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RESEARCH ARTICLE

Evaluation of plant extracts as an efficient source of additives for active food packaging

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Abstract

Natural extracts have been used in several traditional medicine applications and culinary purposes. Their biological properties (antioxidant and antimicrobial) are due to the presence of several active aromatic compounds. Herein, different natural extracts were evaluated, namely structural and thermal characterization and biological activity, in its natural form and incorporated into a polymeric matrix, to assess their effective potential as additives for active food packaging. While rosemary presented the highest thermal stability with a degradation starting at 327°C, lemon balm extract was the less stable (180°C). Regarding the thiobarbituric acid assay, all extracts presented antioxidant activity, in oxidative hemolysis inhibition; anise, cinnamon, and clove extract did not present any action. Overall, the results demonstrated that leaves (rosemary and green tea) and the rhizome (curcumin) are the plant parts with the best performance. Therefore, extracts from aromatic plants are promising natural additives that can be incorporated into polymeric matrices to produce active food packaging film, increasing products shelf-life.

KEYWORDS
antimicrobial, antioxidant, aromatic extracts, cytotoxicity

1 | INTRODUCTION

One of the major food industry worries is related to food safety and apart from spoilage of foodstuff, there are always concerns about the occurrence of foodborne illnesses among food manufactures, regulatory agencies, researchers, and consumers (Khorshidian et al., 2018). Foodborne illness that results from the consumption of contaminated food have been of great concern of public health. Nowadays, consumers are looking for natural and healthier products as an alternative to chemicals preservatives (Román et al., 2017). Therefore, the incorporation of bioactive natural compounds into packaging films is seen as a new trend to preserve food quality and extend its shelf-life (Beya et al., 2021; Estevez-Areco et al., 2018).

Aromatic plants are strongly linked to the human civilization and its evolution. Since ancient times, they have been used in several traditional medicines and also in all cuisines around the world. Several active compounds extracted from these plants exhibited antioxidant and antimicrobial properties, and their constitution are dependent on

the extraction technique, solvent, plant edaphoclimatic conditions, and plant piece among others (Ozdal et al., 2021). Phenolic compounds are present in the majority of edible plants and they account for plants' biological properties; these are part of the secondary plant metabolites, contributing to their protection against ultraviolet radiation, pathogens, parasites, and predators. Moreover, they delineate the color and organoleptic properties contributing to the better taste of fruits, for example. More than 100 plants extracts have been approved by the Food and Drug Administration (FDA) as GRAS (Generally Recognized as Safe). These extracts are prepared in their most purified form to acquire compounds with relevant biological properties. Therefore, in this study, aromatic extracts from different plant parts (leaves, flowers, and seeds) were selected to evaluate their antioxidant and antimicrobial properties.

Rosmarinus officinalis (rosemary) (RE) originated from the Mediterranean region and is categorized as a woody and aromatic plant. Its leaf extracts have been used in traditional medicine to treat several human diseases and for food preservation. The RE bioactivity include antioxidant, anti-inflammatory, antimicrobial, hepatoprotective, and antidiabetic properties. Caffeic acid derivatives, like rosmanaric acid (phenolic compound), lend it medicinal properties (Ribeiro et al., 2016). The European Commission has approved the consumption of RE as food additive E 392 by the Directives 2010/67/EU and 2010/69/EU (Andrade et al., 2018).

Green tea extract (GTE) is taken from the leaves of *Camellia sinensis* L. and is recognized for its antioxidant, antimicrobial, anticarcinogenic, and anti-inflammatory properties. GTE is a plentiful source of polyphenol antioxidants, particularly catechins, which combined with gallic acid are responsible for GTE's antioxidant properties (Özogul et al., 2017). Lemon balm (*Melissa officinalis*) (LBE) is a native plant from the Mediterranean region, it belongs to the Lamiaceae family and was employed in traditional medicine. LBE has high levels of phenolic acids, like rosmarinic acid and it is used as an aromatic, antimicrobial, antioxidant, and antiseptic additive for food and drugs applications (Boneza & Niemeyer, 2018; Özogul et al., 2017).

The most used natural extract preservatives in food are cinnamon (CE) and clove (CLE). CE is mainly composed of cinnamaldehyde and it is a good inhibitor of many food spoilage microorganisms (Ju et al., 2018). Its antioxidant, anti-inflammatory, and antitumor activity are also reported in some studies (Ju et al., 2018; Mulla et al., 2017; Ribeiro-Santos et al., 2017). CE can be applied in traditional kitchen, incenses, perfumes, and pharmaceutical products (Ribeiro-Santos et al., 2017). Eugenol is the major active component of CLE and has strong antibacterial, antioxidant, and insecticidal effect (El-Saber Batiha et al., 2020; Ju et al., 2018; Mulla et al., 2017). Since both CE and CLE are natural preservatives and flavor substances, they are safe to consume and have been approved by the FDA and European Commission as natural food additives (Ju et al., 2018; Mulla et al., 2017; Ribeiro-Santos et al., 2018).

Anise (*Pimpinella anisum*) (AE) is a plant from the Umbelliferae family and a popular aromatic and spice herb from ancient times, its seeds are employed in folk medicine and as a cooking ingredient. Chemical studies demonstrated that AE contains several polyphenols, coumarins, scopoletin, umbelliferone, estrols, terpene hydrocarbons, polenes, and polyacetylenes. Anetholes are the main active compounds responsible for the antimicrobial activity (Mosavat et al., 2019; Topuz et al., 2016).Curcumin extract (CCE) is a natural hydrophobic yellow–orange compound extracted from the *Curcuma Longa* L. root, which is widely used for medicinal and food purposes. CCE shows powerful antioxidant, antitumor, antibacterial, and anticancer properties (Almeida et al., 2018; dos Santos et al., 2019; Luo et al., 2012; Silva de Sá et al., 2019). Clinical trials have shown that CCE consumption is safe, even at a daily dosage of 12 g for 3 months (Luo et al., 2012).

Thus, the purpose of this research is to screen in terms of biological properties (antioxidant and antimicrobial) a set of commercial extracts, available in nature, to select the most promising ones as potential additives to produce active polymeric packaging film. Therefore, proprieties assessment was made both in the extract form and the extract incorporated in a low-density polyethylene (LDPE) matrix. When the film is in contact with the food, the extract that is located at the interface and in the film will diffuse to the food product.

2 | MATERIALS AND METHODS

2.1 | Samples

Low-density polyethylene (LDPE) was kindly provided by Vizelpas. The green tea extract leaves (GTE), rosemary extract leaves (RE), cinnamon extract bark (CE), anise extract seeds (AE), clove extract flowers (CLE) and lemon balm extract leaves (LBE) were acquired from ESSÊNCIAD'UMSEGREDO, LDA. Curcumin extract rhizomes (CCE) (from *C. longa* (Tumeric) powder, ≥65%) was purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.2 | Reagents and standards

Potassium bromide (KBr) (Acros Organics, spectroscopic standard) was used to produce the pellets for Fourier Transform Infrared Spectroscopy (FTIR). Trichloroacetic acid (TCA), ascorbic acid, iron sulfate, sodium chloride, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,2′-azobis(2-methylpropionamidine)dihydrochloride (AAPH), phosphate buffered saline (PBS), hydroxymethylaminomethane buffer (Tris-HCl), dimethyl sulfoxide (DMSO), and 2-thiobarbituric acid (TBA) were purchased from Sigma Aldrich (EUA) and used in oxidative hemolysis inhibition assay (OxHLIA) and TBARS assays. For cytotoxic assay, ellipticine, sulforhodamine B (SRB), Hank's balanced salt solution (HBSS), trypan blue, nonessential amino acid solution (2 mM), tris-(hydroxymethyl)aminomethane (TRIS), RPMI-1640, fetal bovine serum (FBS), and penicillin/streptomycin solution (100 U/ml and 100 mg/ml, respectively) were acquired from Gibco Invitrogen Life Technologies (UK). The culture media Muller Hinton Broth (MHB) and Tryptic Soy Broth (TSB) used for antimicrobial assays, blood agar with 7% sheep blood and Mac Conkey agar plates were purchased from bioMérieux (France).

2.3 | Film preparation and extracts treatment

The extracts were dried at 60°C overnight under vacuum and then incorporated in an LDPE matrix. LDPE compounds with 2 wt.% of each extract were prepared in the Xplore MC15 micro single screw extruder at 145°C and 90 rpm, with a residence time of 2 min. From the prepared materials, films with around 100 μm were produced by compression molding in a hot press at 140°C under a pressure of 10 tons.

For bioactive properties evaluation, and due to compounds' insolubility in water, the dried extracts were dissolved in a PBS/DMSO mixture (95:5, v/v), and the recovered extracts from the films were obtained by grinding followed by stirring during 2 days in 20 ml PBS with 5% DMSO, getting a 10 mg/ml concentration. Successive dilutions were prepared from the stock solutions.

2.4 | Structural and thermal characterization

FTIR analysis of the extracts and films was performed in a 4100 Jasco (Japan) spectrometer in transmittance mode at 32 scans·min^{-1}, 4 cm^{-1} resolution in a wavelength range of 4500–400 cm^{-1}. Each extract (10 wt.%) was mixed with KBr > 99%, to obtain a translucent sample.

Thermogravimetric analysis (TGA) of the extracts was accomplished using a TGA Q500 (TA Instruments, New Castle, EUA) under nitrogen atmosphere at 10°C/min in a temperature range from 40 to 900°C.

2.5 | Antioxidant activity

2.5.1 | Thiobarbituric acid reactive substances formation inhibition assay

The capacity of the extracts and films containing the extracts to prevent the thiobarbituric acid reactive substances (TBARS) formation, such as malondialdehyde (MDA) produced from the ex vivo decomposition of lipid peroxidation products, was assessed with porcine brain cell homogenates, according to the protocol previously reported by Pereira et al. (2014). The color intensity of the malonaldehyde-thiobarbituric acid complex (MDA)-TBA was read at 532 nm and the results were presented as IC_{50} values (μg/ml).

2.5.2 | Oxidative hemolysis inhibition assay

The antihemolytic activity was evaluated using the method described by Takebayashi et al. (2012) with some modifications. Sheep blood samples were gathered from healthy animals and the assay was performed as defined in literature (Lockowandt et al., 2019). The acquired results were expressed as IC_{50} values (μl/ml) at Δt 60 min.

2.6 | Cytotoxic assay

The cytotoxicity was estimated by sulforhodamine B colorimetric assay in a primary culture of porcine liver cells (PLP2), as already illustrated by Abreu et al. (2011). Ellipticine was used as positive control and the results were expressed as GI_{50} values.

2.7 | Antimicrobial activity

Antimicrobial activity was assessed using the microdilution method according to Soković and van Griensven (2006). The antibacterial activity was evaluated using three Gram positive (*Staphylococcus aureus* [ATCC 11632], *Bacillus cereus* [food isolate], and *Listeria monocytogenes* [NCTC 7973]) and three Gram negative (*Escherichia coli* [ATCC 25922], *Enterobacter cloacae* [ATCC 35030], and *Salmonella Typhimurium* [ATCC 13311]) bacteria. The microorganisms used were obtained from the Mycological Laboratory, Department of Plant Physiology, Institute for Biological Research "Siniša Stanković"—National Institute of Republic of Serbia, University of Belgrade. The tested samples were dissolved in 30% ethanol and pipetted into the wells already containing 100 μl of tryptic soy broth (TSB) medium, after which bacterial inocula was added (1.0×10^4 CFU per well). After incubation, (24 h at 37°C), *p*-iodonitrotetrazolium chloride (40 μl, 0.2 mg/ml) was added to each plate well, and further incubated during 60 min at 37°C for color development. The lowest concentrations that showed a distinct reduction in color intensity—light red in comparison to the intensive red in the control well (with no added extracts), or an absence of color, were defined as minimal inhibitory concentration (MICs). The minimal bactericidal concentrations (MBCs) were determined by serial subcultivation of 2 μl into the wells already containing 100 μL of broth and further incubation during 24 h at 37°C. The lowest concentration with no visible growth was defined as the MBC, indicating 99.5% killing of the original inoculum.

The antifungal activity was estimated against six micromycetes: *Aspergillus fumigatus* (ATCC 9197), *Aspergillus versicolor* (ATCC 11730), *Aspergillus niger* (ATCC 6275), *Penicillium funiculosum* (ATCC 36839), *Penicillium verrucosum* var. *cyclopium* (food isolate), and *Trichoderma viride* (IAM 5061). The microorganisms used were from the same laboratory as the previous ones. Prior to the microdilution method, fungal spores were washed from the surface of agar plates with sterile 0.85% saline containing 0.1% Tween 80 (v/v). Samples dissolved in 30% ethanol were added to broth Malt medium, after that fungal inoculum was added. Plates were incubated at 25°C for 5 days. The lowest concentrations with significant reduction in mycelial growth (at the binocular microscope) were defined as MICs. The minimal fungicidal concentrations (MFCs) were determined by serial subcultivation of 2 μl of the tested sample dissolved in medium and further incubated for 72 h at 25°C. The lowest concentration with no visible growth was defined as MFC, indicating killing 99.5% of the original inoculum. In both methods, commercial food preservatives, namely sodium benzoate (E211) and potassium metabisulfite (E224) were used as positive controls.

2.8 | Statistical analysis

The results of each test represent the mean values and standard deviation of three samples, analyzed by analysis of variance (ANOVA)

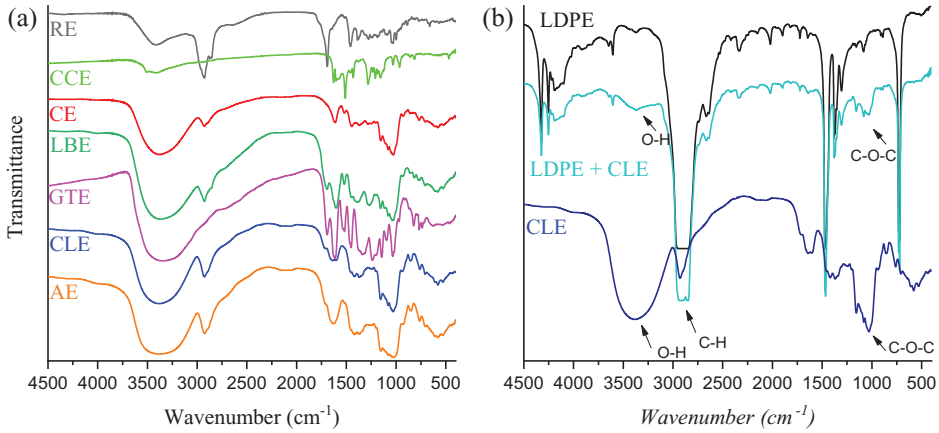

FIGURE 1 FTIR spectra of various extracts (a) and comparison of extract and extract incorporate in LDPE matrix (b)

and post hoc Tukey test, using the SPSS v.22.0 program (IBM Corp., Armonk, New York, USA).

3 | RESULTS AND DISCUSSION

3.1 | Fourier transform infrared spectroscopy

The RE contain several organic molecules which comprise of aromatic and phenolic groups. FTIR spectrum shows (Figure 1a) a strong band corresponding to O–H stretching of phenol group (3000–3600 cm^{-1}), C-H stretching (2932 cm^{-1}), C=C ring stretching (1688 cm^{-1}), (1458 cm^{-1}), and C-OH stretching of phenolic groups (1275 and 1029 cm^{-1}). The spectrum of CCE exhibited the characteristic absorption bands at 3513 (OH stretching vibration), 1622 (carbonyl group [C=O]), 1512 (C=C bonds), 1426 (C-H bending vibration), 1278 (aromatic C-O stretching), and 1032 cm^{-1} (C-O-C stretching vibration). CE presents its characteristic band at 1613 cm^{-1} that indicates the presence of the unsaturated vibration of benzene ring. The band at 1151 cm^{-1} corresponded to the C-O-H stretching of other trace phenolic compounds. The characteristic peaks of LBE were seen at 1448 (aromatic nitro compounds), 1603 (amide, carboxylate, or aromatic ring stretch), 2926 (methylene C-H stretch), and 3372 cm^{-1} (O-H stretching of phenol group). The presence of GTE was confirmed through its characteristic vibrational bands, such as the O-H stretching centered at 3349 cm^{-1}, C=C stretching at 1611 cm^{-1}, O-H bending at 1341 cm^{-1}, and the C-O stretching at 1234 and 1033 cm^{-1}. The FTIR of CLE characterized by eugenol peaks at 3385 (O-H stretching), 1239 (C-O bending), and at 1601 and 1416 cm^{-1} (C-C stretching vibrations in the phenyl ring) and still at 1024 cm^{-1} (C-O-C stretching vibration). In case of AE the FTIR spectrum exhibited characteristic peaks at 3381 cm^{-1} (O-H stretching of phenolic compounds), 2924 (aromatic C-H stretching), 1631 (C=C or COO stretching) and 1034 cm^{-1} (C=O).

As an example of successful incorporation of the extracts in the LDPE matrix, Figure 1b clearly shows the characteristic bands of CLE at 1024 cm^{-1} (C-O-C stretching vibration), perceived among the characteristic LDPE bands.

3.2 | Thermal analysis

The thermograms of all extracts are depicted in Figure 2, with the exception of CCE and RE since they exhibited a weight loss at low temperature (between 70 and 150°C), which can be associated with the presence of moisture and/or traces of ethanol, the solvent used to achieve some of the extracts.

Onset of degradation temperature and the residual mass at 900°C are presented in Table 1. While LBE presents the lower onset temperature (temperature of initial degradation), 180°C, CCE, CLE, and RE are the ones with higher thermal stability, with onset temperatures above 250°C. GTE, CE, and AE exhibit onset values between 210 and 242°C. The differences noticed in the degradation temperatures (temperature of maximum rate decomposition) are due to the volatilization and/or degradation of the compounds contained in each extract. It is possible to observe that LBE presents the lowest thermal stability, showing two degradation peaks, which can be, probably, attributed to the decomposition of bioactive compounds, such as rosmarinic acid. CE, AE, CLE, and RE exhibit a very similar thermal behavior, as can be seen in Figure 2b, with very close onset and degradation temperatures. The greatest loss of mass verified in the thermogram of CE may be due to the decomposition of one of the main constituents, the cinnamaldehyde, both procyanidins, and catechins. The CLE and AE show mass loss with values of approximately 60% that can be associated with the volatilization/decomposition of bioactive constituents, in case of AE probably from anethole or eugenol and for CLE from eugenol. For RE, between 190 and 398°C, it has a mass loss of 86% with onset around at 327°C. This may also attribute to the volatilization/decomposition of bioactive constituents, possibly carnosic acid, and carnosol, as well as from the rosmarinic acid and/or ursolic acid. GTE presented a more complex degradation profile, which is related to the glycosylation of catechins and other components, resulting in a weight loss of 41%. CCE has greater thermal stability, with a mass loss associated to terpenoids present in constitution, as curcuminoids (diphenylheptanoids), demethoxycurcumin, and bisdemethoxycurcumin.

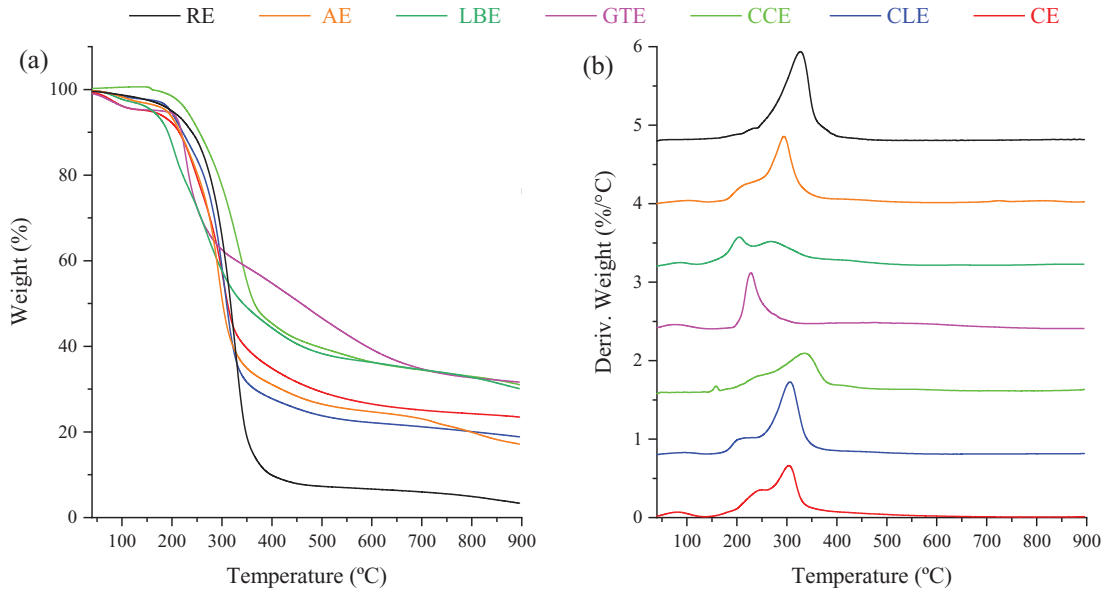

FIGURE 2 Thermogravimetric (TG) (a) and derivative TG (DTG) (b) curves of different extracts

TABLE 1 Onset and degradation temperatures and residual mass of the extracts given by thermal analysis

Extracts	Onset temperature (°C)	Degradation temperature (°C)	Residue mass (%)
LBE	180	268	30
GTE	210	228	32
CE	231	304	24
AE	242	294	17
CCE	255	336	31
CLE	255	306	19
RE	270	327	3

The high values of residual mass at the end of the analysis can be explained by the origin of the extracts, all of them were commercial and were analyzed without further purification.

Therefore, thermal analysis demonstrates that GTE, AE, CE, RE, and CLE are the extracts that exhibit higher thermal stability. Consequently, they can be incorporated in the polymeric matrix of LDPE since the processing temperature is around 180°C, which is lower than the onset decomposition temperature of the extracts.

3.3 | Biological activity

3.3.1 | Antioxidant activity

Even though the most common methods to assess the antioxidant activity are based on chemical mechanisms, in the present work, the methods chosen (TBARS and OxHLIA) are cell-based. These are increasingly employed because the mechanisms involved are believed to be closer to those happening in in vivo systems, which are highly dependent on the oxidizable substrate used in each assay. Therefore, the results of the TBARS and OxHLIA assays, presented in Table 2, allow to recognize the sample concentration providing 50% of the antioxidant activity; lower IC_{50} values correspond to a higher antioxidant capacity. The OxHLIA assay is a suitable method to study free radical-induced oxidative damage of biological membranes and the antioxidant influence of extracts and correspondent films. Sheep erythrocytes were exposed to the oxidizing action of the initiator AAPH, the peroxyl radicals created from the AAPH thermal decomposition attacked the erythrocytes membrane, causing its lysis. Actually, erythrocytes membranes are rich in polyunsaturated fatty acids making them vulnerable to free radical-mediated peroxidation, and consequently membrane attack. As shown in Table 2, RE has the highest antioxidant activity, $IC_{50} = 0.58 \pm 0.04$ μg/ml; this activity was even significantly higher than the one found for the positive control, trolox (85 ± 2 μg/ml). With the exception of AE, CE, and CLE extracts that did not present antioxidant activity, all the extracts revealed a higher antihemolytic capacity than the positive control (1.04 ± 0.07 to 40 ± 3 μg/ml). Instead, most of the films containing the extracts presented the capacity to delay for 60 min the oxidative hemolysis, although in higher concentrations (309 ± 10 to 1603 ± 91 μg/ml). As expected, LDPE did not present any activity and similar observations were also made for LBE and CLE incorporated in the film. Concerning LBE, the fact that the IC_{50} value of the compound extract was the highest one, could possibly explain why the film extract did not present activity at the tested concentrations. On the contrary, films containing AE and CE were revealed to be more active.

The TBARS assay provide information on the compounds capacity to inhibit the formation of thiobarbituric acid reactive substances, such as malondialdehyde generated by the ex vivo decomposition of

TABLE 2 Bioactivity (TBARS and OxHLIA) and cytotoxic activity of the extracts, films incorporated with extracts, and positive control (trolox and ellipticine, respectively)

Extracts	Form	TBARS (IC_{50}; μg/mL)	OxHLIA (IC_{50}; μg/mL)*	Cytotoxic activity (GI_{50}; μg/mL)
AE	extract	447.3 ± 0.2	n.a.	>400
	film	1080 ± 26	432 ± 19.	>400
CE	extract	12.4 ± 0.3	n.a.	263.87 ± 20.37
	film	1086 ± 15	309 ± 10	>400
GTE	extract	0.65 ± 0.03	2.4 ± 0.2	>400
	film	4074 ± 74	704 ± 19	>400
LBE	extract	8.7 ± 0.4	40 ± 3	366.36 ± 25.30
	film	n.a.	n.a.	>400
RE	extract	5.65 ± 0.07	0.58 ± 0.04	>400
	film	n.a.	1603 ± 91	>400
CCE	extract	7.9 ± 0.01	1.04 ± 0.07	141.62 ± 5.01
	film	2706 ± 135	352 ± 11	>400
CLE	extract	8.16 ± 0.02	n.a.	>400
	film	n.a.	n.a.	>400
LDPE	film	n.a.	n.a.	>400
Control		Trolox 139 ± 5	Trolox 85 ± 2	Ellipticine 2.31 ± 0.09

n.a.: no activity.
Results are expressed as mean ± standard deviation.
*Extract concentration required to keep 50% of the erythrocyte population intact for 60 min (Lockowandt et al., 2019).

the lipid peroxidation products. Porcine brain cells are rich in polyunsaturated fatty acids, and therefore, are used for this purpose. The results (Table 2) demonstrate that, with the exception of AE (447.3 ± 0.2 μg/ml), all the extracts show activity, which were higher than the positive control (139 ± 5 μg/ml), GTE presented the highest antioxidant capacity (0.65 ± 0.03 μg/ml). Films with CLE, LBE, and RE did not present capacity to prevent lipid peroxidation.

The results achieved for the extracts are very consistent, since the phenolic compounds are primarily responsible for the antioxidant activity of the extracts, as it is the case of GTE, RE, and CCE, which are richer in phenolic compounds, such as catechins and carnosic acid. Thus, as expected, the antioxidant function of the extracts is affected by presence of these active compounds.

The differences noticed between the antioxidant activity in the extract form or when incorporated into the polymeric LDPE matrix are possibly associated with the main antioxidant compounds degradation at the processing temperature, by its distribution in the matrix, or the inefficiency of the method of extraction of the active compounds from the film.

3.3.2 | Cytotoxic activity

The cytotoxicity results of the extracts and films containing extracts are depicted in Table 2. The extracts revealing toxicity for the primary culture of porcine liver cells were CE, LBE, and CCE, in concentrations of 263.87 ± 20.37, 366.36 ± 25.30, and 141.62 ± 5.01 μg/ml, respectively. Notwithstanding, the GI_{50} values were significantly higher than the concentrations at which these extracts revealed antioxidant properties, meaning that they could be incorporated in lower concentrations. Regarding the films, none of the studied samples presented cytotoxic properties at the tested concentrations.

The cytotoxicity presented by CE, LBE, and CCE is possibly related to their higher concentration of total phenolic acids and flavonoids. Similar observations were previously made with other extracts, such as Portuguese propolis and *Alnus rugosa* L., and reported by Calhelha et al. (2014), Rashed et al. (2014) and Jabeur et al. (2017).

3.3.3 | Antimicrobial activity

The antibacterial activity (Table 3) of the different extracts was evaluated against Gram positive (*S. aureus*, *B. cereus*, and *L. monocytogenes*) and Gram negative (*E. coli*, *E. cloacae*, and *S. Typhimurium*) bacteria. Generally, taking into account a standard deviation of 1.5% for each mean value, LBE was the most active and it was able to inhibit all the bacterial strains in a concentration of 1.09 mg/m;. All the extracts that inhibited the bacterial growth also revealed bactericidal capacity at twice the concentration. CE was the only one that did not show inhibitory nor bactericidal capacity, at the maximum studied concentration (8.50 mg/ml).

Regarding antifungal activity (Table 3), RE showed the lowest MIC and MFC against *A. versicolor* (0.55 and 1.09 mg/ml), *P. funiculosum* (0.27 and 0.55 mg/ml), *P. verrucosum* var. *cyclopium* (0.55 and 1.09 mg/ml), and *A. niger* (0.55 and 1.09 mg/ml), respectively. It was also sensitive to the same concentration of LBE. In the case of *P. funiculosum*, RE demonstrate MIC and MFC values close to the ones found for the positive controls (E211 and E224), (MICs 1.00 and 0.50 mg/ml, and MFCs 2.00 and 0.50 mg/ml, respectively). As presented, all the extracts exhibited inhibitory and fungicidal capacity.

As demonstrated by studies from Stefanovic and Čomić (2012), RE displayed antibacterial activity with MIC values in the range of 5-20 mg/ml for *Bacillus subtilis*, *E. cloacae*, *Klebsiella pneumoniae*, *S. aureus*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, and *E. coli*. Nabavi et al. (2015) demonstrated that CE showed good antibacterial activity against Gram negative bacteria (*E. coli* O157:H7, *Yersinia enterocolitica* O9, *Proteus* spp., and *K. pneumoniae*) with low MIC values (12.5, 6.25, 1.5, and 3.125 μl/ml, respectively). Also, the results reported by Zhang et al. (2016), CE presented MIC and MBC values of 1.0 and 4.0 mg/ml for *E. coli* and 1.0 and 2.0 mg/ml for *S. aureus*.

Xu et al. (2016) verified that CLE revealed strong antibacterial activity against *S. aureus* ATCC 25923 with a MIC of 0.625 mg/ml. The aqueous extracts of *C. longa* rhizome also demonstrate antibacterial activity (MIC = 4–16 mg/ml; MBC = 16-32 mg/ml), mainly against strains, such as, *Staphylococcus epidermidis*, *S. aureus*, *K. pneumoniae*, and *E. coli* (Moghadamtousi et al., 2014).

The results obtained in the present study exhibit higher antibacterial activity than the ones cited in the literature. For *S. aureus* and

TABLE 3 Antibacterial and antifungal activity (mg/ml) of extracts

Antibacterial activity												
	S. aureus (ATCC 11632)		*B. cereus* (food isolate)		*L. monocytogenes* (NCTC 7973)		*S.* Typhimurium (ATCC 13311)		*E. cloacae* (ATCC 35030)		*E. coli* (ATCC 25922)	
Extracts	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
AE	2.19	4.38	2.19	4.38	2.19	4.38	4.38	8.75	4.38	8.75	1.09	2.19
CE	>8.50	>8.50	>8.50	>8.50	>8.50	>8.50	>8.50	>8.50	>8.50	>8.50	>8.50	>8.50
GTE	1.09	2.19	2.19	4.38	1.09	2.19	2.19	4.38	1.09	2.19	2.19	4.38
LBE	1.09	2.19	1.09	2.19	1.09	2.19	1.09	2.19	1.09	2.19	1.09	2.19
RE	1.09	2.19	2.19	4.38	1.09	2.18	1.09	2.18	1.09	2.18	1.09	2.18
CCE	1.09	2.19	2.19	4.38	1.09	2.18	1.09	2.18	1.09	2.18	1.09	2.18
CLE	2.19	4.38	2.19	4.38	2.19	4.38	2.19	4.38	2.19	4.38	1.09	2.19
E211	4.00	4.00	0.50	0.50	1.00	2.00	1.00	2.00	2.00	4.00	1.00	2.00
E224	1.00	1.00	2.00	4.00	0.50	1.00	1.00	1.00	0.50	0.50	0.50	1.00
Antifungal activity												
	Aspergillus fumigatus (ATCC 9197)		*Aspergillus versicolor* (ATCC 11730)		*Aspergillus niger* (ATCC 6275)		*Penicillium funiculosum* (ATCC 36839)		*Penicillium verrucosumvar. cyclopium* (food isolate)		*Trichoderma viride* (IAM 5061)	
Extracts	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC
AE	1.09	2.18	1.09	2.18	1.09	2.18	0.55	1.09	1.09	2.18	0.27	0.55
CE	1.09	2.18	1.09	2.18	1.09	2.18	0.55	1.09	2.18	4.38	0.27	0.55
GTE	0.55	1.09	1.09	2.18	1.09	2.18	0.55	1.09	1.09	2.18	0.27	0.55
LBE	0.55	1.09	1.09	2.18	0.55	1.09	0.55	1.09	1.09	2.18	0.55	1.09
RE	1.09	2.18	0.55	1.09	0.55	1.09	0.27	0.55	0.55	1.09	0.55	1.09
CCE	2.18	4.38	2.18	4.38	1.09	2.18	0.55	1.09	2.18	4.38	1.09	2.18
CLE	2.18	4.38	1.09	2.18	1.09	2.18	0.55	1.09	2.18	4.38	0.55	1.09
E211	1.00	2.00	2.00	2.00	1.00	2.00	1.00	2.00	2.00	4.00	1.00	2.00
E244	1.00	1.00	1.00	1.00	1.00	1.00	0.50	0.50	1.00	1.00	0.50	0.50

E. coli bacteria, according to Thielmann et al. (2019), RE and AE did not present antibacterial activity, which is not in agreement with the results obtained, while LBE revealed a MIC of 100 μg/ml for *S. aureus* and 800 μg/ml for *E. coli*. Nieto et al. (2018) reported that RE did not inhibit *E. coli* ATCC 25922, with the oil showing a MIC > 6.4 mg/L. Tariq and Patole (2015) also tested the antimicrobial activity of GTE and reported MIC values of 11–12 mg/ml and MBC of 12–13 mg/ml, fairly constant for all seven pathogens tested (three *E. coli*, two *K. pneumoniae*, *P. aeruginosa*, and *Citrobacter amalonaticus*).

As stated above, the increased consumers demand has shifted toward use of natural ingredients, as food additives instead of artificial ones. Moreover, the use of plant extracts in food packaging film to decrease the amount of food borne pathogenic microorganisms has become a trend on its own. Successful efforts using plant products have been made in food industry regarding development of new packaging systems, which decrease incidence of microbial spoilage in food products. According to Gyawali et al. (2015), the premise including gradual diffusion of bioactive components from packaging into food has shown an increase in shelf life of certain food products (up to few weeks). However, since natural extracts are of complex chemical nature, additional analyses are a prerequisite for the development of new cost-efficient technologies that will meet the needs of both consumers and food industry.

4 | CONCLUSION

The application of extracts and essential oils of aromatic plants is an innovative approach for active food packaging due to antioxidant and/or antimicrobial properties. Several aromatic natural extracts from different plant parts: leaves (LBE, GTE, and RE), flower (CLE), seed (AE), bark (CE), and rhizome (CCE) were assessed in terms of thermal stability, antioxidant, antimicrobial, and antifungal activities.

While RE presented higher thermal stability, CCE and LBE exhibited lower stability in comparison with the remaining extracts. Based on the results of the OxHLIA assay, RE was the most active extract and CE the most active film, with IC_{50} values of 0.58 ± 0.04 μg/ml and 309 ± 10 μg/ml. The TBARS assay demonstrated that GTE (extract) is the one

with highest activity, (IC_{50} values of 0.65 ± 0.03 μg/ml). Only the CE, LBE, and CCE presented toxicity against the primary cell line (PLP2), however these extracts can be used in packaging if they are incorporated at lower concentrations than GI50 demonstrated. Although all the extracts, except for CE, presented antimicrobial activity, LBE was the most active, with MIC and MBC values of 1.09 and 2.19 mg/ml, respectively. RE was the extract showing the strongest antifungal activity, presenting MIC and MFC values ranging from 0.27 to 0.55 and 0.55 to 1.09 mg/ml, respectively. Generally, the extracts revealed stronger antifungal than antibacterial activity.

Taking into account the different parts of the plants (leaves, flower, seeds, etc.), the results demonstrated that leaves (RE and GTE) and the rhizome (CCE) are the parts with best performance, although they are from different species.

Based on the thermal properties, antioxidant, cytotoxic, antimicrobial activities, it was possible to conclude that RE, GTE, and CCE are the best candidates to be incorporated in the polymeric matrix of LDPE, offering potential to produce active food packaging films that will extend the food shelf-life.

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CONFLICT OF INTEREST

The authors confirm that they have no conflict of interest to declare for this publication.

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