

The plant lyophilized extracts were dissolved in water and successively diluted to obtain the stock solutions (0.125–8 mg/mL). The cytotoxic potential was evaluated using the sulforhodamine B assay (Spréa, Fernandes, Calhelha, et al., 2020) by incubating the extracts with the tested cell lines (190 μL, 10,000 cells/mL) with the final concentrations tested corresponding to 6.25–400 μg/mL. Four human tumour cell lines were tested, namely the human gastric epithelial cell line (AGS), human colorectal adenocarcinoma (CaCo2), breast carcinoma (MCF-7), and non-small cell lung cancer (NCI-H460) (Leibniz-Institute DSMZ - German Collection of Microorganisms and Cell Cultures GmbH). The results were expressed in terms of extract concentration with the ability to inhibit 50 % of the cell growth (GI_{50} value). Ellipticine (Sigma-Aldrich, Saint Louis, MO, USA) was used as a positive control. Fetal bovine serum (FBS), L-glutamine, Hank's balanced salt solution (HBSS), trypsin-EDTA (ethylenediaminetetraacetic acid), penicillin/streptomycin solution (100 U/mL and 100 mg/mL, respectively), RPMI-1640 and DMEM media were from Hyclone (Logan, UT, USA). Acetic acid, formic acid, ellipticine, sulforhodamine B (SRB), trypan blue, trichloroacetic acid (TCA) and Tris were from Sigma-Aldrich.

2.3.3. Antimicrobial activity

The minimum inhibitory, bactericidal and fungicidal concentrations (MICs, MBCs and MFCs, respectively) were determined using 96-well microdilution plates with a flat bottom (Spectar Cacak, Cacak, Serbia) as previously described by Kostić et al. (2017). The following Gram-positive bacteria: *Staphylococcus aureus* (ATCC 11632), *Bacillus cereus* (clinical isolate), *Listeria monocytogenes* (NCTC 7973) and Gram-negative bacteria: *Escherichia coli* (ATCC 25922), *Salmonella* Typhimurium (ATCC 13311) and *Enterobacter cloacae* (clinical isolate) were used to determine the potential antimicrobial activity of the samples. For antifungal assays, six micromycetes were used, namely *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). For positive control, two food grade antioxidant chemical compounds were used, respectively E221: Sodium sulphite; E224:

Table 1
Content of the phenolic compounds identified in the selected Lamiaceae plants.

Peak	Rt (min)	λ^{max} (nm)	$[M-H]^-$ *m/z*	MS^2 (*m/z*)	Tentative Identification	Content (mg/g extract)
***Melissa officinalis* L.**						
1	4.71	327	341	179(100)	Caffeic acid hexoside[7]	1.43 ± 0.01
2	6.6	288sh326	537	493(47), 359(100), 313(3), 295(20), 269 (5),197(42), 179(57)	Lithospermic acid A[4]	1.9 ± 0.2
3	8.59	324	179	135(100)	Caffeic acid[7]	1.8 ± 0.1
4	15.04	330	439	359(100), 179(9), 161(28),135(5)	Sulphated rosmarinic acid isomer *I*[4]	3.9 ± 0.4
5	15.42	331	439	359(100), 179(11), 161(22),135(15)	Sulphated rosmarinic acid isomer II[4]	5.17 ± 0.01
6	16.5	330	439	359(100), 179(10), 161(37),135(26)	Sulphated rosmarinic acid isomer III[4]	4.9 ± 0.4
7	17.19	260sh329	719	539(28), 521(15), 359(100),197(31), 179(4), 161(19), 135(8)	Sagerinic acid isomer *I*[4]	3.1 ± 0.1
8	18.58	258sh330	719	539(20), 521(12), 359(100),197(21), 179(14), 161(29), 135(5)	Sagerinic acid isomer II[4]	2.63 ± 0.04
9	19.45	330	359	197(79), 179(68), 161(100),135(21)	Rosmarinic acid[4]	120. ± 1
10	22.93	345	461	285(100)	Luteolin-*O*-glucuronide[11]	8.13 ± 0.03
11	26.03	289sh326	537	493(53), 359(100), 313(5), 295(18), 269(3), 197(44), 179(64)	Lithospermic acid B[4]	25 ± 1
12	31.96	324	717	537(4), 519(100), 493(7), 359(18), 339(14)	Salvianolic acid[4]	4.53 ± 0.02
					TPA	175 ± 2
					TF	8.13 ± 0.03
					TPC	184 ± 2
***Origanum vulgare* L.**						
1	4.85	310	341	179(100)	Caffeic acid hexoside[7]	0.81 ± 0.04
2	8.72	323	617	287(100)	Eriodictyol-*O*-hexoside-*O*-rutinosideo[2]	1.16 ± 0.01
3	12.07	290	303	285(78), 125(100)	Taxifolin[10]	1.57 ± 0.01
4	13.28	344	537	493(100), 359(88), 313(10), 295(53), 197(16), 179(35), 161(73), 135(50)	Lithospermic acid A[4]	8.76 ± 0.01
5	14.71	265sh290	421	259(21), 153(100)	4-(3,4-Dihydroxybenzoyloxymethyl)-phenyl-*β*-D-glycopyranoside isomer I[6]	5.0 ± 0.1
6	15.24	264sh296	421	259(23), 153(100)	4-(3,4-Dihydroxybenzoyloxymethyl)-phenyl-*β*-D-glycopyranoside isomer II[6]	29 ± 1
7	16.03	265sh294	421	259(32), 153(100)	4-(3,4-Dihydroxybenzoyloxymethyl)-phenyl-*β*-D-glycopyranoside isomer III[6]	6.1 ± 0.2
8	16.92	337	717	537(4), 519(100), 493(7), 359(18), 339(14)	Salvianolic acid A[4]	6.3 ± 0.3
9	19.29	326	719	539(17), 521(15), 359(100), 197(22), 179(26), 161(81), 135(7)	Sangerinic acid isomer *I*[4]	6.33 ± 0.01
10	20.06	343	359	197(49), 179(45), 161(100), 135(21)	Rosmarinic acid[4]	63.4 ± 0.3
11	21.54	330	719	539(17), 521(15), 359(100), 197(22), 179(26), 161(81), 135(5)	Sangerinic acid isomer II[4]	11.9 ± 0.4
12	23.88	288sh325	537	493(67), 359(17), 313(31), 295(100), 269(26), 197(20), 179(77)	Lithospermic acid A isomer [4]	18 ± 1
13	28.46	325	717	537(4), 509(100), 493(7), 359(98), 339(84), 295(12), 197(9), 179(16)	Salvianolic acid B [4]	6.3 ± 0.1
14	31.63	328	577	269(100)	Apigenin-7-*O*-rutinoside[11]	5.31 ± 0.3
					TPA	163 ± 3
					TF	8.0 ± 0.3
					TPC	171 ± 2
***Matricaria chamomilla* L.**						
1	4.7	294sh322	341	179(100)	Caffeic hexoside isomer I[7]	0.59 ± 0.03
2	5.1	293sh321	341	179(100)	Caffeic hexoside isomer II[7]	0.47 ± 0.01
3	5.84	322	377	191(90),173(5),163(100),155(3),137(5),119 (4)	*cis*-3-*p*-Coumarouylquinic acid[1]	0.77 ± 0.02
4	6.37	325	353	191(100), 179(9), 135(3)	5-*O*-Caffeoylquinic acid[9]	11 ± 1
5	7.42	325	381	337(5), 293(12), 251(6), 245(31),201(12), 179 (28), 161(100), 135(8), 133(6)	3-CDOA[7]	2.28 ± 0.04
6	8.76	298sh323	179	135(100)	Caffeic acid[7]	0.72 ± 0.01
7	10.99	326	463	317(100)	Myricetin-3-*O*-rhamnoside[3]	0.54 ± 0.01
8	13.93	351	479	317(100)	Myricetin-3-*O*-glucoside[3]	1.39 ± 0.04
9	15.57	338	639	315(100)	Isorhamnetin dihexoside[3]	0.69 ± 0.01
10	16.61	328	609	301(100)	Quercetin-3-*O*-rutinoside[3]	0.62 ± 0.01
11	17.52	344	725	593(25), 285(100)	Kaempferol-*O*-pentosyl-*O*-deoxysosyl-glucoside[3]	0.77 ± 0.03
12	17.78	342	447	285(100)	Luteolin-6-*C*-glucoside[6]	0.88 ± 0.02
13	18.82	344	493	317(100)	Myricetin-*O*-glucuronide[3]	0.84 ± 0.04
14	20.33	326	543	381(100)	4,9-Di-CDOA (or 3,4 di-CDOA) [7]	1.1 ± 0.1
15	21.67	333	431	269(100)	Apigenin-7-*O*-glucoside[11]	1.25 ± 0.05
16	22.41	330	543	381(62), 319(10), 261(85), 221(24), 203(16), 161(82)	Erigoster B (3,9-di-CDOA) [7]	0.55 ± 0.05
17	24.48	339	519	315(100)	Isorhamnetin-3-*O*-acetyl-glucoside[3]	0.605 ± 0.002

(*continued on next page*)

Table 1 (*continued*)

Peak	Rt (min)	λ^{max} (nm)	$[M-H]^-$ *m/z*	MS^2 (*m/z*)	Tentative Identification	Content (mg/g extract)
18	25.96	326	549	505(100), 301(74)	Quercetin-*O*-malonylglucoside[3]	0.573 ± 0.002
19	26.88	334	473	269(100)	Apigenin-7-*O*-acetylglucoside[11]	1.5 ± 0.1
					TPA	18 ± 1
					TF	8.8 ± 0.1
					TPC	27 ± 1
***Thymus vulgaris* L.**						
1	4.94	263	305	219(45), 179(41), 125(100)	Gallocatechin[8]	3.5 ± 0.1
2	5.92	314	341	179(100)	Caffeic acid hexoside[7]	0.48 ± 0.02
3	6.66	315	387	369(25), 207(100), 163(47)	Caffeic acid acetylhexoside[7]	0.47 ± 0.02
4	8.72	321	593	473(100), 455(9), 383(18), 353(26)	Apigenin-6,8-di-*C*-hexose[6]	1.4 ± 0.01
5	9.29	321	593	503(21), 473(100), 383(17), 353(32)	Apigenin-*C*-Hexoside-*O*-Hexoside[6]	2.5 ± 0.1
6	12.04	330	637	351(100), 285(47)	Luteolin-*O*-diglucuronide isomer I[11]	1 ± 0.1
7	12.85	316	563	473(93), 443(100), 383(27), 353(31), 287(5)	Apigenin-*C*-pentosyl-*C*-glucoside[6]	0.519 ± 0.003
8	13.99	325	595	287(100)	Eriodictyol-7-*O*-rutinoside[8]	0.14 ± 0.01
9	15.01	339	463	301(100)	Quercetin-3-*O*-galactoside[3]	0.81 ± 0.03
10	15.82	322	521	359(100), 323(5), 179(6), 161(7)	Rosmarinic acid hexoside[4]	2.5 ± 0.1
11	17.01	343	461	285(100)	Luteolin-7-*O*-glucuronide[11]	6.3 ± 0.3
12	17.83	340	447	285(100)	Luteolin-*C*-glucoside[6]	2.9 ± 0.2
13	19.07	330	555	493(100), 359(85)	Salvianolic acid K[4]	0.8 ± 0.05
14	19.67	328	359	197(15), 179(21), 161(100)	*cis*-Rosmarinic acid[4]	20.6 ± 0.2
15	20.98	330	359	197(19), 179(23), 161(100)	*trans*-Rosmarinic acid[4]	11.4 ± 0.3
16	22.11	328	539	377(100), 307(92), 275(61)	Yunnaneic acid D[4]	0.758 ± 0.004
17	22.69	333	537	493(100), 359(22), 179(3)	Lithospermic acid A[4]	0.97 ± 0.05
18	23.13	326	537	493(100), 359(12)	Lithospermic acid A isomer[4]	0.93 ± 0.05
19	29.64	328	637	351(6), 285(5), 283(100)	Luteolin-*O*-diglucuronide isomer II[11]	1.06 ± 0.04
					TPA	39 ± 1
					TF	17 ± 1
					TF3O	3.5 ± 0.1
					TPC	59 ± 1
***Ocimum basilicum* L.**						
1	4.37	325	311	179(100), 149(83), 135(72)	Caftaric acid[7]	1.14 ± 0.05
2	5.32	286sh324	539	495(44), 359(28), 297(100), 197(33), 179(31), 161(84), 135(16)	Yunnaneic acid D [4]	2.88 ± 0.1
3	5.71	312	295	163(100), 119(60)	*p*-Coumaroyl pentoside acid[1]	1.39 ± 0.01
4	8.77	324	179	135(100)	Caffeic acid[7]	1.05 ± 0.03
5	14.1	325	473	311(25), 293(20), 179(82), 149(100), 135(14)	*cis*-Chicoric acid[7]	1.8 ± 0.1
6	14.88	328	473	311(20), 293(28), 179(76), 149(100), 135(24)	*trans*-Chicoric acid[7]	2.4 ± 0.1
7	16.48	346	609	301(100)	Quercetin-3-*O*-rutinoside[3]	2.08 ± 0.04
8	17.61	336	717	537(14), 519(100), 493(9), 359(28), 339(8)	Salvianolic acid A[4]	1.82 ± 0.04
9	18.94	337	717	537(8), 519(100), 493(11), 359(19), 339(15)	Salvianolic acid B isomer *I*[4]	3.14 ± 0.04
10	19.6	328	719	539(18), 521(14), 359(100),197(21), 179(24), 161(89), 135(8)	Sagerinic acid[4]	2.01 ± 0.02
11	20.29	280sh326	359	197(71), 179(66), 161(100),135(31)	*cis*-Rosmarinic acid[4]	29.0 ± 0.9
12	23.14	327	359	197(76), 179(45), 161(100),135(21)	*trans*-Rosmarinic acid[4]	1.9 ± 0.2
13	24.72	325	717	537(5), 519(100), 493(8), 359(18), 339(14)	Salvianolic acid B isomer II[4]	3.6 ± 0.1
					TPA	52 ± 2
					TF	2.08 ± 0.04
					TPC	54 ± 2
***Salvia officinalis* L.**						
1	4.87	325	473	311(46), 293(17), 179(81), 149(100), 135(12)	Caftaric acid hexoside[7]	0.54 ± 0.02
2	5.15	324	341	179(100)	Caffeic acid hexoside isomer I [7]	0.52 ± 0.02
3	5.76	315	341	179(100)	Caffeic acid hexoside isomer II[7]	0.46 ± 0.01
4	6.65	282sh313	447	401(71), 269(100)	Apigenin-*O*-glucuronide[5]	1.84 ± 0.1
5	7.4	287sh324	387	369(26), 207(100), 163(47)	Caffeic acid acetylhexoside[7]	0.41 ± 0.03
6	8.75	325	593	473(100), 383(22), 353(41)	Apigenin-*C*-Hexoside-*O*-Hexoside[11]	6.9 ± 0.2
7	10.88	324	537	519(84),341(10),179(32),161(48),135(10)	Salvianolic acid *I*[4]	1.21 ± 0.01
8	12.8	328	637	351(100), 285(47)	Luteolin-*O*-diglucuronide[11]	1.4 ± 0.1
9	13.7	327	533	489(100), 285(18)	Luteolin-*O*-malonylhexoside isomer I[11]	2.5 ± 0.1
10	14.65	339	533	489(100), 285(21)	Luteolin-*O*-malonylhexoside isomer II[11]	2.1 ± 0.1
11	14.84	337	533	489(100), 285(19)	Luteolin-*O*-malonylhexoside isomer III[11]	2.0 ± 0.1
12	16.07	326	521	359(100), 197(22), 179(34), 161(74)	Rosmarinic acid hexoside[4]	2.38 ± 0.01
13	16.82	331	593	285(100)	Luteolin-*O*-rutinoside[11]	1.72 ± 0.04
14	17.57	343	461	285(100)	Luteolin-7-*O*-glucuronide[11]	27 ± 1
15	20.1	328	719	539(9), 521(5), 359(100), 197(8)	Sagerinic acid[4]	6.9 ± 0.1
16	20.31	335	359	197(19), 179(23), 161(100)	Rosmarinic acid[4]	51 ± 1

(*continued on next page*)

Table 1 (*continued*)

Peak	Rt (min)	λ^{max} (nm)	$[M-H]^-$ *m/z*	MS^2 (*m/z*)	Tentative Identification	Content (mg/g extract)
17	21.65	331	401	269(100)	Apigenin-*O*-pentoside[11]	5.3 ± 0.3
18	22.5	331	533	489(100), 285(15)	Luteolin-*O*-malonylhexoside isomer IV[11]	5.1 ± 0.1
19	23.83	331	769	285(100)	Methyl-luteolin-*O*-deoxyhexoside-*O*-glucoside-*C*-glucoside[11]	3 ± 0.1
20	25.82	327	563	545(22), 503(41), 473(100), 443(80), 383(95), 353(74)	Apigenin 6-*C*-pentosyl-8-*C*-hexoside[5]	2.4 ± 0.1
21	27.96	326	563	545(21), 503(34), 473(100), 443(78), 383(94), 353(81)	Apigenin-8-*C*-pentosyl-6-*C*-hexoside[5]	4.93 ± 0.01
					TPA	63 ± 1
					TF	66 ± 2
					TPC	130 ± 3

TPA: total phenolic acids; TF: total flavonoids; TPC: total phenolic compounds, TF3OL: Flavan-3-ol. Calibration curves:1- *p*-coumaric acid ($y = 301950x + 6966.7$; $R^2 = 0.999$; limit of detection (LOD) = 0.68 μg/mL; limit of quantification (LOQ) = 1.61 μg/mL), 2- naringenin ($y = 18433x + 78903$; $R^2 = 0.9998$; LOD = 0.17 μg/mL; LOQ = 0.81 μg/mL); 3- quercetin-3-*O*-glucoside ($y = 34843x - 160173$; $R^2 = 0.9998$; LOD = 0.21 μg/mL; LOQ = 0.71 μg/mL); 4- rosmarinic acid ($y = 191291x - 652903$; $R^2 = 0.999$; LOD = 0.15 μg/mL; LOQ = 0.68 μg/mL); 5- apigenin-7-*O*-glucoside ($y = 10683x - 45794$; $R^2 = 0.999$; LOD = 0.10 μg/mL; LOQ = 0.53 μg/mL); 6-protocatechuic acid ($y = 214168x + 27102$, $R^2 = 0.9999$; LOD = 0.14 μg/mL; LOQ = 0.52 μg/mL); 7- caffeic acid ($y = 388345x + 406369$; $R^2 = 0.994$; LOD = 0.78 μg/mL; LOQ = 1.97 μg/mL); 8- catechin ($y = 84950x - 23200$, $R^2 = 0.9999$; LOD = 0.17 μg/mL; LOQ = 0.68 μg/mL); 9- chlorogenic acid ($y = 168823x - 161172$; $R^2 = 0.9999$; LOD = 0.20 μg/mL; LOQ = 0.68 μg/mL); 10- taxifolin ($y = 203766x - 208,383$; $R^2 = 1$, LOD = 0.67 μg/mL; and LOQ = 2.02 μg/mL) and 11- apigenin 7-*O*-glucoside ($y = 10683x - 45794$; $R^2 = 0.999$; LOD = 0.10 μg/mL; LOQ = 0.53 μg/mL).

Potassium Metabisulphite.

2.4. Statistical analysis

All the analyses were performed at least in triplicate and the results were expressed as mean ± standard deviation (SD). SPSS Statistics software (IBM SPSS Statistics for Windows, v. 23.0.) was used to analyse differences among the extractions by applying the Student's *t-test* at a 95 % confidence level.

3. Results and discussion

3.1. Phytochemical composition

The phenolic composition of the studied plants is shown in Table 1. Overall, *M. officinalis* stood out for its total phenolic content, reaching values higher than 180 mg/g of extract, followed by *O. vulgare* and *S. officinalis* that showed values of 170.5 and 130 mg/g extract, respectively. The remaining extracts presented lower total phenolic content with *T. vulgaris* and *O. basilicum* being lower than 60 mg/g extract and *M. chamomilla* evidencing the lowest value among the 6 extracts (27.6 mg/g extract). In general, all plant extracts revealed a higher content of total phenolic acids compared with total flavonoids. Regarding that, *M. officinalis* and *O. vulgare* stood out with values of 175.89 and 162.54 mg/g extract of total phenolic acids, respectively. Comparatively, the extracts of *S. officinalis* and *O. basilicum* obtained intermediate values (63 and 52 mg/g extract, respectively) while *T. vulgaris* presented 39 mg/g extract and *M. recutita* had the lowest value among the 6 extracts (17.97 mg/g extract). In terms of qualitative profile, *O. basilicum* showed the highest variety of phenolic acids, presenting a total of 12 different acids in the hydroalcoholic extract. Previously, Carocho et al. (2016) identified 11 phenolic acids in *O. basilicum* extracts, several of which in common with the present study. Some phenolic acids were found across most of the different extracts, which can be related with the plants belonging to the same family. In this sense, rosmarinic acid was the major compound in all extracts except for *M. chamomilla* in which it was not identified. Similarly, this plant was the only one for which salvianolic acids were not detected, which agrees with previous studies (Catani et al., 2021; Caleja et al., 2017). The major compounds in *M. chamomilla* were 5-CQA and a derivative of caffeoyl-2,7-anhydro-3-deoxy-2-octulopyranosonic acid (CDOA) as also reported by Caleja et al. (2017). Moreover, caffeic acid or its hexoside were identified in all the evaluated extracts, although in very low amounts, while lithospermic acid was identified *M. officinalis*, *O. vulgare*

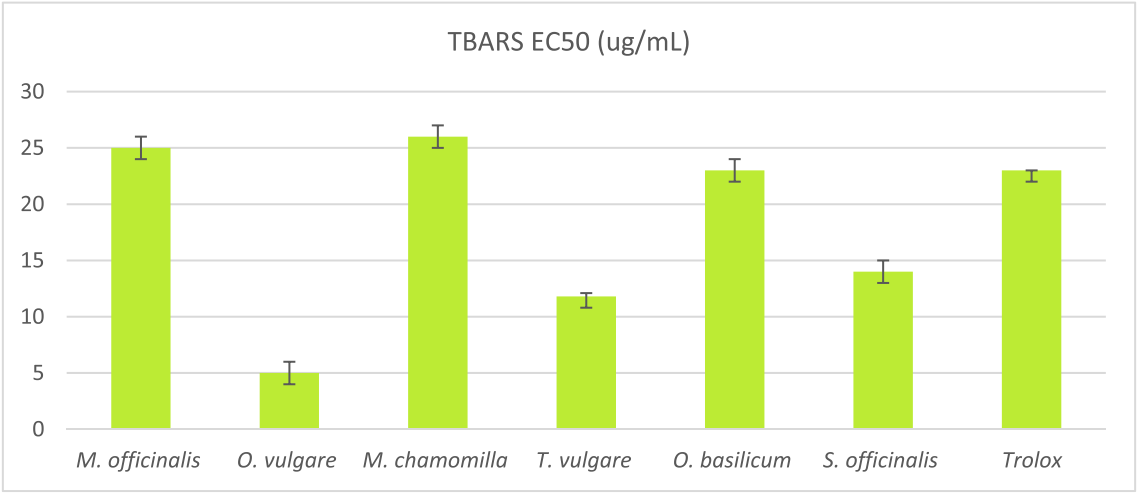

Fig. 1. Antioxidant activity against the lipid peroxidation (TBARS) of the extracts from the selected Lamiaceae plants.

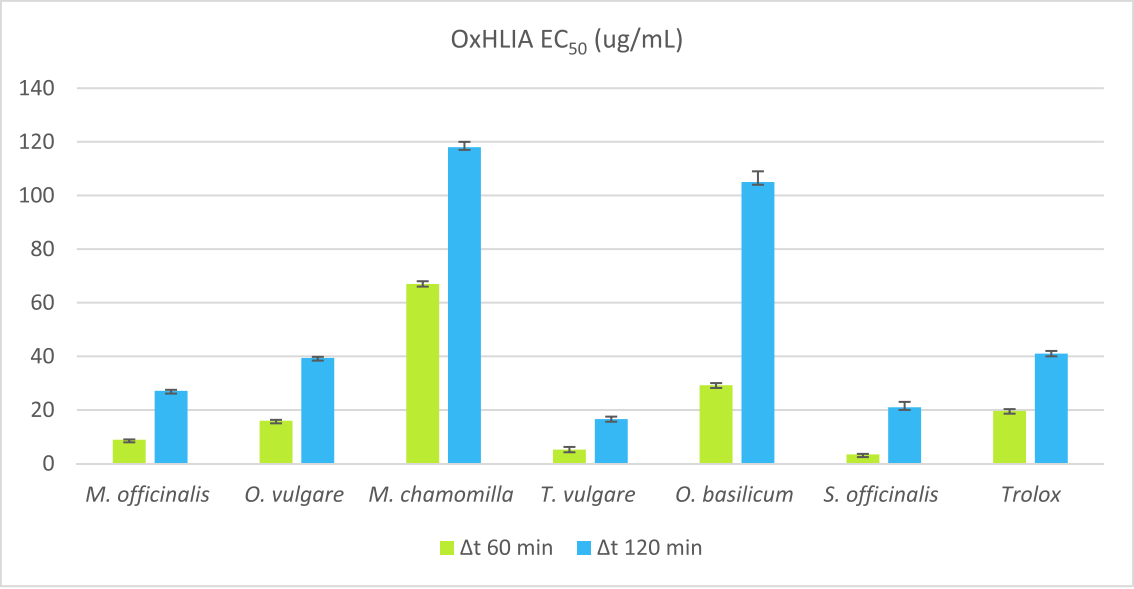

Fig. 2. Antioxidant activity against the oxidative homolysis (OxHLIA) of the extracts from the selected Lamiaceae plants.

and *T. vulgare* as also described by Aubert et al. (2019), Martins et al. (2014) and Martins et al. (2015).

Salvia officinalis was the studied species that presented the highest number of different flavonoids, 13 in total. These were derivates of apigenin and luteolin, with the major compounds being luteolin-*O*-glucuronide (27 mg/g extract) and apigenin-*C*-hexoside-*O*-hexoside (6.9 mg/g extract), which were also the main flavonoids in *T. vulgaris*. Previous studies also described rosmarinic acid followed by luteolin-*O*-glucuronide as the major compounds of these two species (Maliki et al., 2021; Martins, et al., 2015; Pereira et al., 2018). This flavonoid has been also reported to be present in high amounts in *O. vulgare*, but contrary to the results reported by Martins et al. (2014) it was not detected in this study. Derivatives of luteolin and apigenin were also identified in *M. chamomilla,* which in addition presented a great variety of flavonoids as it also evidenced derivatives of myricetin, isorhamnetin and quercetin. Nevertheless, all flavonoids identified in this extract were in concentrations lower than 1.5 mg/g extract. Among the studied plants, *O. vulgare*, *M. officinale* and *O. basilicum* were those that showed the lowest variety of flavonoids, and together with *M. recutita* presented a total flavonoids content < 10 mg/g extract. By the contrary, *S. officinalis* stood out for its high flavonoid content (66 mg/g extract), followed by *T. vulgaris* with 17 mg/g extract of the extract. It is worth mentioning that *T. vulgaris* extract was the only one that presented in its composition flavan-3-ols.

3.2. Bioactive properties

3.2.1. Antioxidant activity

The results of the two cell-based assays performed to evaluate the antioxidant activity of the plant extracts are shown in Figs. 1 and 2. The extracts prepared from *O. vulgare*, *T. vulgaris*, and *S. officinalis* gave the best results in the lipid peroxidation inhibition by the TBARS assay, with IC_{50} values between 5 and 14 μg/mL, in agreement with the results previously reported by Coelho-Fernandes et al. (2021) and Martins et al. (2015), while the extracts of *O. vulgare*, *M. chamomilla*, and *M. officinalis* showed higher IC_{50} values (23–26 μg/mL). In the OxHLIA assay, *T. vulgaris* and *S. officinalis* showed the best results in relation to the antihaemolytic activity, as the obtained IC_{50} values were lower when compared with Trolox, a water-soluble vitamin E analogue widely used as a control antioxidant in biochemical tests. The *M. chamomilla* extract was the only one that showed a relatively higher IC_{50} in OxHLIA compared with Trolox, which can be explained by its lower content of phenolic compounds. Nevertheless, in the TBARS assay, this extract showed similar results to *M. officinalis* and *O. basilicum* (IC_{50} of approximately 25 μg/mL). Overall, these results demonstrate the high antioxidant activity of the selected MAP of the Lamiaceae family.

Table 2
Cytotoxicity activity of the extracts from the selected Lamiaceae plants.

	Tumour cell growth inhibition (GI_{50} values, μg/mL)			
	AGS	Caco2	MCF_7	NCI-H460
M. officinalis	57 ± 5^{c}	96.3 ± 0.4^{c}	372 ± 1^{a}	264 ± 1^{a}
O. vulgare	185 ± 2^{a}	139 ± 7^{b}	219.0 ± 0.3^{c}	186 ± 3^{c}
M. chamomilla	16 ± 1^{d}	$12.4 \pm 0.1^{e,f}$	134 ± 7^{d}	49 ± 3^{e}
T. vulgare	68 ± 2^{b}	21 ± 2^{e}	$224 \pm 17^{b,c}$	58 ± 1^{d}
O. basilicum	21 ± 1^{d}	170 ± 10^{a}	257 ± 11^{b}	196 ± 1^{b}
S. officinalis	19 ± 1^{d}	50 ± 3^{d}	208 ± 5^{c}	24.0 ± 0.3^{f}
Ellipticine	0.9 ± 0.1^{e}	0.8 ± 0.1^{f}	1.020 ± 0.004^{e}	1.01 ± 0.01^{g}

GI_{50} values correspond to the extract concentration responsible for 50 % growth inhibition of tumour cells. Human gastric epithelial cell line (AGS), human colorectal adenocarcinoma (CaCo2), breast carcinoma (MCF-7), and non-small cell lung cancer (NCI-H460). In each column, different letters (a, b, c, d, f and g) mean significant differences ($p < 0.05$) between extracts.

3.2.2. Cytotoxic activity

Regarding cytotoxicity against tumor cell lines (Table 2), in general all the extracts had positive responses against AGS cell line with EC_{50} ranging from 185 μg/mL to 16 μg/mL for *O. vulgare* and *M. chamomilla*, respectively. The extracts of *M. chamomilla*, *T. vulgaris* and *S. officinalis* showed promising results against the NCI-H460 cell line evidenced by their low EC_{50} values (between 24 and 58 μg/mL). Nevertheless, higher concentrations were required for the other 3 extracts. Regarding CaCo2 cells, *O. basilicum*, *T. vulgaris* and *S. officinalis* also showed promising results since they were able to inhibit this cell line with values between 12.4 and 96.3 μg/mL. MCF-7 was the cell line that required the highest concentrations of extracts for its inhibition, the lowest value being obtained by *M. chamomilla* extract (134 μg/mL), being in accordance with.

Overall, the obtained results are in agreement with the literature since positive results for antitumour activity evidenced by these plant's extracts have been described by several authors (Afonso et al., 2019; Al-Dabbagh et al., 2019; Dolghi et al., 2021; Gonçalves et al., 2020; Guran et al., 2021; Taghouti et al., 2020). Moreover, it is worth highlighting

Table 3
Antibacterial and antifungal activity of the extracts from the selected Lamiaceae plants.

	M. officinalis		*O. vulgare*		*M. chamomilla*		*T. vulgare*		*O. basilicum*		*S. officinalis*		Positive controls			
													E211		E224	
Antibacterial activity	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**
Gram-negative bacteria																
Escherichia coli	1	2	1	2	0.5	1	1	2	1	2	0.5	1	1	2	0.5	1
Salmonella typhimurium	1	2	1	2	0.5	1	1	2	1	2	0.5	1	1	2	1	1
Enterobacter cloacae	1	2	2	4	0.5	1	1	2	1	2	0.5	1	2	4	0.5	0.5
Gram-positive bacteria																
Staphylococcus aureus	2	4	1	2	1	2	1	2	2	4	1	2	4	4	1	1
Bacillus cereus	1	2	0.5	1	0.5	1	0.25	0.5	1	2	0.5	1	0.5	0.5	2	4
Listeria monocytogenes	1	2	1	2	0.5	1	0.5	1	1	2	0.5	1	1	2	0.5	1
Antifungal activity	**MIC**	**MFC**	**MIC**	**MFC**	**MIC**	**MFC**	**MIC**	**MFC**	**MIC**	**MFC**	**MIC**	**MFC**	**MIC**	**MFC**	**MIC**	**MFC**
Aspergillus fumigatus	0.5	1	0.5	1	0.5	1	0.5	1	0.5	1	0.5	1	1	2	1	1
Aspergillus niger	0.5	1	1	2	1	2	0.5	1	1	2	0.5	1	1	2	1	1
Aspergillus versicolor	0.25	0.5	0.25	0.5	0.25	0.5	0.25	0.5	0.25	0.5	0.25	0.5	2	2	1	1
Penicillium funiculosum	2	4	0.5	1	2	4	0.5	1	0.5	1	0.25	0.5	1	2	0.5	0.5
Trichoderma viride	0.25	0.5	0.25	0.5	0.25	0.5	0.25	0.5	0.25	0.5	0.25	0.5	1	2	0.5	0.5
Penicillium verrucosum var. *cyclopium*	0.5	1	1	2	1	2	0.5	1	1	2	0.5	1	2	4	1	1

MIC: minimal inhibitory concentration; MBC: minimal bactericidal concentration; MFC: minimal fungicidal concentration; E221: Sodium sulphite; E224: Potassium Metabisulfite.

that the extracts evaluated in this study demonstrated a higher inhibitory capacity as compared to other plants of the Lamiaceae family reported by different authors (Abdallah et al., 2018; Esmaeilbeig et al., 2015).

3.2.3. Antimicrobial activity

Regarding the evaluation of antimicrobial activity, all extracts showed ability to inhibit the six bacteria and six fungi tested, with MIC values $\leq$ 2 mg/mL. For the assayed bacteria, MBC varied from 0.25 mg/mL against *B. cereus* (*T. vulgare*) and 4 mg/mL against *S. aureus* (*O. basilicum*) and *E. cloacae* (*O. vulgare*).

Two food additives, namely sodium benzoate (E211) and potassium metabisulphite (E224), were used as control and its results compared with the extracts, since these additives are used in the food industry. As shown in Table 3, the highest values for bactericidal and bacteriostatic activity (MIC and MBC of 4 mg/mL) were evidenced by the commercial additive E211 against *S. aureus*. Additionally, in general several plant extracts performed better than the commercial additives against some bacteria, particularly against *S. aureus* and *E. cloacae*. With exception of *O. vulgare* extract that obtained identical results to E211, all the other extracts performed better against *E. cloacae* compared with E211 but worse than E224. The same was observed for all extracts against *S. aureus*. By the contrary, all the extracts presented a higher antimicrobial activity against *B. cereus* when compared with E224 but lower that E211. It is worth noticing that *T. vulgare* extract showed a MIC (0.25 mg/mL) lower than both the tested additives. The results obtained against the strains of *L. monocytogenes*, *E. coli* and *S.* Typhimurium, in general were similar to those of the commercial additives with *S. officinalis* and *M. chamomilla* presenting the best results (MIC and MBC of 0.5 and 1 mg/mL, respectively).

Although most reports on the anti-bacterial activity of these plants are mainly focusing on their essential oils rather than on polar extracts, comparing the results with the available literature it is possible to conclude that the MIC values reported for plant extracts of the Lamiaceae family are close to those obtained in this study. Kosakowska et al. (2021) reported MIC values between 2 and 4 mg/mL of *O. vulgare* hydroetanolic extracts against gram-negative bacteria, such as *E. coli, E. coli O157:H7* and *S. enteriditis*, and between 8 and 32 mg/mL for gram-positive bacteria such as *B. cereus, L. monocytogenes* and *S. aureus*. In the study of Assis et al. (2018), *O. basilicum* presented a MIC $>$ 2 mg/mL against *A. baumannii, K. pneumoniae, E. coli* and *P. aeruginosa,* which suggests the inability of the ethanolic extract of the plant to inhibit such strains.

Table 3 also presents the results obtained against the 6 tested strains of fungi, demonstrating an overall good activity for all the extracts when compared to the commercial preservatives tested namely, sodium sulfite (E221) and potassium metabisulfite (E224). Overall, the additive E224 presented a better fungistatic and fungicidal activity than E221 regarding the 6 strains used in this study. Interestingly, all the 6 extracts showed better results than additive E224 against *A. fumigatus*, A. *versicolor* and *T. viridium* and, with values under to 1 mg/mL for MIC and 0.5–1.0 mg/mL for MFC. By the contrary, the best results against *P. verrucosum* were obtained by the additive E224, although *M. officinalis*, *T. vulgare* and *S. officinalis* showed the same MIC value (0.5 mg/mL) as E224. In general, the extracts *of T. vulgaris* and *S. officinalis* stood out both in fungicidal and fungistatic effect as compared to the remaining ones. Also, it is worth noticing the activity against *A. fumigatus* since several strains of the genus *Aspergillus* are known to produce mycotoxins, therefore consisting in a food safety issue.

Up until now, there is still a scarcity of results reported for the antifungal activity exhibited by the extracts of the studied plants, even though several studies highlight the antifungal activity of their essential oils. Nevertheless, Matsubara et al. (2015) reported that the alcoholic extracts of *O. vulgare*, *M. officinalis* and *S. officinalis* showed positive results for the inhibition of *Fusarium oxysporum.*

4. Conclusions

In this work, the extracts of six plants belonging to the Lamiaceae family were studied for their profile in phenolic compounds and *in vitro* biological activities. Considering the phenolic composition, rosmarinic, salvianolic and caffeic acids were the compounds present in most of the extracts. Rosmarinic acid was the major compound in all plants except in *M. chamomilla*. Among the six MAP, *M. officinalis* stood out for its high content of phenolic compounds, followed by *O. vulgare* and *S. officinalis*, while *M. recutita* had the lowest levels. All extracts showed a promising antioxidant activity when compared to Trolox; *S. officinalis*, *T. vulgaris*, *M. officinalis and O. vulgare* stood out in OxHLIA and *O. vulgare* in the TBARS assay. Regarding cytotoxicity, *M. chamomilla* presented the lowest GI_{50} values in all tested tumour cell lines. In addition, all extracts demonstrated an auspicious antimicrobial and antifungal activity compared to the synthetic food preservatives E221 and E224. In general, *M. officinalis* and *S. officinalis* stood out for their *in vitro* bioactivities and phenolic composition. Based on the obtained results, a mixture of different types of extracts may be interesting for possible synergistic effects, bringing additional benefits to consumers' health when incorporated in new products, for example by the development of packaging that prolongs the shelf life of food, and in the incorporation of extracts in the development of new products, as well as in the development of new cosmetics such as anti-ageing creams and products in the beauty industry. Overall, this work demonstrates the usefulness of MAP of the Lamiaceae family as a source of several bioactive compounds with potential interest to be used by different industry as foods and cosmetics.

CRediT authorship contribution statement

Rafael Mascoloti Spréa: Methodology, Investigation, Writing – original draft. **Cristina Caleja:** Methodology, Writing – review & editing. **José Pinela:** Methodology. **Tiane C. Finimundy:** Methodology. **Ricardo C. Calhelha:** Methodology. **Marina Kostić:** Methodology. **Marina Sokovic:** Methodology. **Miguel A. Prieto:** Writing – review & editing. **Eliana Pereira:** Methodology, Writing – review & editing. **Joana S. Amaral:** Conceptualization, Supervision, Writing – review & editing. **Lillian Barros:** Conceptualization, Supervision, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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