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Study of bioactive volatile compounds from different parts of *Pistacia lentiscus* L. extracts and their antioxidant and antibacterial activities for new active packaging application.

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Abstract

Macerates of fruits and leaves of *Pistacia lentiscus* L. were prepared and analysed with the aim of applying them for active packaging. The profile of forty-four different bioactive volatile compounds was obtained by means of gas chromatography-mass spectrometry and

solid-phase microextraction gas chromatography-mass spectrometry. Antioxidant capacity was evaluated by three different methods (2,2-diphenyl-1-picrylhydrazyl, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid and reducing power) which confirmed stronger antioxidant properties in case of leaves macerate. Total phenolic and flavonoids content was determined and showed that macerate leaves presented 15 times more phenolic compounds and 20 times more flavonoids than macerate fruit. Moreover, the analysis of antimicrobial properties of macerate leaves in comparison with macerate fruits revealed very strong antimicrobial properties. Finally, macerate leaves extract was incorporated in an adhesive and a new active multilayer packaging was designed, and its antioxidant capacity as free radical scavenger was confirmed by a method based on in situ hydroxyl radicals generator.

Keywords: *Pistacia lentiscus* L.; antioxidant capacity; antimicrobial capacity; liquid injection GC-MS; HS-SPME-GC-MS; active packaging

1. Introduction

Food safety is being challenged nowadays by strong consumer demands. Food is expected to be primarily safe, then wholesome and nutritious. This led researchers to create a novel concept of packaging that extend the freshness of food products without compromising their quality by incorporating active agents (Wrona & Nerin, 2019).

Undoubtedly, appropriate election and incorporation of active compounds such as antioxidants and antimicrobials are crucial steps during active packaging development. Different active packaging technologies have been designed and applied to reduce foods decay and also to limit environmental pollution connected with packaging. One of the main difficulties when developing an antioxidant material for food protection is the incorporation

of the active agent in an efficient and feasible way, so that the new material can act as an antioxidant without modifying the packaging line or the characteristics of the packaged product (Borzi, Torrieri, Wrona, & Nerín, 2019; Wrona, Cran, Nerín, & Bigger, 2017).

Moreover, the idea of active packaging can be based on compounds from natural resources due to clear trend for substitution of synthetic polymer additives by natural ones. Natural antioxidants are produced in living cells to protect them from the damage due to free radicals produced in chain reactions. In this sense, some fruits and vegetables are good sources of antioxidants (Chang, Alasalvar, & Shahidi, 2016). Moreover, natural extracts can also contain compounds with antimicrobial properties (Gavril et al., 2019).

It is worth drawing attention to plants that are capable of producing from hundreds to thousands of metabolites with a wide range of biological functions. According to literature, the main chemical groups of bioactive compounds present in plants are glycosides, polyphenols and terpenoids (Paulsen, 2010).

Pistacia lentiscus L. is an evergreen plant from Mediterranean area, where is well known because of its nutritional, medicinal and pharmaceutical properties. Several researches have been carried out over time on the different parts of this plant, to determine their antibacterial activity (Alhadi, Omer, Saad, & Yagi, 2018; Missoun, 2017) and antioxidant properties (Bampouli et al., 2014; Benhammou et al., 2018; Bouyahya et al., 2018). Several studies on the composition of the leaves and fruits of *Pistacia lentiscus* L. indicated that this plant contains a wide range of metabolites known for their therapeutic properties (Chekchaki et al., 2017; Rodríguez-Pérez et al., 2013; Sameh et al., 2016). Nevertheless, limited studies on volatile compounds profile characterization have been performed and, to the best of our knowledge, *Pistacia lentiscus* L. extracts have not been tested as potential active agent for active packaging applications.

The aim of this research was to identify bioactive volatile compounds from extracts of fruits and leaves of *Pistacia lentiscus* L. To achieve this goal, two analytical methods were used: a headspace solid-phase microextraction coupled to gas chromatography-mass spectrometry (HS-SPME-GC-MS) and liquid injection (LI) gas chromatography-mass spectrometry (GC-MS). Then, both antioxidant and antimicrobial capacities of active extracts were determined. Finally, a new multilayer active packaging was developed by incorporation of active extracts into a water-based adhesive layer. Finally, a hydroxyl radical generation method, previously developed in our research group, was applied to determine the real antioxidant capacity directly in the active films.

2. Materials and methods

2.1. Reagents

Methanol (HPLC grade, CAS 67-56-1) and ethanol (HPLC grade, CAS 64-17-5) were provided by Scharlau Chemie S.A. (Sentmenat, Spain). Potassium persulfate (>99%, CAS 7727-21-1); potassium ferricyanide (99%, CAS 13746-66-2); trichloroacetic acid (>99%, CAS 76-03-9); iron (III) chloride (>99.99%, CAS 7705-08-0); sodium carbonate (99.99%, CAS 497-19-8); gallic acid ($\geq 98.0\%$, CAS 149-91-7); aluminium chloride (99.99%, CAS 7446-70-0), 2,2-diphenyl-1-picrylhydrazyl (DPPH, CAS 1898-66-4), Folin-Ciocalteu phenol reagent, sodium salicylate (>99.5%, CAS 54-21-7) and 2,5-dihydroxybenzoic acid (>99%, CAS 490-79-9) were supplied by Sigma-Aldrich (Madrid, Spain). Ultrapure water was obtained from a Wasserlab Ultramatic GR system (Barbatáin, Spain).

2.2. Microbial strains

The evaluation of the antibacterial activity relating to the macerates of *Pistacia lentiscus* L. (leaves and fruits) was studied with respect to five Gram-positive bacterial strains:

Staphylococcus aureus (ATCC 6538), *Staphylococcus epidermidis*, *Meticillin-resistant Staphylococcus aureus* (MRSA, ATCC 43300), *Bacillus subtilis* (ATCC 6633), *Listeria innocua* (CLIP 74915) and four Gram-negative bacterial strains: *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Acinetobacter baumannii* (610) and *Salmonella sp* (Hospital strain).

Growth media for the culturing of bacteria such as Nutrient Agar, Mueller Hinton Agar and Mueller Hinton Broth were provided by Sigma-Aldrich (Madrid, Spain).

2.3. Plant Material

The plant material, consisting of leaves and ripe fruit of the medicinal plant *Pistacia lentiscus* L., was collected in the region of Bejaia (Algeria) in January 2018. The specimens of collected samples were identified by the Vegetable Ecological Laboratory of the Algiers University, Algeria. The samples were washed with distilled water, dried in the shade for a week, and then ground in an electric grinder (Sayona SZJ-1306). The powder was passed through standard 120 mesh (125 μ m) sieve and stored in airtight bags in the dark until use.

2.4. Macerates preparation

The extraction of the phenolic compounds was carried out by maceration according to the method developed by Diallo et al. (Diallo et al., 2004). Briefly, 40 g of plant material powder and 200 mL of methanol were macerated for 24 hours at room temperature on a stirring plate protected from light. The extracts were filtered using filter paper and stored at 4 °C for subsequent analysis.

2.5. Active packaging preparation

Solutions (w/w) of macerate *Pistacia lentiscus* L. (either from fruit or from leaves) at concentrations 1% and 2% in water-based adhesive were prepared and vortexed during 2 min. 2% was the maximum possible concentration of that did not compromise the properties of adhesive (homogeneity, adhesion to substrates and high cohesive strength).

Two different active materials were prepared by incorporation of active adhesive (AA) in between of two films: 35 μ m low density polyethylene (LDPE), intended to be in direct contact with foodstuffs, and 12 μ m polyethylene terephthalate (PET) as external layer. This way, a multilayer active material LDPE/AA/PET was prepared, as shown in Figure 1. Application of neat adhesive without active agent (BKA) was used to prepare blank material LDPE/BKA/PET. Films were prepared on laboratory scale by K202 Control Coater 2005 (RK Printcoat Instrument).

Despite its detailed composition is confidential, an acrylic water-based adhesive approved for food contact was provided by a Spanish company for food packaging applications. It means that migration of components of adhesive to food simulants and/or packaged food is below the established limits by European Union according to Commission Regulation 10/2011 (Union Europea, 2011). Therefore, the used adhesive is non-toxic, and no increased risks are expected at all.

<<Figure 1>>

2.6. Sample treatment

Figure 2 shows the hierarchical graph representing the applied experimental design. In the first step, samples of leaves and fruits of *Pistacia lentiscus* L. were collected and macerated. A wide range of different analyses were carried out to determine the chemical properties of the obtained macerates. It included identification of bioactive volatile

compounds by two analytical methods. Liquid injection GC-MS and HS-SPME-GC-MS) were required for these tasks. Also, antioxidant and antimicrobial capacities as well as total phenolic and flavonoid content were determined. Then, the active agent was incorporated into the adhesive and an active packaging was developed. The antioxidant capacity (CAOX) of developed packaging was evaluated by in situ generator of free radicals (Pezo, Salafranca, & Nerín, 2006, 2008).

<<Figure 2>>

2.7. Identification of bioactive volatile compounds

2.7.1. SPME-GC-MS method

Each macerate was diluted 10 times using 20% ethanol and 18 mL of it were placed in a glass vial and directly analysed by SPME-GC-MS method. For this purpose, a CTC Analytics CombiPal coupled to an Agilent Technologies 6890N gas chromatograph with an MS 5975B mass spectrometer (Madrid, Spain) was used. Two different chromatographic columns were tested: HP-5MS (30 m $\times$ 0.25 μ m $\times$ 250 μ m) and BP20 (30 m $\times$ 0.25 μ m $\times$ 250 μ m) due to different polarities. The oven program was as follows: 40 °C for 2 min, with a rate of 10 °C min up to 300 °C for HP-5MS column (200°C for BP20 column), held for 2 min. Carrier gas was helium used with flow 1 mL $\cdot$ min⁻¹. For SPME, 30/50 μ m DVB/CAR/PDMS fibre from Supelco (Bellefonte, PA, USA) was selected. Adsorption was performed at 80 °C during 15 min. Desorption time was 2 min. Temperature of injector was 250 °C. While temperatures of MS Source and MS Quad were 230 °C and 150 °C respectively. The mass detector was used in SCAN mode (in the range of m/z from 45 to 350). Identification was carried out by comparison of the mass spectrum of obtained peaks with NIST library. It was considered that

a candidate was confirmed by NIST peak recognition fitter when match value was higher than 85%.

2.7.2. GC-MS method

0.1% solution of macerate was prepared in methanol and 1 μ L was injected directly in splitless mode (2 min) into GC-MS under the same analytical conditions as previously described. Solvent delay was 4 min.

2.8. Antioxidant capacity of extracts

2.8.1. 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay

Antioxidant capacity of each extract was measured by the procedure described by Brand-Williams et al. (Brand-Williams, Cuvelier, & Berset, 1995). 100 μ L of different concentrations of each macerate were added to 3 mL of the methanolic solution of DPPH. Methanol was used as blank. The absorbance (517 nm) was read against blank using a Shimadzu UV-1700 PharmaSpec spectrophotometer (Duisburg, Germany) after incubation of solutions in the darkness for 30 min at room temperature. Antioxidant capacity was expressed as the percentages of inhibition of the radical IC₅₀.

2.8.2. 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) assay

CAOX of the macerates was assessed by the ABTS test (Spigno & De Faveri, 2009). Free radical aqueous solution (7 mM ABTS) was incubated with 2.45 mM solution of potassium persulfate in the darkness at room temperature for 12-16 h. Solution was then diluted with 50% ethanol to obtain an absorbance value of 0.705 ± 0.02 measured at 734 nm and equilibrated at 30 °C. The reaction was performed by mixing of 2 mL of free radical

solution with 20 μ L of macerate. The absorbance of the three samples was read after 6 min at 734 nm against 50% ethanol. Also blank was prepared and analysed. CAOX was expressed as inhibition percentage of the ABTS radical.

2.8.3. Reducing power

The determination of the ferric reducing antioxidant power (FRAP) was carried out according to the method described by Oyaizu (Oyaizu, 1986): 1 mL of different concentrations of each macerate were mixed with 2.5 mL of the phosphate buffer solution (0.2 M, pH 6.6) and 2.5 mL of 1% solution of potassium ferricyanide. The mixtures were incubated at 50 °C for 30 min. After this time, 2.5 mL of 10% solution of trichloroacetic acid mixed with 2.5 mL of distilled water and 0.5 mL of iron (III) chloride (0.1%) were added. Absorbance was measured against blank at 700 nm using a spectrophotometer. The ferric reducing antioxidant power was expressed as effective concentration IC_{50} .

2.9. Total phenolic content

The determination of the total phenolic content was carried out according to the protocol previously described in literature (George & Bennett, 2005). 500 μ L of macerate were mixed with 2.5 mL of 10-times diluted Folin-Ciocalteu reagent. It was stored during 2 min in the darkness and then 2 mL of 75 g·L⁻¹ solution of sodium carbonate was added. After 15 minutes of incubation at 50 °C, the absorbance was measured against blank at 760 nm using spectrophotometer. The concentrations were expressed as mg gallic acid equivalent per g of powder (GAE).

2.10. Total flavonoids

The total flavonoid content was determined by a colorimetric method described by Serra Bonvehi et al. (Bonvehí, Torrentó, & Centelles, 2001). 1 mL of 2% solution of aluminium chloride was added to 1 mL of macerate. Incubation was carried out during 15 minutes in the darkness. After this time the absorbance was measured against blank at 430 nm using spectrophotometer. The results were expressed as mg quercetin equivalent per g of powder (QE).

2.11. Antimicrobial capacity

2.11.1. Disc diffusion method

The evaluation of the antibacterial activity related to the different macerates of *Pistacia lentiscus* L. was studied with respect to 9 bacterial strains chosen for their high frequency to induce food-borne and gastrointestinal infections. The antimicrobial activity was demonstrated by the diffusion method of the antibacterial compound on the agar medium. The bacterial strains were inoculated into Petri dishes containing agar as nutrient. After 18 h of incubation at 37 °C, microbial suspensions with an optical density of 0.5 Mc Farland were prepared. Whatman paper disks (d = 6 mm) were soaked with 20 µL of macerate of *Pistacia lentiscus* L. Then they were placed on the surface of the dry Muller Hinton agar and after incubation at 37 °C for 24 h. The inhibition halo (mm) was checked (Nalubega, David Kaba, Olila, & Kateregga, 2011).

2.11.2. MIC and MBC

The determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) was as follows: bacteria were suspended into sterile NaCl (0.8–0.9%) to obtain McFarland value of 0.5 (1.0×10^8 CFU·mL⁻¹). Inoculum solution was

diluted to reach 1.0×10^6 CFU·mL⁻¹. Active solutions: in each line of the microplate 100 µL of Muller Hinton medium, 100 µL of macerate and 100 µL of the microbial suspension were deposited. 40 µg·g⁻¹ (v/v) of macerates solutions in Mueller Hinton Broth growing medium (MHB) were prepared. The incubation conditions were 37 °C maintained during 24 h.

Then MBC procedure was based on application of 100 µL of macerate solutions at concentration equal and higher to MIC on agar culture medium. The incubation conditions were 37 °C during 24 h.

2.12. Antioxidant capacity of active materials

Pezo et al. (Pezo et al., 2006, 2008) developed a method for direct analysis of antioxidant capacity of polymers. It consists of a generator of gas-phase hydroxyl free radicals and its quantitative analysis by comparing the antioxidant material (LDPE/AA/PET) vs. blank material (LDPE/BKA/PET). Generation of radicals was performed thanks to photochemical reaction in combination of mist of hydrogen peroxide and UV radiation. In the next step, generated radicals pass through plastic bags (13 × 13 cm) made of potential antioxidant (radical scavenger) and blank material and finally they are bubbled into a solution of sodium salicylate. As a result of reaction of hydroxyl radicals and sodium salicylate, 2,5-dihydroxybenzoic acid is generated and quantified using HPLC (Waters 2795 Series) with fluorescence detector 474 (Milford, USA). Assay was performed during 48 h. Bags were prepared using a sealer PFS-200 Zhejiang Dongfeng Packing Machine Co. (Wenzhou, Zhejiang, China). Antioxidant capacity of active films was tested right after their preparation. Samples were prepared in triplicate. Results were expressed as hydroxylation percentage (H%).

2.13. Statistics

All analyses were carried out in triplicate and experimental data were expressed as mean $\pm$ standard deviation (95% confidence interval).

3. Results and Discussion

3.1. Identification bioactive volatile compounds

The bioactive volatile compounds profile was determined and analysed by GC-MS and SPME-GC-MS. Table 1 presents the identification of compounds, numbered according to their retention time. The type of column and injection mode are indicated for each compound. Also, a list of presence of bioactive analytes in specific macerates is provided. Table 1. Results of identification of bioactive volatile compounds. Compounds present in the different samples are marked with the symbol “■”.

N°	t _R (min)	Retention Index	Compound	CAS	Chemical class	Injection mode	Column	Macerate of <i>Pistacia lentiscus</i> L.	
								Leaves	Fruits
1	8.400	865	2-furanmethanol	98-00-0	alcohol	LI*	HP5	■	
2	8.822	986	phenol	108-95-2	phenol	LI	HP5	■	
3	9.085	1130	2-carene	554-61-0	monoterpene	SPME	BP20	■	
4	9.746	1188	D-limonene	5989-27-5	monoterpene	LI, SPME	BP20	■	
5	10.447	1222	gamma-terpinene	99-85-4	monoterpene	SPME	BP20	■	
6	10.910	1255	o-cymene	527-84-4	monoterpene	SPME	BP20	■	
7	11.615	1300	tridecane	629-50-5	alkane	SPME	BP20		■
8	13.765	1134	2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one	28564-83-2	flavonoid	LI	HP5	■	
9	13.803	1443	decyl nonyl carbonate	1000383-15-8	ester	SPME	BP20		■
10	14.889	1514	1,2,5,5,6,7-hexamethylbicyclo [4.1.0]hept-2-en-4-one	1000110-52-5	ketone	SPME	BP20	■	
11	15.812	1570	beta-caryophyllene	87-44-5	sesquiterpene	SPME	BP20		■
12	16.053	1593	1,2-cyclohexanedione	765-87-7	ketone	LI	BP20	■	■
13	16.910	1696	verbenone	1196-01-6	ketone	SPME	BP20	■	
14	16.993	1369	orcinol	504-15-4	polyphenol	LI	HP5	■	
15	17.414	1507	1,2,3-benzenetriol	87-66-1	polyphenol	LI	HP5	■	
16	17.611	1720	gamma-cadinene	483-76-1	sesquiterpene	LI, SPME	BP20	■	■
17	17.661	1526	hydroxyquinol	533-73-3	polyphenol	LI	HP5		■
18	18.547	1850	trans-calamenene	73209-42-4	sesquiterpene	SPME	BP20	■	■
19	18.720	1874	benzyl alcohol	100-51-6	alcohol	LI	BP20	■	■
20	19.444	1916	alpha-calacorene	21391-99-1	sesquiterpene	SPME	BP20	■	
21	22.421	2203	cadalene	483-78-3	sesquiterpene	SPME	BP20	■	

22	22.454	1927	methyl hexadecanoate	112-39-0	ester	LI	HP5	■	■
23	22.818	2246	ethyl hexadecanoate	628-97-7	ester	SPME	BP20		■
24	23.640	2000	2,3-dihydrobenzofuran	496-16-2	benzofuran	LI	HP5	■	■
25	23.808	2341	2,4-diphenyl-4-methyl-1-pentene	1000111-58-0	aromatic	SPME	BP20	■	
26	23.970	2026	palmitoleic acid	373-49-9	carboxylic acid	LI	HP5	■	■
27	24.149	2040	n-hexadecanoic acid	57-10-3	carboxylic acid	LI	HP5		■
28	24.488	2406	bis(2-ethylhexyl) adipate	103-23-1	ester	SPME	BP20	■	
29	24.931	2431	apiol	523-80-8	propenylphenol derivative	SPME	BP20	■	■
30	24.939	2446	ethyl octadecanoate	111-61-5	ester	LI	BP20	■	■
31	25.014	2109	(Z,Z)-1,3-cyclooctadiene	3806-59-5	diene	LI	HP5	■	
32	25.297	2113	(Z,Z)-9,12-octadecadienoic acid	60-33-3	carboxylic acid	LI	HP5	■	
33	25.352	2515	nootkatone	4674-50-4	sesquiterpene	SPME	BP20	■	
34	25.502	2124	octadecanoic acid	57-11-4	carboxylic acid	LI	HP5	■	
35	25.544	2536	ethyl linoleate	544-35-4	ester	LI	BP20	■	
36	25.819	2141	(E)-9-octadecenoic acid	112-79-8	carboxylic acid	LI	HP5		■
37	26.299	2227	columbianetin	3804-70-4	heterocyclic	LI	HP5	■	
38	26.861	2255	ethyl linolenate	1191-41-9	ester	LI	HP5	■	■
39	27.126	2294	3-tridecylphenol	72424-02-3	phenol	LI	HP5		■
40	27.644	2661	catechol	120-80-9	polyphenol	LI	BP20	■	■
41	28.458	2458	2-palmitoylglycerol	23470-00-0	monoglyceride	LI	HP5	■	■
42	28.679	2479	(Z)-3-(pentadec-8-en-1-yl)phenol	501-26-8	phenol	LI	HP5		■
43	28.817	2499	3-pentadecyl-phenol	501-24-6	phenol	LI	HP5		■
44	30.208	2520	(Z)-3-(heptadec-10-en-1-yl)phenol	111047-33-7	phenol	LI	HP5		■

*LI – Liquid Injection

The profile of forty-four different bioactive volatile compounds was obtained. Thirty-three compounds eluted from the sample of macerate leaves and twenty-three compounds from one of macerate fruit (Catalani, Palma, Battistelli, & Benedetti, 2017; Djenane, Yangüela, Montañés, Djerbal, & Roncalés, 2011; Yosr, Imen, Rym, Chokri, & Mohamed, 2018). There are very few publications about the identification of volatile compounds from *Pistacia lentiscus* L. and they are focused on essential oils obtained from leaves and fruits. Few compounds determined in this work are in common with those articles, among them: d-limonene, o-cymene, gamma-terpinene, caryophyllene and gamma-cadinene. It indicates that obtained extract are reach in different volatile compounds.

Chemical classes of identified compounds are also presented in table 1. The two largest classes of compounds detected in this investigation are phenols/polyphenols and monoterpenes/sesquiterpenes. Natural antioxidants are produced in living cells to protect them from the damage due to free radicals chain reactions. Among the natural antioxidants from plant origin the most common compounds are phenols and polyphenols. They can block radical chain reactions, work as enzyme inhibitors or as metal-chelating agents. Among this group, the detected compounds included phenol, orcinol, 1,2,3-benzenetriol, hydroxyquinol, catechol, (Z)-3-(pentadec-8-en-1-yl)phenol, 3-pentadecyl-phenol and (Z)-3-(heptadec-10-en-1-yl)phenol.

Monoterpenes and sesquiterpenes are widely present in the plants because they act as allelopathic agents. Their antioxidant capacity has been studied and proven. They can act as free radical quenchers and they can function through either the hydrogen donor or electron donor mechanism. In this study 10 different monoterpenes and sesquiterpenes were identified: 2-carene, d-limonene, gamma-terpinene, o-cymene, caryophyllene, gamma-cadinene, trans-calamenene, alpha-calacorene, cadalene and nootkatone.

Different classes of bioactive volatile compounds, which can be characterized by different antioxidant mechanisms, were identified. As a result, *Pistacia lentiscus* L. macerate can be considered as a very good potential active agent for incorporation into active packaging for food applications.

In terms of antimicrobial compounds, it has been proven that some detected phenols and terpenes have antimicrobial activity (Ultee, Bennis, & Moezelaar, 2002), such as d-limonene, effective against *Staphylococcus aureus*, and gamma-terpinene, effective against *Listeria innocua*, *Pseudomonas aeruginosa*, and *Salmonella*.

3.2. Antioxidant capacity of extracts

Figure 3 shows a bar graph that is a summary of results of antioxidant capacities of samples of leaf and fruit macerates measured using three different methods.

<<Figure 3>>

3.2.1. DPPH assay

As shown in Figure 3, there is significant difference between the antioxidant activity of macerates of leaves and fruits, although both macerates exhibited antioxidant activity. DPPH scavenging activity is usually presented as the concentration of the antioxidant providing 50% inhibition of DPPH in the test solution (IC_{50}). The lower IC_{50} value is obtained, the higher the CAOX of the analysed sample. *Pistacia lentiscus* L. leaves macerate showed higher capacity by DPPH (%) than fruits (58.68 ± 0.54 and $193.48 \pm 3.16 \mu\text{g}\cdot\text{mL}^{-1}$, respectively). DPPH assay exhibits the H-donating capacity of compounds. Moreover, free radical scavenging phenomena based on removal of hydrogen by antioxidants is a mechanism of prevention of lipids against oxidation. Obtained results demonstrate that leaves macerate shows 3 times strongest H-donating capacity than fruits macerate.

3.2.2. ABTS assay

In order to validate the results of antioxidant capacity of macerates of two parts of *Pistacia lentiscus* L. obtained by the DPPH method, a second test based on the proton trapping ability of the cationic radical $ABTS^+$ was carried out. Here, also lower IC_{50} value indicates a higher free radical scavenging activity. Macerate leaves of *Pistacia lentiscus* L. ($IC_{50} 99.72 \pm 1.82 \mu\text{g}\cdot\text{mL}^{-1}$) had greater radical scavenging activity than the macerate fruits ($IC_{50} 155.92 \pm 1.09 \mu\text{g}\cdot\text{mL}^{-1}$). The measured antioxidant capacity can be justified by the

presence of terpenoids, polyphenols and flavonoids (Schreier, 1987) as commented in the identification of bioactive compounds.

3.2.3. Reducing power

Reducing power of macerates was analysed to distinguish the most active solution. The reducing power shows another mechanism of action different than DPPH and ABTS methods. It is based on the behaviour of hydroxyl groups in the active compounds which can act as electron donors (Siddhuraju & Becker, 2007). Method of FRAP is selective and cannot detect compounds which act by radical quenching by hydrogen transfer mechanism. According to the obtained results, the leaves and the fruits macerates of *Pistacia lentiscus* L. have a very considerable antioxidant power (IC_{50} 19.99 ± 0.76 and $55.54 \pm 1.41 \mu g \cdot mL^{-1}$, respectively), however better results were obtained for macerate leaves. The results of antioxidant capacity carried out by the three different methods were coherent and showed that the best CAOX can be attributed to macerates leaves.

3.3. Total phenolic content

The concentration of both phenolic compounds and flavonoids was determined from the linear regression equation of each calibration curve expressed successively in mg equivalent of gallic acid and mg equivalent of catechin per gram of dry matter. According to obtained data the total amount of phenolic compounds (TPC) in the case of leaves was very high $192.5 \pm 7.9 \text{ mg GAE} \cdot g^{-1}$ powder while TPC for fruits was $179.6 \pm 6.4 \text{ mg GAE} \cdot g^{-1}$. The obtained results are very important, and they confirm the properties of the various extracts of this medicinal plant already described in literature, such as TPC values of $114.95 \pm 12 \text{ mg GAE} \cdot g^{-1}$ powder (Missoun, 2017) and $185.69 \pm 18.35 \text{ mg GAE} \cdot g^{-1}$ powder (Dahmoune et al., 2014).

3.4. Total flavonoids

The contents of flavonoids (TFC) recorded for the analysed two macerates were 12.6 ± 4.1 mg QE·g⁻¹ powder for the leaves and 9.0 ± 4.1 mg QE·g⁻¹ powder (Dahmoune et al., 2014) for the fruits.

The amount of total flavonoid compounds quantified in samples of leaves was 3 times higher than in samples of fruits. The obtained results are coherent with those obtained in literature, where TFC yield was 5.16 ± 0.22 mg QE·g⁻¹ powder and 12.93 ± 1.69 mg QE·g⁻¹ powder (Atmani et al., 2009), respectively.

3.5. Antimicrobial capacity

3.5.1. Disc diffusion method

The antibacterial activity was justified by the appearance of an inhibition halo of microbial growth around the disc containing active macerate. According to Sousa et al. (Sousa et al., 2006), different levels of activity can be distinguished on the basis on the diameter of the zones of inhibitions: $2 \leq d \leq 3$ mm indicates low activity; $4 \leq d \leq 5$ mm, intermediate activity; $6 \leq d \leq 9$ mm, strong activity and finally > 9 mm means very strong activity. The results related to the antibacterial activity revealed that both *Pistacia lentiscus* L. macerates exert an antibacterial effect with respect to most of the tested bacterial strains (Table 2).

Table 2. Diameters of inhibition halos of microbial growth for different macerates of *Pistacia lentiscus* L. (mm).

Microbial strain

Macerate of *Pistacia lentiscus* L.

	Leaves	Fruits
<i>S. aureus</i>	8.50 ± 0.50	5.00 ± 1.00
<i>S. epidermidis</i>	9.50 ± 0.50	6.00 ± 1.00
<i>MRSA</i>	7.50 ± 0.50	4.50 ± 0.50
<i>B. subtilis</i>	10.50 ± 1.50	8.00 ± 0.00
<i>A. baumannii</i>	3.00 ± 1.00	4.00 ± 2.00
<i>L. innocua</i>	9.00 ± 1.00	5.00 ± 1.00
<i>P. aeruginosa</i>	3.50 ± 0.50	4.00 ± 1.00
<i>Salmonella sp</i>	4.50 ± 0.50	3.50 ± 0.50
<i>E. coli</i>	4.00 ± 1.00	4.00 ± 1.00

Observed antibacterial effects of the different macerates can be explained by the presence of biologically active compounds such as alcohols, aldehydes, esters and terpenes that can significantly contribute to the antimicrobial effect (Derwich, Manar, Benziane, & Boukir, 2010).

The recorded inhibition halos demonstrate a significant inhibitory effect on the growth of the strains tested with *B. subtilis* as the most sensitive and *A. baumannii* the least sensitive species. The obtained results are consistent with previous studies, indicating that monoterpene-rich macerates such as α -pinene and limonene, which are present among the major components of *Pistacia lentiscus* L., have a strong antibacterial activity (Benhammou et al., 2018). According to the obtained results for the strains *B. subtilis*, *S. epidermidis* and *L. innocua*, significant differences were found between *Pistacia lentiscus* L. macerates. The macerate of leaves has shown the largest inhibition halos; therefore, it has reflected the best antibacterial effect with respect to these three strains. These levels of antimicrobial activity can be classified as very strong. Also, strong antimicrobial properties were observed against *S. aureus* and *MRSA*. On the other hand, no significant difference was observed between the macerate leaves and that of the fruits concerning the other strains: *B. subtilis*, *MRSA*, *E. coli*, *P. aeruginosa*, *A. baumannii* and *Salmonella*. Macerate *Pistacia lentiscus* L. fruits showed the

largest diameter of inhibition halo in case of *S. epidermidis* and *B. subtilis* which however was smaller than respective diameter of leaf macerate.

3.5.2. MIC and MBC

The results of MIC and MBC were shown in Table 3.

Table 3. MIC and MBC of the different macerates of *Pistacia lentiscus* L.

Microbial strain	Macerate of <i>Pistacia lentiscus</i> L.			
	Leaves		Fruits	
	MIC (mg·mL ⁻¹)	MBC (mg·mL ⁻¹)	MIC (mg·mL ⁻¹)	MBC (mg·mL ⁻¹)
<i>S. aureus</i>	2.23	4.46	4.46	8.92
MRSA	4.46	8.92	4.46	8.92
<i>S. epidermidis</i>	8.92	17.85	2.23	4.46
<i>B. subtilis</i>	4.46	8.92	4.46	8.92
<i>L. innocua</i>	2.23	4.46	1.11	2.23

In case of *B. subtilis* and MRSA strains, the obtained results showed the same MIC and MBC values for both types of macerates of *Pistacia lentiscus* L. (4.46 and 8.92 mg·mL⁻¹, respectively).

For the *S. aureus* strain, macerate from leaves showed lower values of MIC (2.23 mg·mL⁻¹) and MBC (4.46 mg·mL⁻¹), while MIC and MBC results for fruit macerate were, respectively, 4.46 and 8.92 mg·mL⁻¹. The results obtained are more interesting than those obtained in the literature, where the values of MIC and MBC are 50 mg·mL⁻¹ (Missoun, 2017). The highest MIC and MBC values of 8.92 mg·mL⁻¹ and 17.85 mg·mL⁻¹ were observed for the leaves macerate in case the *S. epidermidis* strain, whereas the results for the same microbial strain in case of fruit macerate were considerably lower (2.23 and 4.46 mg·mL⁻¹). The lowest results were obtained in case of *L. innocua* strain where the MIC and the MBC were, respectively, 2.23 and 4.46 mg·mL⁻¹ for leaves macerate, and 1.11 and 2.23 mg·mL⁻¹ for fruits macerate.

3.6. Antioxidant capacity of active materials

The multilayer plastic films containing 1% and 2% of *Pistacia lentiscus* L. leaves and fruits macerates were exposed to an atmosphere enriched in hydroxyl free radicals generated in situ for a duration of 48 hours. In this case, indirect methods of evaluation of CAOX are not effective, since their primary disadvantage is the impossibility of evaluating real antioxidant mechanisms in the polymer, without applying any extraction procedure. In addition, the multilayer material can be tested *in vivo* (with food), mimicking the real situation of food packaging.

The results of the antioxidant capacity of the multilayer active film and extracts were presented as a percentage of hydroxylation in comparison to the blank sample, for which H%=100%. The values obtained for the macerates of *Pistacia lentiscus* L. leaves incorporated into the multilayer at the concentrations of 1% and 2% were $25.4 \pm 9.6\%$ and $11.8 \pm 1.1\%$ respectively. On the other hand, H% equal to $38.89 \pm 9.84\%$ and $20.28 \pm 0.6\%$ were obtained for the same concentrations of fruit macerates incorporated into the multilayer packaging. The film containing 2% of *Pistacia lentiscus* L. leaves macerates gave the best results; therefore, the leaves extract proved to be the most antioxidant with H% of 11.8%. All the results showed a significant difference ($p < 0.05$) in comparison to blank .

4. Conclusions

Forty-four different bioactive volatile compounds have been detected in *Pistacia lentiscus* L. macerates, thirty-three of them in leaves macerate, whereas twenty-three were present in the fruit macerate. The two main chemical classes of identified compounds were phenols/polyphenols and monoterpenes/sesquiterpenes. Since some of these compounds are

natural antioxidants, several assays were carried out demonstrating that the strongest antioxidant properties corresponded to the leaves macerate, containing 15 times more phenolic compounds and 20 times more flavonoids than the fruit macerate.

In addition, the antimicrobial activity of macerates was also assessed for the strains *B. subtilis*, *S. epidermidis* and *L. innocua*. The leaves macerate showed the largest inhibition halos with very strong antimicrobial activity levels. The highest MIC ($8.92 \text{ mg} \cdot \text{mL}^{-1}$) and MBC ($17.85 \text{ mg} \cdot \text{mL}^{-1}$) values were observed in the case of *S. epidermidis* strain.

Finally, new multilayer active films were designed and prepared. The one containing 2% of *Pistacia lentiscus* L. leaves macerate gave the best antioxidant results with a hydroxylation percentage of 11.8%, according to the in situ generator of hydroxyl radicals procedure.

This work provided new information on the main active compounds present in the different parts of the medicinal *Pistacia lentiscus* L. plant. Specifically, its leaves macerate constitutes a very good agent for incorporation into active packaging for food applications.

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Declaration of competing interest

The authors declare no competing financial interest.

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Figure 1. Scheme of developed active material: LDPE/AA/PET.

Figure 2. Workflow of applied experimental design.

Figure 3. Results of three methods for determination of CAOX (DPPH, ABTS, Reducing power) of *Pistacia lentiscus* L. macerates.

Figure 1.

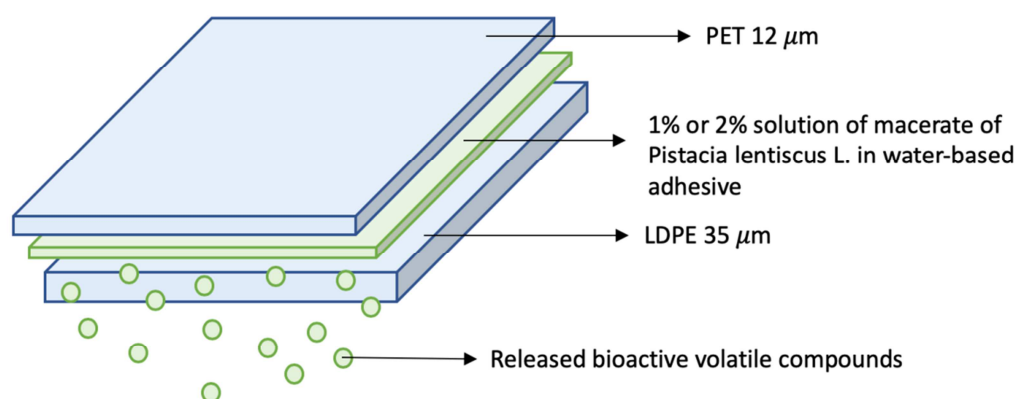


Figure 2

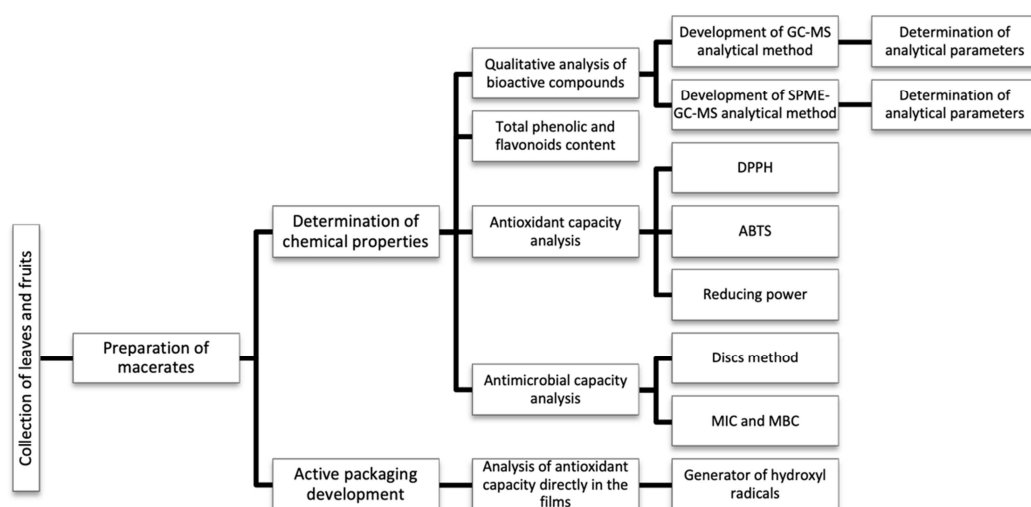
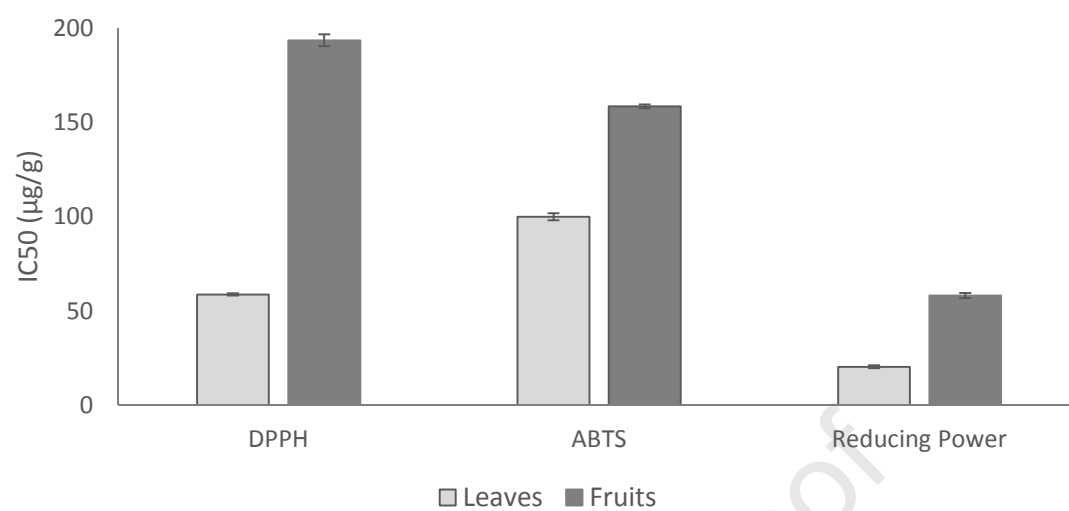


Figure 3.



HIGHLIGHTS

- 44 bioactive compounds were determined in samples of *Pistacia lentiscus L.* plant;
- GC-MS and SPME-GC-MS were used for identification of volatile compounds;
- Antioxidant and antimicrobial capacities were determined;
- New active multilayer packaging LDPE/AA/PET was prepared.

CRediT author statement:

Sabrina Djebari: Methodology, Validation, Investigation, Data Curation, Writing - Original Draft; **Magdalena Wrona:** Conceptualization, Validation, Formal analysis, Investigation, Data Curation, Writing - Original Draft, Writing - Review & Editing, Supervision; **Asma Boudria:** Methodology, Validation, Investigation, Data Curation; **Jesús Salafranca:** Conceptualization, Data Curation, Writing - Review & Editing; **Cristina Nerin:** Conceptualization, Resources, Writing - Review & Editing, Supervision, Project administration, Funding acquisition; **Kenza Bedjaou:** Conceptualization, Resources, Supervision, Project administration, Funding acquisition; **Khodir Madani:** Conceptualization, Resources, Supervision, Project administration, Funding acquisition.

Declaration of interests

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

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: