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Effects of gamma radiation on the bioactivity of medicinal and aromatic plants: *Mentha* × *piperita* L., *Thymus vulgaris* L. and *Aloysia citrodora* Paláu as case studies

Eliana Pereira, [a] Andreia I. Pimenta,[b] Lillian Barros, [a] Ricardo C. Calhelha, [a] Amilcar L. Antonio, [a] Sandra Cabo Verde [b] and Isabel C. F. R. Ferreira *[a]

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Irradiation is a feasible and safe decontamination technique, being applied to several types of foods including edible and medicinal plants. The aim of this study was to evaluate the effects of different gamma radiation doses (1, 5 and 10 kGy) on the individual profile of phenolic compounds determined by HPLC-DAD-ESI/MS, and the bioactive potential (cytotoxic, virucidal, and antimicrobial activities) of *Aloysia citrodora* Paláu (lemon verbena), *Mentha* × *piperita* L. (peppermint) and *Thymus vulgaris* L. (thyme). The observed cytotoxic activity varied with the plant and with the applied dose, being higher in *Thymus vulgaris* irradiated with 10 kGy. The virucidal activity was also dependent on the radiation dose, but was preserved with irradiation treatment. Gamma rays had no effect on the antimicrobial activity of the studied plants. Otherwise, the effects of gamma radiation on the phenolic profile were heterogeneous, with an increase in some compounds and decrease in others, depending on the species and on the radiation dose.

Introduction

The demand for a diet rich in functional foods leads to a greater consumption of plants because they represent a source of bioactive compounds, which confer important properties for the prevention and/or treatment of several diseases.[1] It has been proven, through several studies, that plants possess different molecules with antioxidant, anti-inflammatory, antimicrobial, hepatoprotective and antitumor activities, acting essentially at the level of several biological systems, such as nervous, digestive, hepatic and immune systems.[2] Some of these molecules are vitamins (A, B, C, D, E and K), carotenoids (lycopene, β-carotene and xanthophylls) and polyphenols (flavonoids such as flavonols, flavones, flavanones, flavanols, anthocyanins, and isoflavones, phenolic alcohols, phenolic acids, tannins, stilbenes and lignans).[3,4]

These molecules interact differently in the body, intervening as neutralizers of the oxidation processes. These compounds extinguish free radicals and act as chelators of metal ions, catalyzing the oxidative reactions.[5] Their putative beneficial effects on lipid metabolism, antidiabetic efficacy, ability to stimulate digestion, and antioxidant and anti-inflammatory potential confer to plant bioactive compounds the character of nutraceuticals.[6]

Taking into account all these benefits, plants are incorporated in several industrial sectors, such as food, cosmetic and pharmaceutical, which leads to the obligation of complying with all phytosanitary safety standards to not damage the final product, and consequently, consumer's health.[7] Thus, irradiation appears as a food processing technique supported by several globally recognized organizations such as the FAO, WHO, IAEA and others.[6] This type of processing is used in several countries and is suitable for different food matrices, extending not only the food shelf life, but also providing its microbiological decontamination.[8]

In this study, it was intended to give continuity and deepen the knowledge of previous studies that evaluated the effects of gamma radiation on the chemical composition and bioactive properties of methanolic and aqueous extracts of different plant species used in traditional medicine.[9–14] This study allows one to understand if the extractability of the compounds depends only on the irradiation process or if the solvent used in the extraction procedure could be more relevant in the extractability of the bioactive compounds. The heated aqueous extracts (used in a previous study[11]) could

[a]*Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal. E-mail: iferreira@ipb.pt; Fax: +351273325405; Tel: +351273303219*

[b]*Centro de Ciências e Tecnologias Nucleares, Instituto Superior Técnico, Universidade de Lisboa, Estrada Nacional 10 (km 1397), 2695-066 Bobadela LRS, Portugal*

affect the extractability of phenolic compounds, due to the fact that some compounds are sensitive to temperature and could undergo degradation. Also, hydroalcoholic extracts, generally, have a higher extractability without presenting loss of compounds; nonetheless, until now only methanolic extracts were explored. Moreover, it is important to explore the use of green solvents (solvents that reduce the impact on the environment and on human health), because not only do they reduce the impact on the environment and reduce human exposure to hazardous chemicals, but they also facilitate the incorporation of these extracts in products within different industrial sectors (pharmaceutical, cosmetic and food). Therefore, the present study evaluates an environmentally friendly solvent (ethanol/water), in order to estimate the extractability of phenolic compounds and its relation with the irradiation process applied. Furthermore, this study reveals the antimicrobial potential of hydroalcoholic extracts in both anti-viral and antibacterial assays, and evaluates this new extraction approach as a process to add an improvement value to aromatic plants as virucidal and antibacterial agents.

For that, *Mentha* × *piperita* L., *Thymus vulgaris* L. and *Aloysia citrodora* Paláu were exposed to gamma radiation and further extracted with a hydroethanolic solution, in order to evaluate the effects on phenolic compounds, cytotoxicity, virucity and antimicrobial activity.

Experimental

Dried samples and gamma radiation

Aloysia citrodora Paláu (lemon verbena), *Mentha* × *piperita* L. (peppermint) and *Thymus vulgaris* L. (thyme) were provided by Lecifarma, Loures, Portugal. For the gamma radiation procedure, dried samples were divided into four groups: control (non-irradiated, 0 kGy) and samples irradiated with three different doses (1, 5 and 10 kGy).

The irradiation was performed as described by Pereira *et al.*[9] using a Co-60 experimental chamber (Precisa 22, Graviner Manufacturing Company Ltd, London, UK) with a total activity of 177 TBq (4.78 kCi), in September 2013. To estimate the dose rate of irradiation, a Fricke dosimeter was used, and to monitor the absorbed dose, a routine dosimeter Amber Perspex (batch X, from Harwell Company, U.K.) was applied, following the procedures previously described by Pereira *et al.*[9]

The estimated dose rate was 1.7 kGy h^{-1}, and the average absorbed doses by the samples were 1.0 ± 0.1 kGy, 5.6 ± 0.2 kGy and 10.5 ± 0.6 kGy. The dose uniformity ratio (D_{max}/D_{min}) was 1.1. In the text and tables, for simplicity, the values were considered as 0, 1, 5 and 10 kGy, for the doses of non-irradiated and irradiated groups, respectively.

Sample extraction

The hydroethanolic extracts (ethanol/water 80 : 20, v/v) were obtained from the dried plant material and the extraction procedure was made according to a study performed by Pereira *et al.*[12] The dried extracts were re-dissolved at different concentrations, for further *in vitro* bioactivity evaluation assays and phenolic compound identification and quantification.

Evaluation of the cytotoxic activity

For cytotoxicity, the extracts were re-dissolved in water at 8.0 mg mL^{-1} and the assay was performed according to a procedure previously described by Pereira *et al.*[13]

Four human tumor cell lines, breast carcinoma (MCF-7), non-small cell lung cancer (NCI-H460), cervical carcinoma (HeLa) and hepatocellular carcinoma (HepG2), were used to determine the cytotoxicity. The cell growth inhibition was measured using the sulforhodamine B assay (SRB, Sigma-Aldrich, St Louis, MO, USA), where the amount of pigmented cells is directly proportional to the total protein mass and therefore to the number of bounded cells. For non-tumor cell line evaluation, a primary culture cell line (PLP2) was used. This culture was obtained from porcine liver in the laboratory and the SRB assay was employed to evaluate the growth inhibition.

The results are expressed as GI_{50} values (μg mL^{-1}, sample concentration that inhibited 50% of cell growth) and ellipticine was used as the positive control.

Evaluation of the virucidal activity

Viruses and cell cultures were prepared according to a procedure described by Pereira *et al.*[13] Human adenovirus type 5 (HAdV-5, ATCC®VR-1516™) and murine norovirus type 1 (MNV-1) strain P3 (kindly provided by Dr Christiane E. Wobus at the University of Michigan Medical School, USA) were used and viral stocks were prepared on confluent A549 cells and Raw 264.7, respectively.

For the virucidal efficacy assay, the plant extracts were evaluated in separate experiments at concentrations of 4.0 and 8.0 mg mL^{-1} in sterile ultrapure water, following a procedure described by Pereira *et al.*[13] and the virus titer was expressed in PFU per milliliter of substrate (PFU mL^{-1}).

The anti-viral neutralization experiments were performed by neutralization assays following a procedure reported by Gilling *et al.*[15] with some modifications, which are reported by Pereira *et al.*[13] In this assay two concentrations of lemon verbena, thyme and peppermint (4 mg mL^{-1} and 8 mg mL^{-1}) were tested in order to achieve the anti-viral neutralization activity. The results represent the log 10 reductions in cell culture infectivity of murine norovirus and human adenovirus and are expressed in PFU mL^{-1}.

Evaluation of the antimicrobial activity

The antibacterial activity was studied using Gram-negative bacteria: *Escherichia coli* (ATCC® 8739™) and *Salmonella enterica* Typhimurium (ATCC® 14028™) and Gram-positive bacteria: *Staphylococcus aureus* (ATCC® 6538™), *Bacillus cereus* (SSIC® 1/1), *Listeria monocytogenes* (ATCC® 15313™) and *Enterococcus faecalis* (ATCC® 29212™) following a procedure previously performed by Sokovic *et al.*[16] The results are expressed as minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC). Streptomycin and penicillin were

used as the standard in the antibacterial assays and sterile ultrapure water was used as a negative control.

Analysis of phenolic compounds

The extracts at a concentration of 10 mg mL^{-1} were analyzed using a Dionex Ultimate 3000 UPLC chromatographic system (Thermo Scientific, San Jose, CA, USA). This system consists of a diode array detector (using 280, 330 and 370 nm as wavelengths) coupled to an electrospray ionization mass detector working in negative mode (Linear Ion Trap LTQ XL mass spectrometer, Thermo Finnigan, San Jose, CA, USA), following a procedure previously performed by the authors.[17] Calibration curves of the available phenolic standards (apigenin-6-*C*-glucoside, caffeic acid, chlorogenic acid, hesperetin, luteolin-7-*O*-glucoside, naringenin, quercetin-3-*O*-rutinoside and rosmarinic acid, Extrasynthese, Genay, France) were constructed based on the UV signal to perform quantitative analysis. In the case of the unavailable commercial standards, the compounds were quantified *via* the calibration curve of the most similar standard available. The results are expressed as mg per g of dry extract.

Statistical analysis

For each one of the plant species three samples were used and all the assays were carried out in triplicate. The results are expressed as mean values and standard deviation (SD), and were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's HSD test with $\alpha = 0.05$ (IBM SPSS Statistics for Windows, Version 23.0., IBM Corp., Armonk, NY, USA).

Results and discussion

Cytotoxic properties of hydroethanolic extracts from non-irradiated and irradiated samples

The results obtained from the cytotoxic evaluation are presented in Table 1. A primary cell culture (PLP2) and four human tumor cell lines (MCF-7, NCI-H460, HeLa and HepG2) were tested using hydroethanolic extracts (ethanol/water, 80 : 20, v/v) prepared from non-irradiated (0 kGy) and irradiated (1, 5 and 10 kGy) lemon verbena, peppermint and thyme samples. All samples revealed antiproliferative activity in all studied cell lines, with GI_{50} values ranging between 57–263, 61–271, and 31–273 μg mL^{-1} in peppermint, thyme and lemon verbena, respectively. The results obtained show a clear difference in comparison with the positive control (ellipticine – values oscillated between 0.91 and 3.22 μg mL^{-1}). However, the study extracts do not represent one isolated compound but a mixture of compounds; therefore, the GI_{50} values presented for these extracts revealed good cytotoxic potential. Regarding the effects of gamma radiation, significant differences ($p < 0.05$) were observed among the tested doses, with thyme revealing a higher activity with a dose of 10 kGy, and presenting antiproliferative activity in most of the studied cell lines (MCF-7, HeLa and HepG2). Otherwise, for peppermint, the control sample (0 kGy) showed lower GI_{50} (higher cytotoxic activity) than the irradiated samples, in MCF-7, NCI-H460 and HeLa cell lines (154, 156 and 242 μg mL^{-1}, respectively). For the HepG2 cell line, no significant differences were observed between the control sample (0 kGy) and the samples subjected to 1 and 10 kGy doses (GI_{50} values ranging between 57 and 71 μg mL^{-1}). Concerning lemon verbena extracts, significant

Table 1 Cytotoxicity (GI_{50} values, μg mL^{-1}) of lemon verbena, peppermint and thyme hydroethanolic extracts prepared from non-irradiated and irradiated samples

	Doses			
	0 kGy	1 kGy	5 kGy	10 kGy
Peppermint				
MCF-7 (breast carcinoma)	154 ± 16c	197 ± 14b	229 ± 17a	201 ± 2b
NCI-H460 (non-small cell lung cancer)	156 ± 10c	191 ± 11b	201 ± 16a	192 ± 1b
HeLa (cervical carcinoma)	242 ± 14c	254 ± 4ab	245 ± 16bc	263 ± 10a
HepG2 (hepatocellular carcinoma)	71 ± 16b	64 ± 21b	110 ± 22a	57 ± 15b
Hepatotoxicity PLP2 (non-tumor cells)	326 ± 19b	313 ± 20b	321 ± 20b	343 ± 27a
Thyme				
MCF-7 (breast carcinoma)	271 ± 26a	235 ± 23b	213 ± 17c	201 ± 3c
NCI-H460 (non-small cell lung cancer)	94 ± 2c	249 ± 5a	228 ± 2b	224 ± 14b
HeLa (cervical carcinoma)	222 ± 7a	228 ± 19a	204 ± 9b	211 ± 16b
HepG2 (hepatocellular carcinoma)	126 ± 46a	74 ± 6b	61 ± 12b	64 ± 6b
Hepatotoxicity PLP2 (non-tumor cells)	>400	>400	>400	>400
Lemon verbena				
MCF-7 (breast carcinoma)	252 ± 4b	192 ± 4c	250 ± 10b	273 ± 5a
NCI-H460 (non-small cell lung cancer)	176 ± 1b	175 ± 8b	234 ± 23a	231 ± 29a
HeLa (cervical carcinoma)	245 ± 3a	233 ± 22b	232 ± 9b	249 ± 14a
HepG2 (hepatocellular carcinoma)	31 ± 10d	47 ± 3c	70 ± 7b	83 ± 18a
Hepatotoxicity PLP2 (non-tumor cells)	349 ± 11b	358 ± 2b	353 ± 26b	371 ± 10a

Positive control (ellipticine) – MCF-7: 0.91 ± 0.04; NCI-H460: 1.03 ± 0.09; HeLa: 1.91 ± 0.06; HepG2: 1.14 ± 0.21; PLP2: 3.22 ± 0.67. GI_{50} values (μg mL^{-1}) correspond to the sample concentration achieving 50% of growth inhibition in human tumor cell lines or in liver primary culture PLP2. In each row different letters mean significant differences ($p < 0.05$).

differences ($p < 0.05$) were observed in the antiproliferative activity of all tested cell lines and within the doses of gamma rays applied. The sample irradiated with 1 kGy stands out in MCF-7, NCI-H460 and HeLa cell lines with higher cytotoxicity (GI_{50} = 192, 175 and 233 μg mL^{-1}, respectively). On the other hand, in the HepG2 cell line, the GI_{50} value was lower in the control sample (31 μg mL^{-1}).

These results evidence heterogeneities related with the irradiation process, highlighting that the antiproliferative activity is linked with the plant species and with the irradiation dose.

Regarding the assays in non-tumor cells, thyme was the only species that did not show toxicity (GI_{50} > 400 μg mL^{-1}), while in peppermint the values ranged between 313 and 343 μg mL^{-1}, and in lemon verbena between 349 and 371 μg mL^{-1}. These results imply a low toxicity index, since the values are very near the maximal tested concentration (400 μg mL^{-1}), and higher than those present for tumor cell lines.

Pereira *et al.*[12] have previously reported the antiproliferative activity of the methanolic extracts of *T. vulgaris* and *M. piperita*, demonstrating that gamma radiation did not produce significant effects on the cytotoxicity of these plants. Nevertheless, when low doses were applied the antiproliferative activity against tumor cells undergoes a slight increase, which could be considered a positive effect of this technology. Furthermore, Pereira *et al.*[13] described the cytotoxicity of infusions prepared from non-irradiated and irradiated *A. citrodora* and *M. piperita*, also verifying that these samples exhibited antiproliferative activity in all tested cell lines. *A. citrodora* samples irradiated with different doses (0, 1 and 10 kGy) did not reveal significant differences. However, *M. piperita* irradiated with 10 kGy stood out with the highest cytotoxic activity among all tested cell lines.

Virucidal activity of hydroethanolic extracts from non-irradiated and irradiated samples

The virucidal efficacy of hydroethanolic extracts from irradiated and non-irradiated samples of lemon verbena, peppermint and thyme was evaluated in two enteric viruses – human adenovirus type-5 (HAdV-5) and murine norovirus type-1 (MNV-1, as a human norovirus surrogate). Both viruses were exposed to two concentrations of extracts (4 and 8 mg mL^{-1}) for 24 h. The resulting data are expressed in log 10 variation ($t_0 - t_{24}$) of the viral titer (PFU ml^{-1}) as shown in Table 2. The obtained results showed a viral titer variation lower than 1 log 10 PFU mL^{-1} for all of the analyzed samples, irrespective of the extract concentration, gamma radiation dose and exposed virus. An exposure control was made where the viruses were incubated for 24 h in the presence of PBS (phosphate-buffered saline) to access the natural loss of infectivity. The obtained values were very similar for both HAdV and MNV. Significant differences between the natural loss of infectivity and the antiviral effect of some extracts were achieved. In the case of HAdV, the virucidal effect of lemon verbena appears to be enhanced after irradiation (5 and 10 kGy). Concerning MNV, the exposure to the lemon verbena non-irradiated extract causes a significant reduction of the viral titer in comparison with the control, even at the lowest concentration. The same virucidal effect was attained with lemon verbena extracts from irradiated samples (5 and 10 kGy).

Unlike lemon verbena, that seems to cause a major effect on reducing the MNV infectious state, peppermint extracts (4 mg mL^{-1}) only lead to significant titer reductions of HAdV-5 (for the non-irradiated and 5 kGy irradiated samples). From all

Table 2 Antiviral efficacy of lemon verbena, peppermint and thyme hydroethanolic extracts prepared from non-irradiated and irradiated samples

	Human adenovirus 5	
	Log_{10} reduction (mean ± SE)	
	4 mg mL^{-1}	8 mg mL^{-1}
Peppermint		
0 kGy	0.23 ± 0.05^{a}	0.03 ± 0.01
1 kGy	0.010 ± 0.005^{c}	0.062 ± 0.004
5 kGy	0.29 ± 0.01^{a,b,d}	0.001 ± 0.006^{b}
10 kGy	0.075 ± 0.005^{c}	−0.10 ± 0.01^{c}
Thyme		
0 kGy	0.11 ± 0.04	0.15 ± 0.03^{d}
1 kGy	−0.10 ± 0.01^{b,c,d}	0.14 ± 0.03^{b}
5 kGy	0.10 ± 0.05^{d}	−0.12 ± 0.08^{c,d}
10 kGy	−0.10 ± 0.02^{c}	0.07 ± 0.03
Lemon verbena		
0 kGy	0.14 ± 0.04	0.12 ± 0.06
1 kGy	0.17 ± 0.07^{d}	0.12 ± 0.06
5 kGy	0.18 ± 0.04	0.19 ± 0.01^{a}
10 kGy	0.22 ± 0.02^{a}	0.19 ± 0.06^{a}
Control	0.07 ± 0.01	
	Murine norovirus 1	
	Log_{10} reduction (mean ± SE)	
	4 mg mL^{-1}	8 mg mL^{-1}
Peppermint		
0 kGy	0.08 ± 0.04	0.10 ± 0.03
1 kGy	0.04 ± 0.01	0.02 ± 0.01
5 kGy	0.053 ± 0.003^{d}	0.08 ± 0.06
10 kGy	−0.038 ± 0.005	−0.129 ± 0.003^{c}
Thyme		
0 kGy	0.014 ± 0.03	−0.02 ± 0.01^{d}
1 kGy	0.06 ± 0.04^{d}	0.11 ± 0.03
5 kGy	−0.16 ± 0.06^{a,b,c}	0.15 ± 0.03^{b,d}
10 kGy	−0.024 ± 0.002	0.09 ± 0.02
Lemon verbena		
0 kGy	0.24 ± 0.06^{a}	0.24 ± 0.05^{a}
1 kGy	0.06 ± 0.03^{b,c,d}	0.18 ± 0.07^{b}
5 kGy	0.22 ± 0.07^{a}	0.23 ± 0.03^{a}
10 kGy	0.21 ± 0.07^{a}	0.25 ± 0.02^{a}
Control	0.13 ± 0.05	

The results represent the log 10 reductions in cell culture infectivity of murine norovirus and human adenovirus (initial titer, 10^5–10^6 PFU mL^{-1}) after 24 h of exposure to lemon verbena, thyme and peppermint extracts (irradiated and non-irradiated) at two concentrations. The experiment was conducted in duplicate ($p \leq 0.05$). [a] Reduction was statistically significant in comparison with the control (with no extract) at the same time exposure. [b] Reductions were significantly different between 4 mg mL^{-1} and 8 mg mL^{-1} of the hydroethanolic extracts. [c] Reductions were significantly different between the doses of irradiation. [d] Reductions were significantly different between the viruses.

the evaluated samples, the viral exposure to thyme hydroethanolic extracts causes the lowest virucidal effect on both viruses, regardless of the different irradiation doses and concentrations.

The viral exposure to some of the extracts seems to cause a negative variation on viral titer, revealing that the virus load after 24 h of contact was superior to the initial one. These results may suggest an increase of the infectious state of the viruses that could be a consequence of stabilization of the viral particle or an enhancement of the host–pathogen interaction and further infection.

Despite being non-enveloped type viruses and being quite similar in terms of environmental resistance, HAdV and MNV demonstrated different behaviors when in contact with some herbal hydroethanolic extracts, although it was not possible to determine any trend in terms of comparable resistance between both viruses.

The radiation treatment of the plant prior to extraction procedures seems to cause different effects on the virucidal potential of the extract depending on the plant, the virus and the absorbed dose. Almost all of the significant variations associated with the radiation processing of the plants seem to cause an increase in the virucidal potential of the extracts, namely, after HAdV incubation with 4 mg mL^{-1} of the irradiated extract of peppermint (5 kGy) and lemon verbena (10 kGy), and with 8 mg mL^{-1} of the irradiated thyme extracts (5 and 10 kGy). For MNV, these differences were only detected after incubation with both concentrations of irradiated lemon verbena samples (5 and 10 kGy). In contrast, the incubation of MNV with 4 mg ml^{-1} of irradiated thyme extract (5 kGy) seems to cause an increase in the infectious potential of the virus.

Overall, the data suggest that the irradiation treatment of lemon verbena, peppermint and thyme seems to preserve the natural properties of the plant against enteric viral pathogens.

The results indicated that 4 mg mL^{-1} of the hydroethanolic extract from peppermint samples non-irradiated and irradiated at 5 kGy had the most virucidal effect on HAdV-5. For MNV-1, lemon verbena extracts (irradiated (5 and 10 kGy) and non-irradiated) appear to be the most effective anti-viral tested samples at both concentrations.

The virucidal properties of plants processed as extracts, infusions or even essential oils have been studied over the years.[13,18–20] The anti-viral properties of herbal samples like peppermint were mostly revealed in enveloped viruses or

Table 3 Antimicrobial efficacy of lemon verbena, peppermint and thyme hydroethanolic extracts prepared from non-irradiated and irradiated samples

	E. faecalis	*E. coli*	*B. cereus*	*S.* Typhimurium	*S. aureus*	*L. monocytogenes*
	MIC[a] (mg mL^{-1})					
Peppermint						
0 kGy	≥50	≥50	4.16	≥50	12.5	≥50
1 kGy	≥50	≥50	4.69	≥50	≥50	≥50
5 kGy	≥50	≥50	4.16	≥50	≥50	≥50
10 kGy	≥50	≥50	3.12	≥50	≥50	≥50
Thyme						
0 kGy	≥50	≥50	9.38	≥50	25	≥50
1 kGy	≥50	≥50	5.21	≥50	25	≥50
5 kGy	≥50	≥50	5.21	≥50	25	≥50
10 kGy	≥50	≥50	4.16	≥50	25	≥50
Lemon verbena						
0 kGy	≥50	≥50	9.38	≥50	12.5	≥50
1 kGy	≥50	≥50	6.25	≥50	12.5	≥50
5 kGy	≥50	≥50	6.25	≥50	12.5	≥50
10 kGy	≥50	≥50	6.25	≥50	12.5	≥50
PEN/STREP	0.002	0.009	≤0.001	0.12	≤0.001	≤0.001
	MBC[a] (mg mL^{-1})					
Peppermint						
0 kGy	≥50	≥50	3.12	≥50	12.5	≥50
1 kGy	≥50	≥50	3.12	≥50	12.5	≥50
5 kGy	≥50	≥50	3.12	≥50	≥50	≥50
10 kGy	≥50	≥50	3.12	≥50	≥50	≥50
Thyme						
0 kGy	≥50	≥50	3.12	≥50	25	≥50
1 kGy	≥50	≥50	3.12	≥50	25	≥50
5 kGy	≥50	≥50	3.12	≥50	25	≥50
10 kGy	≥50	≥50	3.12	≥50	25	≥50
Lemon verbena						
0 kGy	≥50	≥50	11.98	≥50	20.83	≥50
1 kGy	≥50	≥50	3.12	≥50	16.67	≥50
5 kGy	≥50	≥50	3.12	≥50	16.67	≥50
10 kGy	≥50	≥50	3.12	≥50	20.83	≥50

[a] Concentrations of PEN/STREP are expressed in 10^3 U mL^{-1} of penicillin and mg mL^{-1} of streptomycin.

viruses exclusively related with respiratory tract infection.[21,22] Enteric viruses due to their structural resistance and stability to environmental conditions are more able to survive the herbal extract exposure. Nevertheless, considering the viral titer reductions achieved with lemon verbena, peppermint and thyme extracts, the combination of phytotherapies with classic anti-viral treatments could be a field to explore.

Antibacterial activity of hydroethanolic extracts from non-irradiated and irradiated samples

The antibacterial activity of lemon verbena, peppermint and thyme extracts was evaluated against a set of Gram-positive (*S. aureus*, *B. cereus*, *L. monocytogenes* and *E. faecalis*) and Gram-negative (*E. coli* and *S. enterica* serotype Typhimurium) bacteria. Considering the obtained data, it seems that lemon verbena, peppermint and thyme extracts up to 50 mg mL^{-1} have no inhibitory effect on the growth of *E. faecalis*, *E. coli*, *S.* Typhimurium and *L. monocytogenes* (Table 3). Also, gamma irradiation treatment of the plants had no effect on the antibacterial potential of the studied plants against these microbial agents.

The Gram-positive *B. cereus* and *S. aureus* were the only species that seem to be susceptible to all tested extracts. Regarding *B. cereus*, the peppermint extract appears to be the most effective antimicrobial agent tested, with the lowest MIC value of 3.12 mg mL^{-1}. The increasing absorbed dose of radiation appears to cause a gradual enhancement of the antibacterial effect of the three herbal extracts against *B. cereus*.

Concerning *S. aureus*, lemon verbena and peppermint seem to be the most effective agents with a MIC of 12.5 mg mL^{-1}. The effect of lemon verbena extract was not affected by gamma irradiation treatment. The same observation was obtained for thyme extracts. In the case of peppermint extracts, when the samples were irradiated at 1, 5 or 10 kGy, the inhibitory effect on *S. aureus* decreased (MIC $\geq$ 50 mg mL^{-1}). The same effect of gamma radiation on peppermint antimicrobial properties was previously reported for the inhibitory effect of peppermint infusions on *S. aureus*.[13]

Shalayel *et al.*[23] tested the effect of different peppermint extracts (ethanol, methanol, ethyl acetate and chloroform) on several bacterial species including *E. coli*, *E. faecalis* and multi-drug resistant *S. aureus*. Pereira *et al.*[13] studied the effect of lemon verbena and peppermint infusions against a set of both Gram-positive and Gram-negative bacteria. Their results are not in total agreement with the data presented in this study, although it is important to note that different antimicrobial effects could be achieved when different extraction methods are performed.

In general, it was possible to observe that despite the fact that the hydroethanolic extracts of lemon verbena, peppermint and thyme revealed low antibacterial potential for the majority of the tested bacteria, their activity against *B. cereus* and

Table 4 Retention time (R_t), wavelengths of maximum absorption in the visible region (λ_{max}), mass spectral data, identification and tentative quantification of phenolic compounds in peppermint hydroethanolic extracts prepared from non-irradiated and irradiated samples

Peak	R_t (min)	λ_{max} (nm)	$[M-H]^-$ (*m*/*z*)	MS^2 (*m*/*z*)	Tentative identification	Dose of gamma radiation (kGy) (concentration, mg g^{-1} extract) 0	1	5	10
1	12.8	348	637	285(100)	Luteolin-7-*O*-di-glucoronide[a]	1.461 ± 0.002c	1.60 ± 0.02a	1.58 ± 0.01b	1.594 ± 0.003a
2	13.8	288, 330sh	537	493(45), 313(18), 295 (36), 269(55), 197(36), 179(64), 135(100)	Caffeic acid trimer[b]	0.150 ± 0.004c	0.25 ± 0.01b	0.248 ± 0.004b	0.26 ± 0.01a
3	14.5	284, 332sh	595	287(100)	Eriodictyol-7-*O*-rutinoside[c]	17.67 ± 0.01c	18.5 ± 0.1b	19.02 ± 0.02a	19.1 ± 0.1a
4	17.3	350	593	285(100)	Luteolin-7-*O*-rutinoside[a]	10.6 ± 0.1d	12.1 ± 0.1c	12.50 ± 0.01a	12.387 ± 0.004b
5	17.8	350	593	285(100)	Luteolin-*O*-rutinoside[a]	4.9 ± 0.1c	5.4 ± 0.1b	5.4 ± 0.1b	5.524 ± 0.005a
6	19.4	278, 338sh	717	537(34), 519(50), 493 (39), 339(29), 321(37), 313(6), 295(100), 197(3), 179(11), 161(5), 135(11)	Salvianolic acid B/E/L[b]	0.169 ± 0.002b	0.192 ± 0.004a	0.135 ± 0.002d	0.163 ± 0.003c
7	20.6	286, 338sh	609	301(100)	Hesperetin-*O*-rutinoside[c]	tr	0.40 ± 0.02a	0.40 ± 0.02a	0.27 ± 0.01b
8	21.1	330	359	197(13), 179(20), 161 (100), 135(21)	Rosmarinic acid[d]	4.00 ± 0.04c	5.0 ± 0.2b	5.3 ± 0.1a	5.08 ± 0.04b
					TPA	4.32 ± 0.04c	5.5 ± 0.2b	5.7 ± 0.2a	5.50 ± 0.04b
					TF	34.6 ± 0.2c	38.0 ± 0.3b	38.9 ± 0.1a	38.8 ± 0.1a
					TPC	39.0 ± 0.2c	43.5 ± 0.5b	44.5 ± 0.2a	44.3 ± 0.1a

tr – traces; TPA – total phenolic acids; TF – total flavonoids; TPC – total phenolic compounds. Standard calibration curves: [a] Apigenin-7-*O*-glucoside ($y = 10\,683x - 45\,794$; $R^2 = 0.996$). [b] Caffeic acid ($y = 388\,345x + 406\,369$; $R^2 = 0.994$). [c] Hesperetin ($y = 34\,156x + 268\,027$; $R^2 = 0.999$). [d] Rosmarinic acid ($y = 191\,291x - 652\,903$; $R^2 = 0.999$). In each row, for each dose applied, different letters mean significant differences among total compounds ($p < 0.05$).

S. aureus was prominent. Considering the public health risk associated with these microorganisms, some of them largely associated with multidrug resistance, the use of herbal extracts and the identification of novel potential antibacterial active molecules could become a helpful strategy.

Comparative analysis of the phenolic compounds in hydroethanolic extracts from non-irradiated and irradiated samples

Phenolic compound identification (retention time, λ_{max} in the visible region, pseudomolecular ion, main fragment ions in MS^2, and tentative identities) and quantification in all plant species are presented in Tables 4–6.

Eight compounds were detected in the hydroethanolic extracts of peppermint (Table 4 and Fig. 1), which were identified as three phenolic acids (peaks 2, 6 and 8) and five flavonoids, namely, 2 flavanones (peaks 3 and 7) and 3 flavones (peaks 1, 4 and 5). All these compounds were identified by the authors in a previous study.[12] Eriodictyol-7-*O*-rutinoside (peak 3) was the main compound found and the values range between 17.67 and 19.1 mg g^{-1} of extract, in the control samples and samples irradiated with 10 kGy, respectively. This study showed that gamma irradiation affects significantly the concentration of all compounds, evidencing a higher amount of compounds in irradiated samples. Regarding TPA (total phenolic acids), TF (total flavonoids) and TPC (total phenolic compounds), it was evident that the highest content of polyphenols was observed in samples irradiated with 5 and 10 kGy, in comparison with the control sample (0 kGy). The increase in total phenolic and flavonoid contents with the ionizing radiation could be related to the release of these compounds from the matrix structures, increasing extractability of certain molecules and degradation of larger compounds into smaller ones.[24,25] The higher extractability can be explained by the changes in cellular structures, namely by the depolymerization and dissolution of the cell wall by irradiation.[26] Pereira *et al.*[12] evaluated the effects of gamma radiation on the phenolic composition of methanolic extracts of the mentioned plant species and concluded that the irradiation procedure, at a 10 kGy dose, did not affect the phenolic composition.

Table 5 Retention time (R_t), wavelengths of maximum absorption in the visible region (λ_{max}), mass spectral data, identification and tentative quantification of phenolic compounds in thyme hydroethanolic extracts prepared from non-irradiated and irradiated samples

Peak	R_t (min)	λ_{max} (nm)	$[M-H]^-$ (*m*/*z*)	MS^2 (*m*/*z*)	Tentative identification	Dose of gamma radiation (kGy) (concentration, mg g^{-1} extract) 0	1	5	10
1	6.3	284	611	449(100), 287(12)	Eriodictyol-*O*-di-hexoside[a]	0.35 ± 0.01c	0.48 ± 0.02b	0.62 ± 0.03a	0.430 ± 0.002d
2	9.2	325	593	503(29), 473(100), 383 (12), 353(22)	Apigenin-6,8-*C*-dihexoside[b]	10.2 ± 0.2d	11.3 ± 0.3c	11.6 ± 0.4b	12.1 ± 0.2a
3	10.1	284	449	287(100)	Eriodictyol-*O*-hexoside[a]	2.3 ± 0.1d	2.9 ± 0.1b	2.63 ± 0.02c	3.2 ± 0.1a
4	11.6	284	449	287(100)	Eriodictyol-7-*O*-glucuronide[a]	8.4 ± 0.2d	10.31 ± 0.02b	9.7 ± 0.3c	10.7 ± 0.2a
5	15.3	285	463	287(100)	Eriodictyol-*O*-glucuronide[a]	2.9 ± 0.1d	3.0 ± 0.1c	3.9 ± 0.2a	3.3 ± 0.1b
6	16.0	341	447	285(100)	Luteolin-7-*O*-glucoside[c]	14 ± 1a	14.5 ± 0.1a	14.4 ± 0.2a	14.7 ± 0.1a
7	16.7	332	521	359(100), 197(5)	Rosmarinic acid hexoside[d]	1.43 ± 0.04a	1.3 ± 0.1b	1.39 ± 0.04a	1.4 ± 0.1a
8	17.3	330	593	285(100)	Luteolin-7-*O*-rutinoside[c]	2.36 ± 0.02c	2.695 ± 0.002a	2.7 ± 0.2a	2.62 ± 0.02b
9	17.8	340	461	285(100)	Luteolin-7-*O*-glucuronide[c]	17 ± 1c	17.3 ± 0.1c	22.2 ± 0.2a	20.7 ± 0.1b
10	18.1	340	461	285(100)	Luteolin-*O*-glucuronide[c]	15 ± 1b	14.2 ± 0.1c	16.1 ± 0.3a	14.26 ± 0.01c
11	20.9	328	359	197(13), 179(20), 161 (100), 135(21)	Rosmarinic acid[d]	74 ± 4c	74.9 ± 0.1c	82.5 ± 0.4a	79 ± 2b
12	22.0	286, 328sh	555	493(100), 359(17), 225(5)	Salvianolic acid K[e]	1.02 ± 0.04c	1.01 ± 0.03c	1.7 ± 0.1a	1.33 ± 0.04b
13	22.5	278, 338sh	717	537(34), 519(50), 493(39), 339(29), 321(37), 313(6), 295(100), 197(3), 179(11), 161(5), 135(11)	Salvianolic acid B/E/L[e]	2.4 ± 0.1d	2.8 ± 0.1c	3.0 ± 0.1b	3.20 ± 0.03a
14	24.3	324	537	493(100), 359(10), 215(3), 179(5)	Lithospermic acid A[e]	5.1 ± 0.3b	4.6 ± 0.1d	5.68 ± 0.02a	5.0 ± 0.2c
					TPA	84 ± 4c	84.6 ± 0.2c	94.3 ± 0.2a	90 ± 2b
					TF	73 ± 3d	77 ± 1c	83.9 ± 0.3a	82.0 ± 0.4b
					TPC	157 ± 7d	161 ± 1c	178.2 ± 0.1a	172 ± 2b

TPA – total phenolic acids; TF – total flavonoids; TPC – total phenolic compounds. Standard calibration curves: [a] Hesperetin ($y = 34\,156x + 268\,027$; $R^2 = 0.999$). [b] Apigenina-6-*C*-glucosido ($y = 107\,025x + 61\,531$; $R^2 = 0.999$). [c] Apigenin-7-*O*-glucoside ($y = 10\,683x - 45\,794$; $R^2 = 0.996$). [d] Rosmarinic acid ($y = 191\,291x - 652\,903$; $R^2 = 0.999$). [e] Caffeic acid ($y = 388\,345x + 406\,369$; $R^2 = 0.994$). In each row, for each dose applied, different letters mean significant differences among total compounds ($p < 0.05$).

Table 6 Retention time (R_t), wavelengths of maximum absorption in the visible region (λ_{max}), mass spectral data, identification and tentative quantification of phenolic compounds in lemon verbena hydroethanolic extracts prepared from non-irradiated and irradiated samples

Peak	R_t (min)	λ_{max} (nm)	$[M-H]^-$ (m/z)	MS^2 (m/z)	Tentative identification	Dose of gamma radiation (kGy) (concentration, mg g^{-1} extract) 0	1	5	10
1	4.2	280	461	315(8), 135(28)	Verbascoside[a]	tr	0.0151 ± 0.0004b	0.0204 ± 0.0003a	0.0014 ± 0.0001c
2	12.9	344	637	351(100), 285(89)	Luteolin-7-*O*-di-glucuronide[b]	2.6 ± 0.1b	2.34 ± 0.02c	2.52 ± 0.04b	2.87 ± 0.001a
3	16.0	330	623	461(18), 315(5)	Verbascoside[a]	9.0 ± 0.3b	9.72 ± 0.05a	8.8 ± 0.1c	8.44 ± 0.01d
4	18.7	330	623	461(18), 315(5)	Isoverbascoside[a]	0.401 ± 0.004a	0.34 ± 0.01b	0.283 ± 0.004d	0.31 ± 0.01c
5	20.4	330	637	491(5), 461(60), 315(13)	Eukovoside[a]	0.121 ± 0.002a	0.091 ± 0.003c	0.079 ± 0.005d	0.098 ± 0.004b
6	26.7	330	651	505(7), 475(22)	Martinoside[a]	0.047 ± 0.001a	tr	0.0140 ± 0.0005b	0.0042 ± 0.0002c
					TCP	9.6 ± 0.3b	10.2 ± 0.1a	9.2 ± 0.1c	8.853 ± 0.003d
					TF	2.6 ± 0.1b	2.34 ± 0.02c	2.52 ± 0.04b	2.875 ± 0.001a
					TPC	12.2 ± 0.4b	12.51 ± 0.04a	11.7 ± 0.2c	11.729 ± 0.004c

tr – traces. TCP – total caffeoyl phenylethanoid derivatives (including verbascoside); TF – total flavonoids; TPC – total phenolic compounds. Standard calibration curves: [a] Caffeic acid ($y = 388\,345x + 406\,369$; $R^2 = 0.9939$). [b] Apigenin-7-*O*-glucoside ($y = 10\,683x - 45\,794$; $R^2 = 0.996$). In each row, for each dose applied, different letters mean significant differences among total compounds ($p < 0.05$).

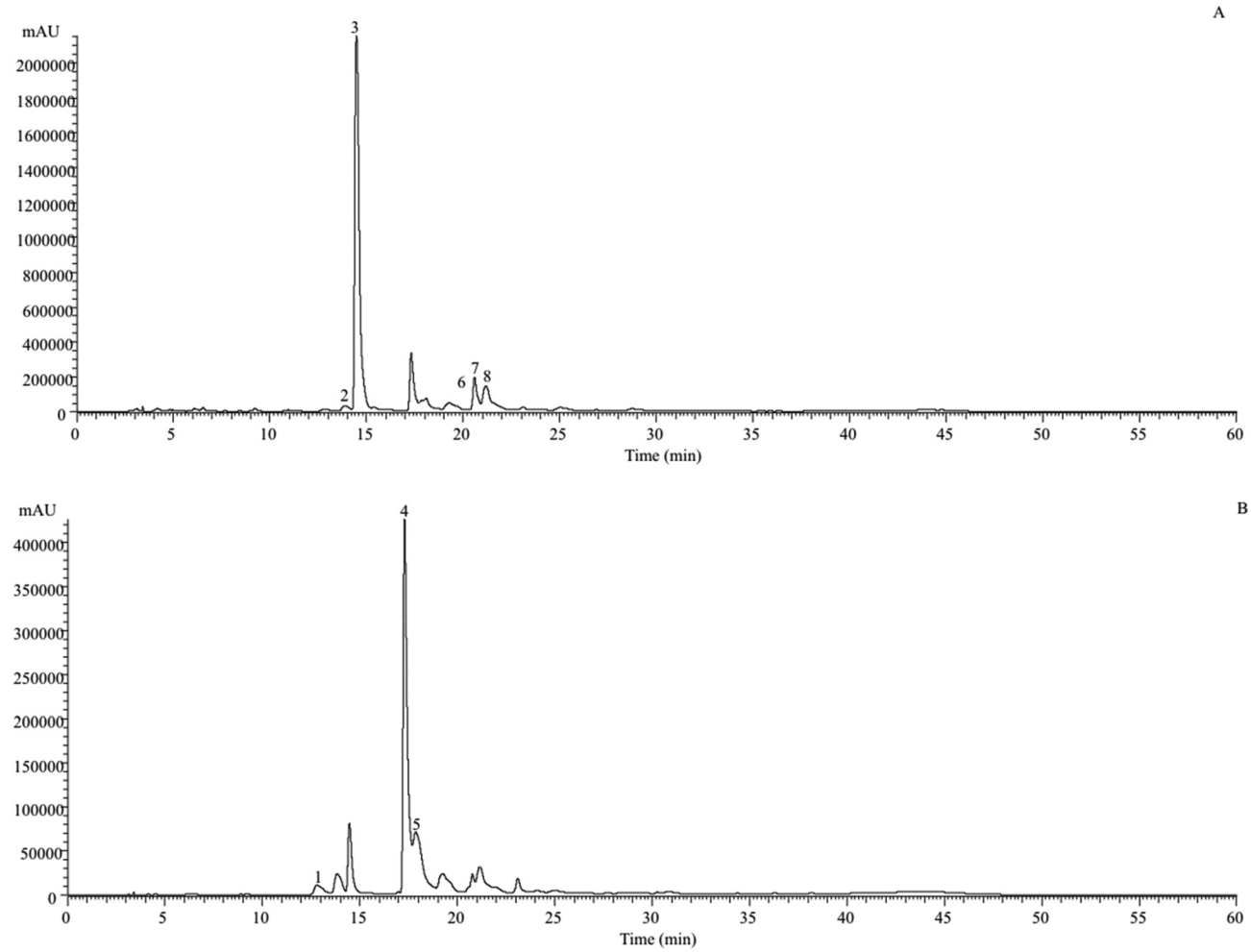

Fig. 1 Phenolic compound profile of peppermint irradiated at 10 kGy, recorded at 280 (A) and 370 (B) nm. The numbers on the chromatograms correspond to the peaks identified in Table 4.

Considering the thyme samples (Table 5 and Fig. 2), fourteen compounds were detected, which were identified as five phenolic acids (peaks 7, 11, 12, 13 and 14) and nine flavonoids, namely, 5 flavones (peaks 2, 6, 8, 9 and 10) and 4 flavanones (peaks 1, 3, 4 and 5). Most of the identified compounds were previously described in studies performed by Pereira *et al.*[11,12] and Martins *et al.*[27] Rosmarinic acid (peak 11) was the main compound, followed by luteolin-7-*O*-glucuronide (peak 9), luteolin-*O*-glucuronide (peak 10), and luteolin-7-*O*-glucoside (peak 6). With the application of gamma radiation, it was evident that in most of the compounds, there were significant differences, taking into account the dose applied. Peaks 2, 3, 4 and 13 showed a higher concentration with the applied dose of 10 kGy. On the other hand, peaks 1, 5, 9, 10, 11, 12 and 14 evidenced an increase in samples irradiated with 5 kGy. Peak 8 showed a higher concentration with the doses of 1 and 5 kGy, and peaks 6 and 7 did not demonstrate significant differences with the different doses applied. Pereira *et al.*[11] studied the effects of gamma rays on the infusions extracts of *T. vulgaris* and considering the obtained results, the dose of 10 kGy caused relevant changes in the concentration of several compounds, namely higher total phenolic compounds and flavonoids. Pereira *et al.*[12] also studied the effects of gamma rays (1 and 10 kGy) on the methanolic extract of *T. vulgaris*, demonstrating that irradiation with 10 kGy did not affect the phenolic composition of the sample, compared to the non-irradiated control sample (0 kGy).

These heterogeneous results could be explained by the different geographic origins of the species used, the different types of extracts studied, the different irradiation doses applied and the different amounts of water present in the sample, because although all samples are dry, they always contain a certain percentage of humidity, which could cause differences in their contents.[28,29]

Concerning lemon verbena (Table 6 and Fig. 3), six phenolic compounds were detected, which were identified as one flavonoid (peak 2) and five caffeoyl phenylethanoid derivatives (peaks 1, 3, 4, 5 and 6). All mentioned compounds were pre-

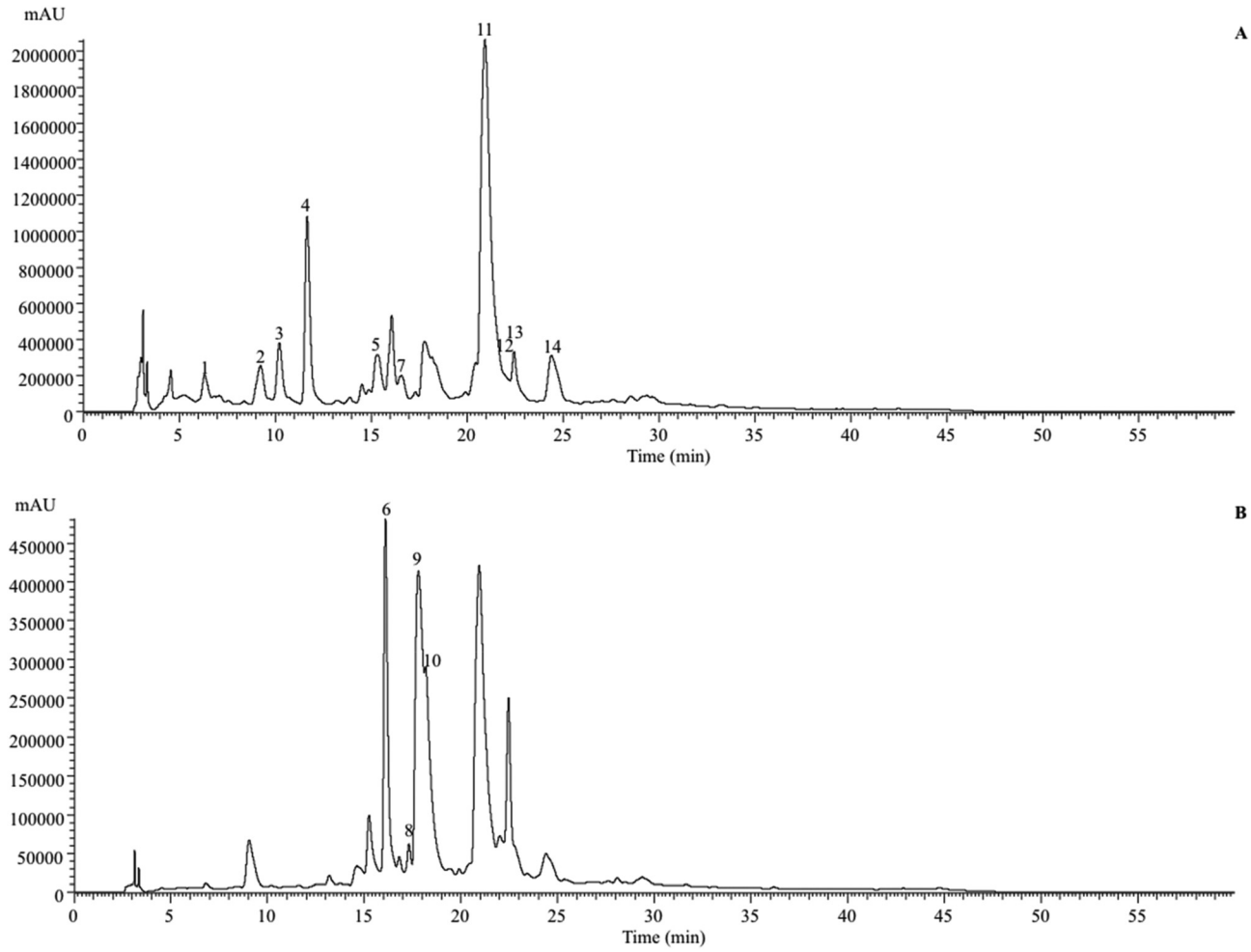

Fig. 2 Phenolic compound profile of thyme irradiated at 10 kGy, recorded at 280 (A) and 370 (B) nm. The numbers on the chromatograms correspond to the peaks identified in Table 5.

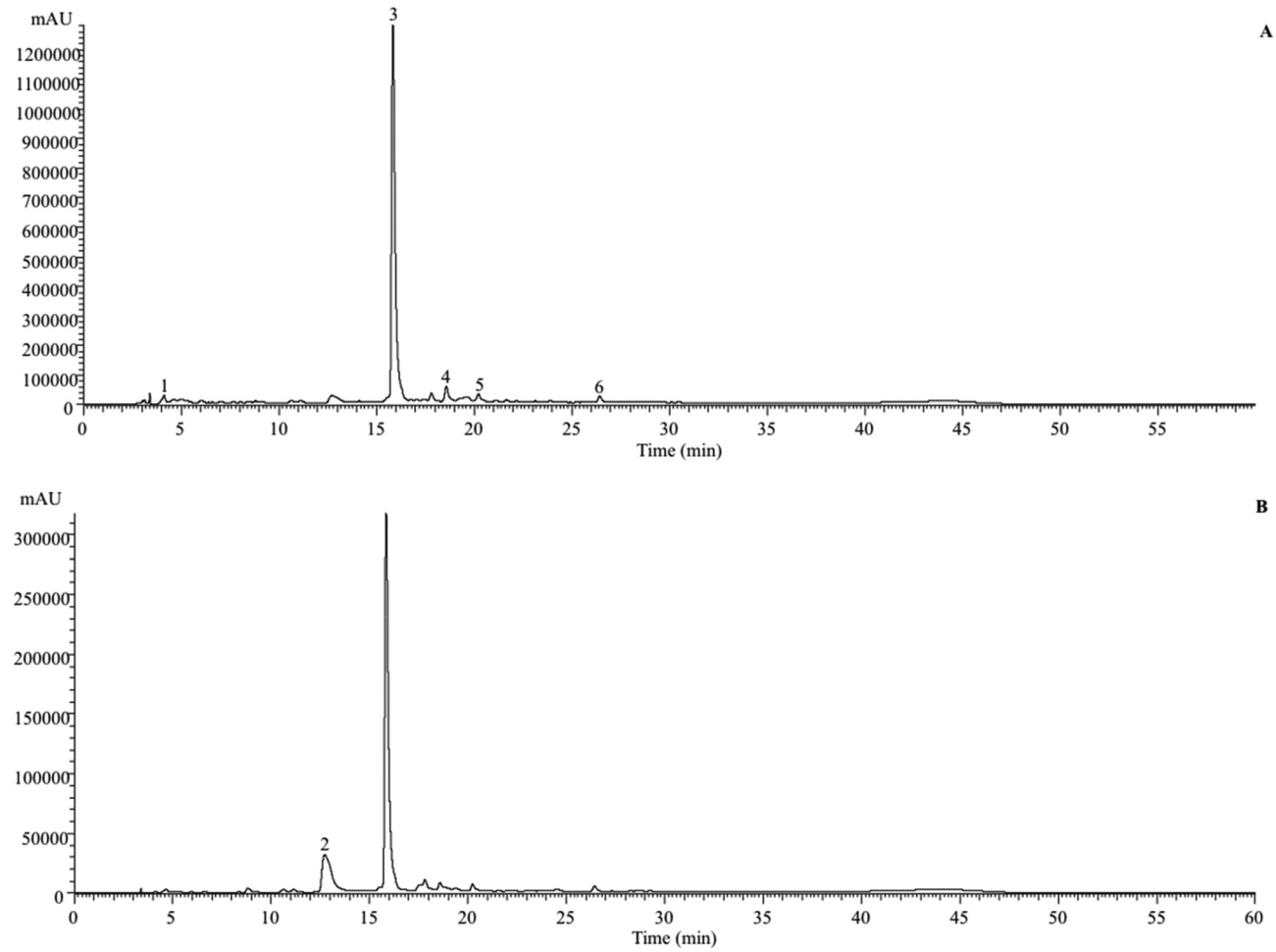

Fig. 3 Phenolic compound profile of lemon verbena irradiated at 10 kGy, recorded at 280 (A) and 370 (B) nm. The numbers on the chromatograms correspond to the peaks identified in Table 6.

viously identified in a study performed by Pereira *et al.*[14] Verbascoside (peak 3) was highlighted as the main compound, presenting values ranging from 8.44 to 9.72 mg g^{-1} of extract, for 10 and 1 kGy doses, respectively. This compound has numerous biological properties, including antioxidant, anti-inflammatory, hepatoprotective, immunoregulatory, and neuroprotective activity.[30] In all the applied doses, significant differences ($p < 0.05$) were verified, which were evident with a higher concentration of these molecules in the control sample (0 kGy) and in the samples irradiated with 1 kGy. On the other hand, TPA and TPC revealed higher amounts in the samples irradiated with 1 kGy, while TF was higher in the samples irradiated with 10 kGy. These results are in agreement with those previously reported by Pereira *et al.*,[14] which evaluated the effects of gamma radiation on the phenolic profile of the methanolic extracts of *Aloysia citrodora* Paláu (lemon verbena). The results also presented some heterogeneity, with the increase in the concentration of some compounds and decrease of others, depending on the applied dose (0, 1 and 10 kGy) and the respective compounds.

Conclusion

Irradiation is a food processing technology increasingly recognized and supported by several international organizations. Over time, studies have been carried out to better apply this technology, evaluating the occurrence of possible changes in the chemical and nutritional profiles of food. In this study, the variability of the bioactive properties was evaluated, taking into account the effects of gamma radiation on hydroethanolic extracts obtained from irradiated (1, 5 and 10 kGy) and non-irradiated (0 kGy) plants that are commonly used in food and for therapeutic purposes.

In general, antiproliferative activity was evident in all samples and in all analysed doses; however, upon evaluating each plant species individually and comparing the GI_{50} values at all applied doses, significant differences ($p < 0.05$) were shown, and in some cases higher cytotoxic activity was present in irradiated samples. For the virucidal activity, the data suggested that the irradiation treatment of all species can preserve the natural properties of the plant against enteric viral

pathogens. Regarding the antibacterial activity, gamma irradiation treatment did not affect this bioactivity in most of the studied plant species. In the case of *B. cereus*, gamma radiation seems to potentiate the antibacterial capacities of all of the tested extracts. Finally, regarding the phenolic profile, all species revealed to be a good source of bioactive compounds, and in some cases, the increase of the amount of several compounds was demonstrated in the irradiated samples. However, the lack of linearity in these results could be directly related to the differences found in the phenolic profile of the analysed species and the applied doses. Thus, this study contributed to better knowledge in the application of irradiation technology to thyme, lemon verbena and peppermint, allowing one to define the influence of this treatment on their bioactive properties and phenolic composition.

Conflicts of interest

The authors declare no conflict of interest.

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References

1 S. Wang, P. Shena, J. Zhou and Y. Lu, Diet phytochemicals and cutaneous carcinoma chemoprevention: A review, *Pharmacol. Res.*, 2017, **119**, 327–346.
2 H. Tapiero, K. D. Tew, N. Ba and G. Mathé, Polyphenols: do they play a role in the prevention of human pathologies?, *Biomed. Pharmacother.*, 2002, **56**, 200–207.
3 M. Carocho and I. C. F. R. Ferreira, The Role of Phenolic Compounds in the Fight against Cancer – A Review, *Anti-Cancer Agents Med. Chem.*, 2013, **13**, 1236–1258.
4 L. Tian, Y. Hu and X.-Y. Chen, Advancing Human Health through Exploration of Plant Metabolism and Reaping the Benefits of Edible Medicinal Plants, *Mol. Plant*, 2017, **10**, 533–536.
5 E. D. Cömert and V. Gökmen, Evolution of food antioxidants as a core topic of food science for a century, *Food Res. Int.*, 2018, **105**, 76–93.
6 K. Srinivasan, Spices as influencers of body metabolism: an overview of three decades of research, *Food Res. Int.*, 2005, **38**, 77–86.
7 I. Ihsanullah and A. Rashid, Current activities in food irradiation as a sanitary and phytosanitary treatment in the Asia and the Pacific Region and a comparison with advanced countries, *Food Control*, 2017, **72**, 345–359.
8 P. B. Roberts, Food irradiation: Standards, regulations and world-wide trade, *Radiat. Phys. Chem.*, 2016, **129**, 30–34.
9 E. Pereira, A. L. Antonio, J. C. M. Barreira, L. Barros, A. Bento and I. C. F. R. Ferreira, Gamma irradiation as a practical alternative to preserve the chemical and bioactive wholesomeness of widely used aromatic plants, *Food Res. Int.*, 2015, **67**, 338–348.
10 E. Pereira, L. Barros, M. Dueñas, A. L. Antonio, C. Santos-Buelga and I. C. F. R. Ferreira, Gamma irradiation improves the extractability of phenolic compounds in *Ginkgo biloba* L., *Ind. Crops Prod.*, 2015, **74**, 144–149.
11 E. Pereira, L. Barros, A. L. Antonio, S. Cabo Verde, C. Santos-Buelga and I. C. F. R. Ferreira, Infusions from *Thymus vulgaris* L. treated at different gamma radiation doses: Effects on antioxidant activity and phenolic composition, *LWT–Food Sci. Technol.*, 2016, **74**, 34–39.
12 E. Pereira, A. I. Pimenta, R. C. Calhelha, A. L. Antonio, S. Cabo-Verde, L. Barros, C. Santos-Buelga and I. C. F. R. Ferreira, Effects of gamma irradiation on cytotoxicity and phenolic compounds of *Thymus vulgaris* L. and *Mentha x piperita* L., *LWT–Food Sci. Technol.*, 2016, **71**, 370–377.
13 E. Pereira, A. I. Pimenta, R. C. Calhelha, A. L. Antonio, L. Barros, C. Santos-Buelga, S. Cabo Verde and I. C. F. R. Ferreira, Infusions of gamma irradiated *Aloysia citrodora* L. and *Mentha x piperita* L.: Effects on phenolic composition, cytotoxicity, antibacterial and virucidal activities, *Ind. Crops Prod.*, 2017, **97**, 582–590.
14 E. Pereira, L. Barros, A. L. Antonio, S. Cabo Verde, C. Santos-Buelga, I. C. F. R. Ferreira and P. Rodrigues, Is Gamma Radiation Suitable to Preserve Phenolic Compounds and to Decontaminate Mycotoxins in Aromatic Plants? A Case-Study with *Aloysia citrodora* Paláu, *Molecules*, 2017, **22**, 347.
15 D. H. Gilling, M. Kitajima, J. R. Torrey and K. R. Bright, Mechanisms of antiviral action of plant antimicrobials against murine norovirus, *Appl. Environ. Microbiol.*, 2014, **80**, 4898–4910.
16 M. Sokovic, J. Glamočlija, P. D. Marin, D. Brkic and L. J. van Griensven, Antibacterial effects of the essential oils of commonly consumed medicinal herbs using an in vitro model, *Molecules*, 2010, **15**, 7532–7546.
17 S. M. F. Bessada, J. C. M. Barreira, L. Barros, I. C. F. R. Ferreira and M. B. P. P. Oliveira, Phenolic profile and antioxidant activity of *Coleostephus myconis* (L.) Rchb.f.: An underexploited and highly disseminated species, *Ind. Crops Prod.*, 2016, **89**, 45–51.

18 M. Sökmen, J. Serkedjieva, D. Daferera, M. Gulluce, M. Polissiou, B. Tepe, H. A. Akpulat, F. Sahin and A. Sokmen, In vitro antioxidant, antimicrobial, and antiviral activities of the essential oil and various extracts from herbal parts and callus cultures of *Origanum acutidens*, *J. Agric. Food Chem.*, 2004, **2**, 3309–3312.

19 B. Glatthaar-Saalmüller, A. M. Fal, K. Schönknecht, F. Conrad, H. Sievers and A. Saalmüller, Antiviral activity of an aqueous extract derived from *Aloe arborescens* Mill. against a broad panel of viruses causing infections of the upper respiratory tract, *Phytomedicine*, 2015, **15**, 911–920.

20 S. Rebensburg, M. Helfer, M. Schneider, H. Koppensteiner, J. Eberle, M. Schindler, L. Gürtler and R. Brack-Werner, Potent *in vitro* antiviral activity of Cistus incanus extract against HIV and Filoviruses targets viral envelope proteins, *Sci. Rep.*, 2016, **2**(6), 20394.

21 S. Geuenich, C. Goffinet, S. Venzke, S. Nolkemper, I. Baumann, P. Plinkert, J. Reichling and O. T. Keppler, Aqueous extracts from peppermint, sage andlemon balm leaves display potent anti-HIV-1 activity by increasing the viriondensity, *Retrovirology*, 2008, **5**, 27.

22 M. Loolaie, N. Moasefi, H. Rasouli and H. Adibi, Peppermint and Its Functionality: A Review, *Arch. Clin. Microbiol.*, 2017, **8**, 4–54.

23 A. M. A. Shalayel, M. A. Qureshi and A. B. Elhussein, Antibacterial activity of peppermint (*Mentha piperita*) extracts against some emerging multi-drug resistant human bacterial pathogens, *J. Herb. Med.*, 2017, **7**, 27–30.

24 M. Polovka and M. Suhaj, The effect of irradiation and heat treatment on composition and antioxidant properties of culinary herbs and spices - A Review, *Food Rev. Int.*, 2010, **26**, 138–161.

25 S. Taheri, T. L. Abdullah, E. Karimi, E. Oskoueian and M. Ebrahimi, Antioxidant capacities and total phenolic contents enhancement with acute gamma irradiation in *Curcuma alismatifolia* (Zingiberaceae) leaves, *Int. J. Mol. Sci.*, 2014, **15**, 13077–13090.

26 R. Bhat, K. R. Sridhar and K. Tomita-Yokotani, Effect of ionizing radiation on antinutritional features of velvet bean seeds (Mucuna pruriens), *Food Chem.*, 2007, **103**, 860–866.

27 N. Martins, L. Barros, C. Santos-Buelga, S. Silva, M. Henriques and I. C. F. R. Ferreira, Decoction, infusion and hydroalcoholic extract of cultivated thyme: Antioxidant and antibacterial activities, and phenolic characterization, *Food Chem.*, 2015, **167**, 131–137.

28 J. Sádecká, Irradiation of spices - A review, *Czech J. Food Sci.*, 2007, **25**, 231–242.

29 J. V. Tezotto-Uliana, P. P. M. Silva, R. A. Kluge and M. H. F. Spoto, Radiação Gama em Produtos de Origem Vegetal, *Rev. Virtual Quim.*, 2015, **7**, 267–277.

30 Y. Wan, B. Zou, H. Zeng, L. Zhang, M. Chen and G. Fu, Inhibitory effect of verbascoside on xanthine oxidase activity, *Int. J. Biol. Macromol.*, 2016, **93**, 609–614.