Evaluation of Plant Extracts as an Efficient Source of Additives for Active Food Packaging

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ABSTRACT

Natural extracts have been used, around the world, in several traditional medicine application and also for culinary purposes. Their biological properties, such as, antioxidant and antimicrobial, are due to the presence of several active aromatic compounds in their composition. Herein, different natural extracts (rosemary, anise, lemon balm, green tea, curcumin, clove, and cinnamon) were evaluated, in its natural form and incorporated in 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 ºC, lemon balm extract was the less stable, with a degradation onset temperature at 180 ºC. While, in the thiobarbituric acid reactive substances assay, all extracts presented antioxidant activity, in oxidative hemolysis inhibition, anise, cinnamon and clove extract did not present any action.

Taking into account the different parts of the plants (leaves, flower, seeds, etc), the results demonstrated that leaves (rosemary and green tea) and the rhizome (curcumin) are the parts with best performance. Therefore, extracts from aromatic plants are promising antioxidant and antimicrobial natural additives that can be incorporated into polymeric matrices to produce active food packaging film, which will increase products shelf-life.

Keywords: Aromatic extracts; Antioxidant; Antimicrobial; Cytotoxicity; Active food packaging

1. Introduction

One of the major food industry worry 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 materials is seen as a new trend to preserve food quality and extend its shelf-life (Estevez-Areco et al., 2018, Beya et al., 2021).

Aromatic plants are strongly linked to the human civilization and its evolution. Since ancient times, they have been applied in several traditional medicine applications and also in all cuisines around the world. Several active compounds extracted from these plants are responsible for antioxidant and antimicrobial properties, and their constitution are dependent on the extraction technique, solvent, plant edaphoclimatic conditions, plant piece, among others (Ozdal et al., 2021). Phenolic compounds are present in the majority of edible plants and are responsible for plants biological properties, these are part of the secondary plant metabolites, contributing for their protection against ultraviolet radiation, pathogens, parasites and predators. Moreover, they are responsible for the color and organoleptic properties contributing to the better taste of fruits, for example. More than one hundred of plants extracts have been approved by the Food and Drug Administration (FDA) as GRAS (Generally Recognized as Safe). These extracts are prepared in its 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. Table 1 presents the

selected plants and the part from which the extract was taken, as well as, its main properties described in literature.

Rosmarinus officinalis (rosemary) (RE) is originated from the Mediterranean region and categorized as a woody and aromatic plant. Their leaf extracts have been used in traditional medicine to treat several human diseases and in food preservation. The RE bioactivity include antioxidant, anti-inflammatory, antimicrobial, hepatoprotective, and antidiabetic properties. Caffeic acid derivatives, like rosmanaric acid (phenolic compound), is responsible for the 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 by 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 antioxidant properties (Özogul et al., 2017).

Lemon balm (*Melissa officinalis*) (LBE) is a native plant from the Mediterranean region, it belongs to Lamiaceae family and is employed in traditional medicine. LBE have high levels of phenolic acids, like rosmarinic acid and it is used as 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 good inhibitor on many food spoilage microorganisms (Ju et al., 2018). The 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 pharmaceuticals 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 secure, even at a daily dosage of 12 g for 3 months (Luo et al., 2012).

Table 1 - The main characteristics of selected extracts.

Extract	Latin Name	Plant Part	Activity	References
LBE	*Melissa officinalis L.*	Leaves	Antimicrobial, Antioxidant and Antiseptic	(Boneza & Niemeyer, 2018; Özogul et al., 2017)
GTE	*Camellia sinensis L.*	Leaves	Antioxidant, Antimicrobial, Anticarcinogenic and Anti-inflammatory	(Martins et al., 2018)
RE	*Rosmarinus officinalis L.*	Leaves	Antioxidant, Antimicrobial, Anti-inflammatory, Hepatoprotective and Antidiabetic	(Andrade et al., 2018; Ribeiro et al., 2016)
CLE	*Eugenia carvophyllata*	Flowers	Antibacterial, Antioxidant, Anti-inflammatory, Analgesic, Anti-stress, Antiseptic and Insecticidal	(Ju et al., 2018; Mulla et al., 2017)
AE	*Pimpinella anisum*	Seeds	Antioxidant and Antimicrobial	(Mosavat et al., 2019; Topuz et al., 2016)
CE	*Cinnamomium zeylanicum*	Bark	Antimicrobial, Antioxidant, Anti-inflammatory and	(Ju et al., 2018; Ribeiro-Santos et

			Antitumoral		al., 2017)
CCE	*Curcuma longa*	Rhizome	Antioxidant, Antibacterial and Anticarcinogenic	Antitumoral,	(Almeida et al., 2018; dos Santos et al., 2019; Luo et al., 2012; Silva de Sá et al., 2019)

Thus, the purpose of this research is to screen in terms of biological properties (antioxidant and antimicrobial) a set of commercial extracts to select the most promising ones as a potential additive to produce active polymeric packaging. Therefore, proprieties assessment was made both in the extract form and the extract incorporated in a polymeric matrix.

2. Materials and methods

2.1 Samples

Low-density polyethylene (LDPE) was kindly provided by Vizelpas. The green tea extract (GTE), rosemary extract (RE), cinnamon extract (CE), anise extract (AE), clove extract (CLE) and lemon balm extract (LBE) were acquired from ESSÊNCIAD'UMSEGREDO, LDA. Curcumin extract (CCE) (from *Curcuma 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. 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 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 $U.mL^{-1}$ and 100 mg mL^{-1}, 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). Antifungal agents, ketoconazole and bifonazole, were purchased by Srbolek, and Zorkapharma (Serbia), respectively.

2.3 Film and extracts preparation

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 ºC and 90 rpm, with a residence time of 2 min. From the prepared materials, thin films were produced by compression molding in a hot press at 140 ºC 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 $mg.mL^{-1}$ concentration. Successive dilutions were prepared from the stock solutions.

2.4 Structural and Thermal characterization

Infrared Fourier Transform Spectroscopy (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 ºC/min in a temperature range from 40 to 900 ºC.

2.5 Antioxidant Activity

2.5.1 Thiobarbituric acid reactive substances (TBARS) formation inhibition assay

The capacity of the extracts and films contining the extracts to prevent the 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 ($\mu g.mL^{-1}$).

2.5.2 Oxidative hemolysis inhibition assay (OxHLIA)

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 perfomed as defined in literature (Lockowandt et al., 2019). The acquired results were expressed as IC_{50} values ($\mu L.mL^{-1}$) 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 depicted by Abreu et al., 2011. Ellipticine was used as positive control and the results were expressed as GI_{50} values.

2.7 Antimicrobial activity

The extracts were dissolved in 30% ethanol solution and added to TSB medium. The antibacterial activity was assessed against 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 microdilution method, which consists in the adjustment of bacterial cultures to a concentration of $1x10^5$ CFU/ mL through a spectrophotometer at 625 nm (OD 625), was established the minimum inhibitory (MIC) and minimum bactericidal (MBC) concentrations. Extract's testing was accomplished in different

dilutions of TSB (100mL) and further, 10 µL of the bacterial suspension was added to all the microplates and incubated for 24 hours at 37 ºC. The MIC concentrations were reached by adding iodonitrotetrazolium chloride (INT) (40 mL, 0.2 $mg.mL^{-1}$) and left for incubation during 30 min at 37 ºC. The lowest concentration affording a considerable inhibition (around 50%) of the bacterial growth, comparing with the positive control, was considered as the MIC. The MBC were defined by sub-cultivation of 10 mL into microplates containing 100 mL of TSB and the lowest concentration that shows no bacterial growth is considered as the MBC value. Standard antibiotics, specifically streptomycin and ampicillin, were used as positive controls whereas 5% DMSO was used as the negative control.

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 MIC and MFC (minimum fungicidal concentrations) were determined by using the microdilution method, which consists in dissolving the rice extracts in a DMSO solution that was then added to a broth malt medium containing fungal inoculum. After that, the microplates were incubated for 72 h at 28 ºC. MFCs were defined by serial subcultivation of 2 mL in microtiter plates containing 100 mL of malt broth and further incubation for 72 h at 28 ºC. Moreover, MFC is described as the lowest concentration with no visible growth, suggesting 99.5% killing of the original inoculum. DMSO 5% solution, bifonazole and ketoconazole were used as negative and positive control, respectively.

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) 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 comprising aromatic and phenolic groups. FTIR spectrum shows (Figure 1 - A) 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 cm^{-1} and 1029 cm^{-1}). The spectrum of CCE exhibited the characteristic absorption bands at 3513 cm^{-1} (OH stretching vibration), 1622 cm^{-1} (carbonyl group (C=O)), 1512 cm^{-1} (C=C bonds), 1426 cm^{-1} (C-H bending vibration), 1278 cm^{-1} (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 cm^{-1} (aromatic nitro compounds), 1603 cm^{-1} (amide, carboxylate or aromatic ring stretch), 2926 cm^{-1} (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 cm^{-1} and 1033 cm^{-1}. The FTIR of CLE characterized by eugenol peaks at 3385 cm^{-1} (O-H stretching), 1239 cm^{-1} (C-O bending) and at 1601 cm^{-1} 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 cm^{-1} (aromatic C-H stretching), 1631 cm^{-1}(C=C or COO stretching) and 1034 cm^{-1} (C=O).

Figure 1 - B clearly shows the successful incorporation of the CLE, through the evidence of its characteristic bands at 1024 cm^{-1} (C-O-C stretching vibration), perceived among the characteristic LDPE bands.

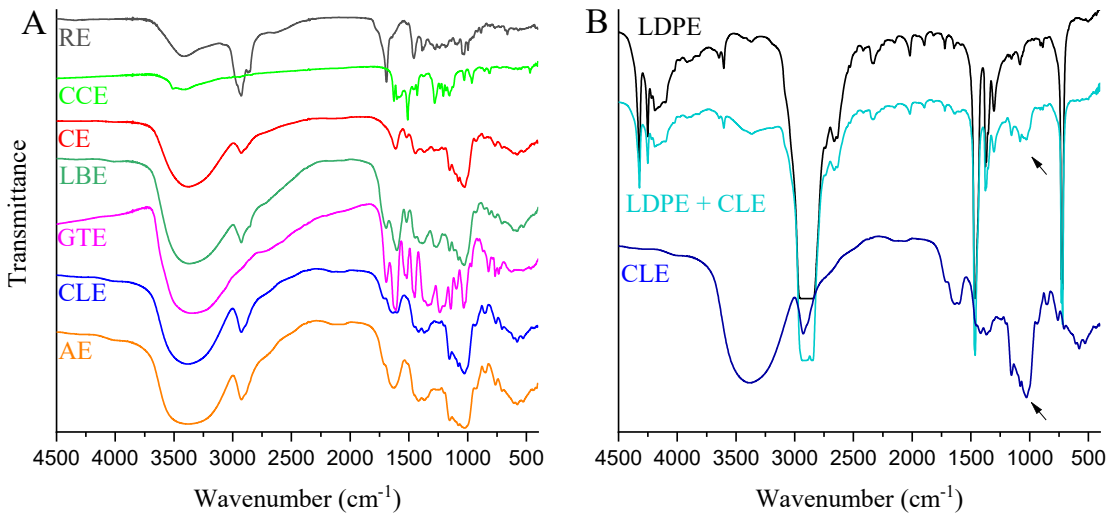

Figure 1 - FTIR spectra of various extracts (A) and comparison of extract and extract incorporate in LDPE matrix (B).

3.2 Thermal Analysis

The thermograms of all extracts are depicted in

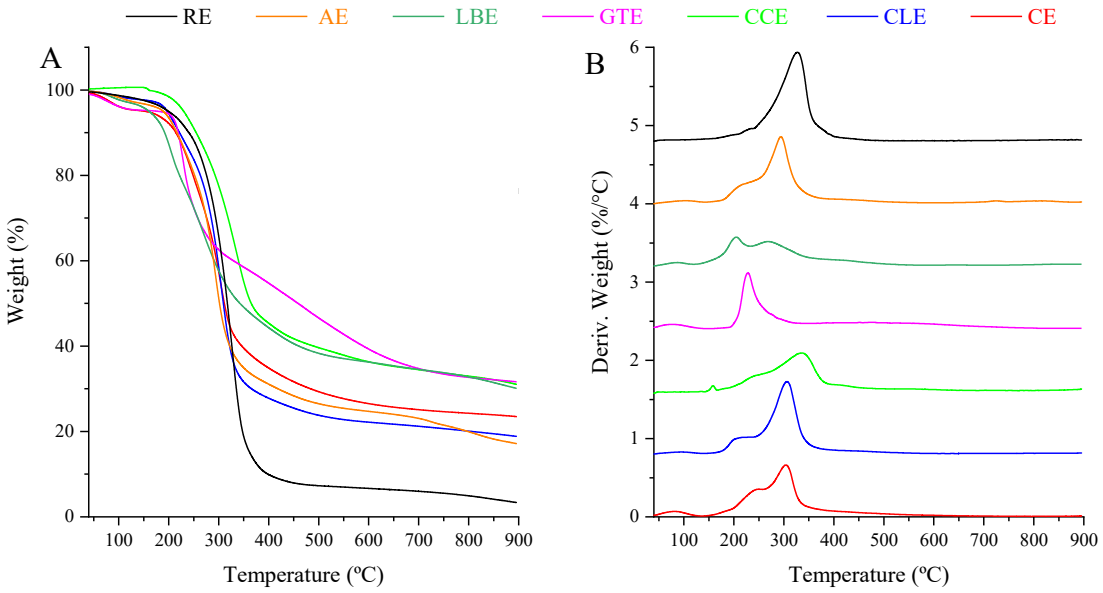

Figure 2, with the exception of CCE and RE since they exhibited a weight loss at low temperature (between 70 and 150 ºC), which can be associated to the presence of moisture and/or traces of ethanol, the solvent used to achieve some the extracts.

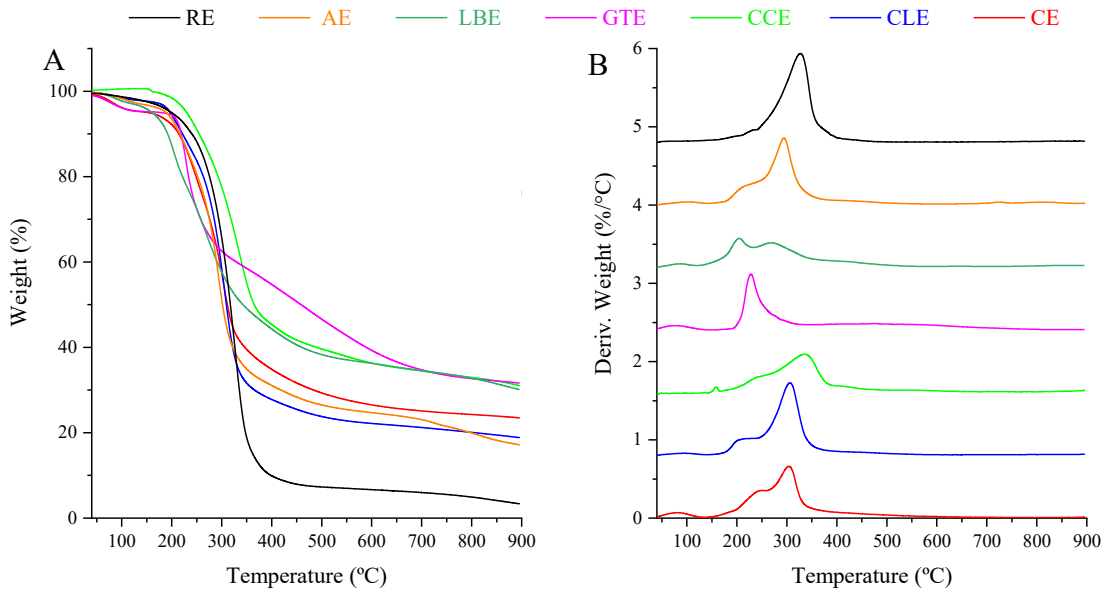

Figure 2 - Thermogravimetric (TG) (A) and derivative TG (DTG) (B) curves of different extracts.

Onset of degradation temperature and the residual mass at 900 ºC are presented in Table 2. While LBE presents the lower onset temperature (temperature of initial degradation), 180ºC, CCE, CLE and RE are the ones with higher thermal stability, with onset temperatures above 250ºC. GTE, CE and AE exhibit onset values between 210 and 242ºC. 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

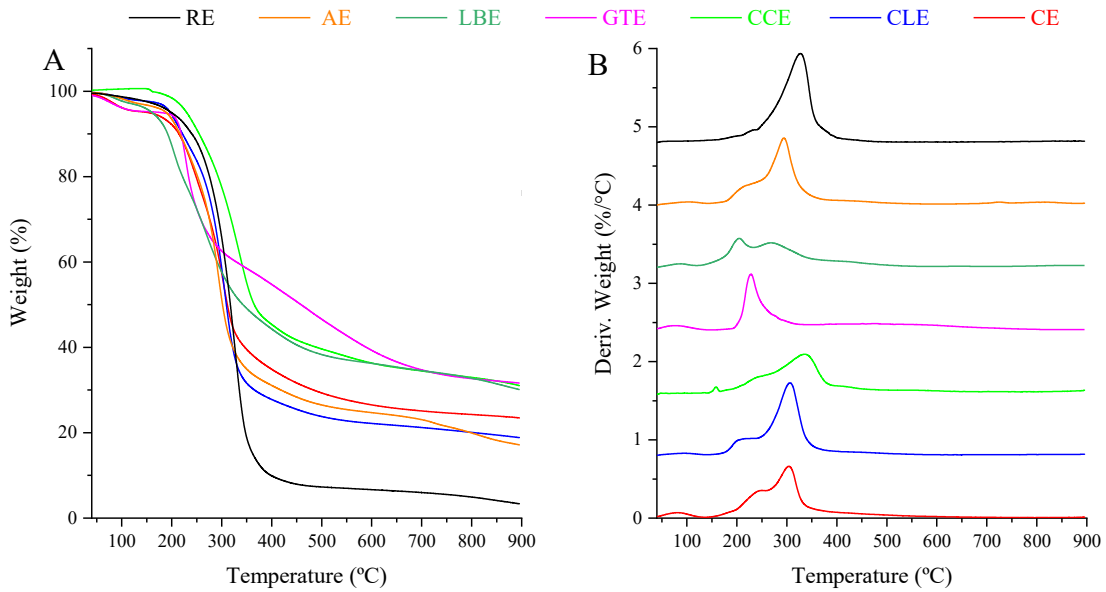

Figure 2 - B, with very close onset and degradation temperatures. The greatest loss of mass verified in the thermogram of CE may be due to the decomposition of one of the main constituents, the cinnamaldehyde, both procyanidins, and catechins. The CLE and AE show mass loss with values of approximately 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 to 398 ºC, it has a mass loss of 86% with onset around at 327 ºC. 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 presents in your constitution, as curcuminoids (diphenylheptanoids), demethoxycurcumin, and bisdemethoxycurcumin.

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.

Table 2 – 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

Therefore, thermal analysis demonstrate 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 ºC, which is lower than the onset decomposition temperature of the extracts.

3.3 Biological activity

3.3.1 Antioxidant activity

The results of the TBARS and OxHLIA assays, presented in Table 3, 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 3, RE has the highest antioxidant activity, IC_{50}=0.58±0.04 μg.mL^{-1}; this activity was even significantly higher than the one found for the positive control, trolox (85±2 μg.mL^{-1}). 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^{-1}). 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^{-1}). 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 that the film extract did not present activity at the tested concentrations. On the contrary, films containing AE and CE 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 the lipid peroxidation products. Porcine brain cells are rich in polyunsaturated fatty acids, and therefore, are used for this purpose. The results (Table 3) demonstrate that, with the exception of AE (447.3±0.2 $\mu g.mL^{-1}$), all the extracts show activity, which were higher than the positive control (139±5 $\mu g.mL^{-1}$), GTE presented the highest antioxidant capacity (0.65±0.03 $\mu g.mL^{-1}$). 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 the ones 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 to the main antioxidant compounds degradation at the processing temperature, by its distribution in the matrix or to the inefficiency of the method of extraction of the active compounds from the film.

Table 3 - Bioactivity (TBARS and OxHLIA) of different extracts, films containing the extracts, and positive control (trolox).

Extracts	**Form**	**TBARS (IC_{50}; $\mu g.mL^{-1}$)**	**OxHLIA (IC_{50}; $\mu g.mL^{-1}$)***
AE	extract	447.3±0.2	n.a.
	film	1080±26	432±19.
CE	extract	12.4±0.3	n.a.
	film	1086±15	309±10
GTE	extract	0.65±0.03	2.4±0.2
	film	4074±74	704±19

LBE	extract	8.7±0,4	40±3
	film	n.a.	n.a.
RE	extract	5.65±0.07	0.58±0.04
	film	n.a.	1603±91
CCE	extract	7.9±0.01	1.04±0.07
	film	2706±135	352±11
CLE	extract	8.16±0.02	n.a.
	film	n.a.	n.a.
LDPE	film	n.a.	n.a.
Trolox		139±5	85±2

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).

3.3.2 Cytotoxic activity

The cytotoxicity results of the extracts and films containing extracts are depicted in Table 4. The only extracts revealing toxicity for the primary culture of porcine liver cells are CE, LBE, and CCE, in concentrations of 263.87±20.37, 366.36±25.30, and 141.62±5.01 µg.mL^{-1}, 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 in total phenolic acids and flavonoids. Similar observations were previously made with other extracts, as Portuguese propolis and *Alnus rugosa* L., and reported by Calhelha, et al. (2014), Rashed et al. (2014), and Jabeur et al. (2017).

Table 4 - Cytotoxic (GI_{50} values µg.mL^{-1}) activity of the extracts, films incorporated with extracts, and positive control (ellipticine).

Extracts	**Form**	**Cytotoxic activity (GI_{50}; µg.mL^{-1})**
AE	extract	>400
	film	>400
CE	extract	263.87±20.37
	film	>400
GTE	extract	>400

	film	>400
LBE	extract	366.36±25.30
	film	>400
RE	extract	>400
	film	>400
CCE	extract	141.62±5.01
	film	>400
CLE	extract	>400
	film	>400
LDPE	film	>400
Ellipticine		2.31±0.09

Results are expressed as mean ± standard deviation.

3.3.3 Antimicrobial activity

The antibacterial activity (Table 5) of the different extracts was evaluated against Gram positive (*Staphylococcus aureus*, *Bacillus cereus*, and *Listeria monocytogenes*) and Gram negative (*Escherichia coli*, *Enterobacter cloacae*, and *Salmonella typhimurium*) bacteria. Generally, LBE was the most active, it was able to inhibit all the bacterial strains in a concentration of 1.09 $mg.mL^{-1}$. 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 $mg.mL^{-1}$).

Regarding antifungal activity (Table 6), RE showe the lowest MIC and MFC against *A. versicolor* (0.55 and 1.09 $mg.mL^{-1}$), *P. funiculosum* (0.27 and 0.55 $mg.mL^{-1}$), *P. verrucosum* (0.55 and 1.09 $mg.mL^{-1}$), and *A. niger* (0.55 and 1.09 $mg.mL^{-1}$), respectively. This latest 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 control, the antifungal drug ketoconazole (0.20 and 0.50 $mg.mL^{-1}$). As presented, all the extracts exhibited inhibitory and fungicidal capacity.

Studies from literature by Stefanovic and Čomić (2012), RE displayed antibacterial activity with MIC values in the range of 5-20 $mg.mL^{-1}$ for *Bacillus subtilis, Enterobacter cloacae, Klebsiella pneumoniae, Staphylococcus aureus, Pseudomonas aeruginosa, Proteus mirabilis* and *Escherichia coli* (Stefanovic & Čomić, 2012). 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 *Klebsiella pneumonia*) with low MIC values (12.5 µL.mL^{-1}, 6.25 µL.mL^{-1}, 1.5 µL.mL^{-1} and 3.125 µL.mL^{-1}, respectively) (Nabavi et al., 2015). Also, the results reported by Zhang, Liu, Wang, Jiang, & Quek (2016), CE presented MIC and MBC values of 1.0 and 4.0 mg.mL^{-1} for *E. coli* and 1.0 and 2.0 mg.mL^{-1} for *S. aureus* (Zhang et al., 2016).

Xu, Liu, Hu, & Cao (2016) verified that CLE revealed strong antibacterial activity against *Staphylococcus aureus* ATCC 25923 with a MIC of 0.625 mg.mL^{-1} (Xu et al., 2016). The aqueous extracts of *Curcuma longa* rhizome also demonstrate antibacterial activity (MIC=4-16 mg.mL^{-1}; MBC=16-32 mg.mL^{-1}), mainly against strains, such as, *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, and *Escherichia coli* (Moghadamtousi et al., 2014).

The results obtained in the present study exhibit higher antibacterial activity than the ones cited in the literature. For *Staphylococcus aureus* and *Escherichia coli* bacteria, according to Thielmann, Muranyi, & Kazman (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^{-1} for *S. aureus* and 800 µg.mL^{-1} for *E. coli* (Thielmann et al., 2019). Nieto, Ros, & Castillo (2018) reported that RE did not inhibit *E. coli* ATCC 25922, with the oil showing a MIC >6.4 mg.L^{-1} (Nieto et al., 2018). Tariq and Patole (2015) also tested the antimicrobial activity of GTE and reported MIC values of 11-12 mg.mL^{-1} and MBC of 12-13 mg.mL^{-1}, fairly constant for all seven pathogens tested (3 *Escherichia coli, 2 Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Citrobacter amalonaticus*) (Tariq & Patole, 2015).

Table 5 - Antibacterial activity ($mg.mL^{-1}$) of extracts.

Extracts	*S. aureus* (ATCC 11632)		*B. cereus* (food isolate)		*L. monocytogenes* (NCTC 7973)		*S. typhimurium* (ATCC 13311)		*E. cloacae* (ATCC 35030)		*E. coli* (ATCC 25922)	
	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
Streptomycin	0.006	0.012	0.10	0.20	0.20	0.30	0.20	0.30	0.003	0.006	0.20	0.30
Ampicilin	0.012	0.025	0.25	0.40	0.40	0.50	0.75	1.20	0.006	0.012	0.40	0.50

Table 6 - Antifungal activity ($mg.mL^{-1}$) of extracts.

Extracts	*Aspergillus fumigatus* (ATCC 9197)		*Aspergillus versicolor* (ATCC 11730)		*Aspergillus niger* (ATCC 6275)		*Penicillium funiculosum* (ATCC 36839)		*Penicillium verrucosum* var. *cyclopium* (food isolate)		*Trichoderma viride* (IAM 5061)	
	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
Ketoconazole	0.20	0.50	0.20	0.47	0.20	0.50	0.20	0.50	0.20	0.30	0.20	0.30

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. According to the OxHLIA assay, RE and CE were the most active extract and film, respectively, with IC_{50}values of 0.58±0.04 µg.mL^{-1} and 309±10 µg.mL^{-1}. The TBARS assay demonstrated that GTE (extract) is the one with highest activity, (IC_{50} values of 0.65±0.03 µg.mL^{-1}). Only the CE, LBE, and CCE presented toxicity. 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^{-1}, 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^{-1}, 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.

Based on the thermal properties, antioxidant, cytotoxic, antimicrobial and antifungal 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 increase the food shelf-life.

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