

Phenolic Profile, Antioxidant and Antimicrobial Activities of Algerian Pistacia Lentiscus L. Leaf Extract

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Abstract:

The objective of this study was to determine the composition of the phenolic extract from Pistacia lentiscus leaves (PLLPE) and to evaluate its antioxidant and antimicrobial activities.

The total polyphenol and flavonoid contents of P. lentiscus were determined using the Folin-Ciocalteu and AlCl₃ methods, respectively. The total polyphenol content was determined to be 193.88 mg gallic acid equivalent, while flavonoids were determined to be 18.96 mg quercetin equivalent.

HPLC/MS revealed the presence of eight phenolic compounds, six flavonoids, and two phenolic acids. The most abundant compounds were kaempferol-3-O-glucoside (1.003 mg/mL) and quercetin-3-O-glucoside (0.865 mg/mL).

The extract showed significant antioxidant activity with a ability of PLLPE to scavenge DPPH (CI₅₀ = 119.94 µg/mL) and reduce Fe²⁺ (CI₅₀ = 2.18 mM Fe²⁺/mL) was concentration-dependent. The CI₅₀ for β-carotene bleaching (298.07 µg/mL) was higher than that of the standards (i.e., ascorbic acid and quercetin). The FRAP assay showed that the antioxidant activity of PLLPE was lower than that of ascorbic acid but higher than that of quercetin.

The diameters of the inhibition zones generated by the antimicrobial activity of PLLPE against the different strains ranged from 5.13 mm (for the beneficial strain *Lactobacillus rhamnosus*) to 35.12 mm (for the pathogenic strain *Staphylococcus aureus*).

Keywords: *Pistacia lentiscus* L. leaf, polyphenols, Antioxidant activity, Antimicrobial activity.

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1. Introduction

Food safety and public health are facing increasing demands from consumers today. Consumers are increasingly turning to products containing natural additives and prefer herbal medicine to synthetic drugs (Djebari et al., 2021; Tabuti et al., 2003). The use of chemicals is increasingly being blamed for human toxicity (Edziri, 2012). Plants are capable of producing hundreds to thousands of bioactive metabolites, including polyphenols (Paulsen et al., 2010).

Natural antioxidants are produced in living cells to protect them from damage caused by free radicals produced in chain reactions. In this sense, plant extracts are good sources of antioxidants and compounds with antimicrobial properties (Chang et al., 2016; Gavril et al., 2019).

Polyphenols are organic compounds found in plants. They are being studied for their potential to prevent chronic diseases, including cardiovascular disease, cancer, osteoporosis, diabetes, and neurodegenerative diseases. Their protective activity is attributed to their antioxidant properties, which protect cells from damage caused by free radicals (Daglia, 2012). In addition, polyphenols have antimicrobial properties, which can be used to develop new food preservatives or innovative therapies for the treatment of microbial infections (Saavedra et al., 2010).

Algeria is the largest country bordering the Mediterranean. It is recognized for its varietal diversity of medicinal and aromatic plants (Ilbert et al., 2016), including *Pistacia lentiscus* L., commonly known as lentisk or "darw" in the Anacardiaceae family. It is a wild, thermophilic, aromatic, and medicinal species widely distributed in the country from humid to arid areas (Rauf et al., 2017; Benhammou et al., 2008).

Lentisk contains many active chemical substances. Many studies on the composition of the leaves reveal a significant richness in phenolic compounds such as phenolic acids (gallic acid and its glycosylated derivatives), flavonoids, especially flavones (luteolin, tricetin, and chrysoeriol), flavonols (myricetin, quercetin, and kaempferol), heterosides (orientin, isoorientin, vitexin, and rutin), anthocyanins (delphinidin 3-O-glycoside and cyanidin 3-O-glucoside), and tannins (Djeridane et al., 2006; Abdelwahed et al., 2007; Benhammou et al., 2008; Hamlat and Hassani, 2008; Rogosic et al., 2008; López-Lázaro, 2009).

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This study is a contribution that scientifically complements the knowledge held, in an empirical way, by the Algerian population on the antioxidant and antimicrobial activities of *Pistacia lentiscus*, a plant of great interest that deserves to be valued.

2. Materials and methods

2.1. Plant material

In May 2022, leaves of *Pistacia lentiscus* (*P. lentiscus*) were collected in the region of Aïn Temouchent, located in western Algeria at 35°14'06" North, 1°05'57" East, and at an altitude of 50 meters. The climate in the region is Mediterranean, with hot, dry summers and mild, wet winters (Köppen climate classification: Csa). Botanical identification of the plant material was performed at the Laboratory of Beneficial Microorganisms, Functional Foods, and Health at the University Abdelhamid Ibn Badis in Mostaganem, Algeria. After collection, the leaves were carefully washed, dried, and crushed.

2.2. polyphenol extraction

Phenolic compounds were extracted according to Belabbas et al. (2020). Two successive 30-minute macerations at room temperature with stirring were performed using a sample/solvent ratio (g/mL) of 3. The solvent in the first maceration was methanol: 0.05% (v/v) aqueous HCl (90/10, v/v). In the second maceration, after filtering the extract with Whatman paper (No. Z146374100EA), the methanol was replaced with ethyl acetate. The two extracts were combined and evaporated to dryness with a rotary evaporator at a temperature of 45°C. The extraction was repeated three times.

2.3. Analysis of polyphenols by HPLC/MS

Chromatographic analysis of *P. lentiscus* leaf phenolic extract (PLLPE) was performed using a Thermo Finnigan Surveyor HPLC instrument equipped with an Advantage Max Ion Trap LCQ mass spectrometer (Thermo Fisher Scientific, Waltham, USA). The separation was carried out on a C18 110 Å column (150 x 2 mm, 5 µm). The mobile phase consisted of water (A) and methanol (B), both acidified with 0.075% (V/V) formic acid. The composition of the mobile phase varied over time according to the following elution gradient: 2% of solvent B during the first 5 minutes, then a linear gradient of solvent B from 2 to 100% during the following 115 minutes. The volume of injected sample was 5 µL and the flow rate was 1 mL/min (Belabbas et al., 2020).

Chromatograms were recorded at 280 nm, and spectral data were collected in the range 200-800 nm for all peaks. HPLC/MS data were acquired in positive and negative ionization modes. The quantification of each phenolic compound present in the extract studied was determined using the regression equations of the calibration standard curves.

2.4. Colorimetric determination of phenolic compounds

2.4.1. Total Phenolic Content (TPC)

The content of total polyphenols was determined as described by Gutfinger (1981), using Folin Ciocalteu's reagent. 100 μ L of PLLPE were mixed with 5 mL of distilled water and 500 μ L of Folin Ciocalteu's reagent. After 3 min, 1 mL of a 35% (w/v) sodium carbonate solution was added to the mixture which was shaken and incubated in the dark for 20 min at room temperature; absorbance was measured at 725 nm. The total phenolic content of PLLPE was expressed in terms of mg gallic acid equivalent per g extract (mg EGA/g).

2.4.2. Total flavonoid content (TFC)

The determination of the total flavonoid content was carried out according to the method of Arvouet-Grand et al. (1994). 500 μ L of PLLPE was mixed with 500 μ L of 2% (w/v) aluminum trichloride solution. After stirring, the mixture was incubated for 10 min at room temperature in the dark. Absorbance was measured at 415 nm. The total flavonoid content was expressed as quercetin equivalent per g of extract (mg EQ/g).

2.5. Evaluation of the Antioxidant Activity of *P. lentiscus* leaf phenolic extract (PLLPE)

2.5.1. DPPH-Scavenging Assay

DPPH-scavenging activity of *P. lentiscus* leaf phenolic extract (PLLPE) was carried out using the Yang et al. (2008) method. One milliliter of the methanolic solution of the 430 sample at different concentrations (from 0.125 to 1.5 mM) was added to 3 mL of a 0.1 mmol/L MeOH solution of DPPH and incubated in the dark at room temperature for 30 minutes. The absorbance was measured at 517 nm against a negative control (methanol without DPPH) and compared to the positive control prepared with the same concentrations of ascorbic acid and quercetin. The percentage of inhibition was calculated using formula (1) as follows:

$$I\% = 100 \times (A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}} \quad (1)$$

Where: I%: Percentage of Inhibition, A_{control} : Absorbance of the negative control, A_{sample} : Absorbance of the PLPE (sample) or the positive controls (ascorbic acid and quercetin).

The concentration of PLLPE, acid ascorbic or quercetin required to scavenge 50% of DPPH radical (IC_{50}) was determined graphically from the function: % inhibition = f (antioxidant concentration).

2.5.2. Ferric Reducing Antioxidant Power assay (FRAP)

The ferric reducing antioxidant power (FRAP) of *P. lentiscus* leaf phenolic extract (PLLPE) was determined as described by Oyaizu (1986), where ferric iron (Fe^{3+}) of potassium hexacyanoferate (III) $K_3Fe(CN)_6$ is reduced to ferrous iron (Fe^{2+}) in a complex potassium hexacyanoferate (II) $K_4Fe(CN)_6$ of Prussian blue color whose absorbance measured at 700 nm is proportional to the

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reducing power of the extract. 400 μ L of the extract (PLLPE) at different concentrations (from 0.075 to 2 mM) are mixed with 2.5mL of 0.2M phosphate buffer solution (pH 6.6) and 2.5mL of a 1% (w/v) $K_3Fe(CN)_6$ solution, and then incubated 20 min at 50 °C in a water bath before voluntarily stopping the reaction by addition of 2.5mL of 10% (w/v) trichloroacetic acid. The mixture was centrifuged (3000 rpm/10 min) and 2.5 mL of the supernatant were mixed with 2.5 mL of distilled water and 0.5 mL of a freshly prepared 0.1% (w/v) ferric chloride solution. Absorbance of the sample (PLLPE) and the standards (positive controls: ascorbic acid and quercetin) was measured at 700 nm against a negative control (free from PLLPE). The results were expressed in mM Fe^{2+} /g of extract.

2.5.3. β -carotene bleaching test

The β -carotene bleaching test was carried out according to the method of Yu et al. (2006). An emulsion was prepared as follows: 0.5 mg of β -carotene was dissolved in 1 mL of chloroform and mixed with 25 μ L of linoleic acid and 200 μ g of Tween-20. The chloroform was removed under vacuum at 50°C, then 100 mL of distilled hydrogen peroxide was slowly added with vigorous stirring to form an emulsion. 5mL of this emulsion was added to 1 mL of the antioxidant solution (PLLPE sample or ascorbic acid and quercetin as a positive controls) or to 1mL distilled water (negative control), and an initial absorbance was measured immediately at 470 nm. The tubes were then placed in a water bath at 50°C and the absorbance was measured again at 470 nm after 2h. The antioxidant activity was determined using the following formula (2):

$$AA (\%) = [1 - (A_{S_0} - A_{S_{120}}) / (A_{C_0} - A_{C_{120}})] \times 100 \quad (2)$$

Where, AA is the antioxidant activity, A_{S_0} is the initial absorbance, $A_{S_{120}}$ is the absorbance after 2h, A_{C_0} is the initial absorbance of the negative control and $A_{C_{120}}$ is the absorbance of the negative control after 2h.

2.6. Antibacterial and antifungal activity of *P. lentiscus* leaf phenolic extract (PLLPE)

2.6.1. Microbial strains

The microbial strains used were *Bifidobacterium animalis sbsp lactis* Bb12, *Lactobacillus rhamnosus* LbRE-LSAS, *Staphylococcus aureus* (*S. aureus*) (ATCC 29213), *Escherichia coli* (*E. coli*) (ATCC 25922), *Bacillus cereus* (*B. cereus*) (ATCC 10876), *Proteus mirabilis* (*P. mirabilis*) (ATCC 13315), *Klebsiella pneumoniae* (*K. pneumoniae*) (ATCC 13883), *Candida albicans* (*C. albicans*) (ATCC 10231), *Aspergillus niger* ATCC 1688 these strains were obtained at the LMBAF laboratory (University of Mostaganem, Algeria).

2.6.2. Antimicrobial Activity by Disc Diffusion Method

The antimicrobial activity of *P. lentiscus* leaf phenolic extract (PLLPE) at a concentration of 200 mg/mL in 10% DMSO was evaluated using the agar diffusion method described by the Institute of Clinical Laboratory Standards (CLSI, 2008). The bacterial strains were pre-cultured for 16

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hours, adjusted to a concentration of 108 CFU/mL, and seeded in 20 mL of culture medium. After 15 minutes, sterile discs impregnated with 10 µL of PLLPE were placed on the medium.

Vancomycin and gentamicin (30 µg/mL) were used as positive controls, and miconazole (40 µg/mL) was used as an antifungal agent. A disc soaked in 10% (v/v) DMSO served as the negative control. The plates were incubated at 37°C for 24 hours, and the diameters of the inhibition zones were measured. All tests were performed in quintuplicate, and the antibacterial activity was expressed as the mean of the inhibition diameters (mm) produced ± SD (standard deviation).

2.6.3. Determination of Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) of the samples was determined using the Muller Hinton broth microdilution method (CLSI, 2009) in a 96-well microplate. Each well received 100 µL of bacterial suspension (105 CFU/mL) and 100 µL of PLLPE studied at different dilutions (from 0.009 to 20 mg/mL). Wells containing Muller Hinton broth alone were used as a negative control, while wells containing Mueller Hinton inoculated with each strain and without extract were used as positive controls. All tests were performed in triplicate, and the MIC was defined as the lowest concentration at which no bacterial growth was observed after incubation at 37°C for 24 hours.

After each MIC identification, 100 µL of the contents of the MIC well were seeded on Mueller Hinton solid medium and incubated at 37°C for 16 hours to assess the bactericidal or fungicidal effect of PLLPE: no visible growth was considered bactericidal or fungicidal, while colony formation was considered bacteriostatic or fungistatic.

2.7. Statistical analysis

The set of experiments was repeated three times and the data obtained were analyzed using IBM SPSS statistics, version 26.0. Results were reported as mean ± SD.

The level of statistical significance was set at $p < 0.05$, for a two-tailed test. One-way ANOVA followed by Tukey's test was used to compare the antioxidant activity results of PLLPE obtained in the three tests (FRAP, TEAC, DPPH and β-carotene bleaching inhibition test) with those of ascorbic acid and quercetin. The multidirectional MANOVA followed by Tukey's test was used to compare the results of the antimicrobial activity of the EAEMPPF obtained in the test of inhibition of the growth zone of the strains with those of reference antimicrobials.

3. Results and Discussion

3.1. *Pistacia lentiscus* HPLC/MS phenolic profile

The polyphenols present in the *P. lentiscus* leaves phenolic extract (PLLPE) were identified by PDA spectroscopy and MS data. The identified phenolic compounds were numbered and quantified based on their elution order and the interpolation of calibration curves (Table 1).

Table 1: Identification of *Pistacia lentiscus* leaf phenolic extract (PLLPE) by HPLC/MS.

N	Retention time (min)	Molecular formula	MS ²	Compounds	Concentration (µg/mL)
1	9,464	C ₇ H ₆ O ₄	155	Protocatechuic acid	76.22
2	14,448	C ₁₇ H ₂₆ O ₁₃	379	Galloyl quinic acid	78.36
3	19,321	C ₁₅ H ₁₄ O ₆	289	Catechin	231.06
4	32,150	C ₁₅ H ₁₄ O ₇	305	Gallocatechin	248.89
5	34,769	C ₁₅ H ₁₀ O ₈	319	Myricetin	167.55
6	36,407	C ₂₁ H ₂₀ O ₁₂	463	Quercetin-3-Oglucoside	865.11
7	38,973	C ₂₁ H ₂₀ O ₁₁	447	Kaempferol-3-O-glucoside	1003.24
8	46,032	C ₂₇ H ₃₀ O ₁₆	593	Luteolin-7-O-rutinoside	512.68

The identified phenolic compounds belong to two classes, namely flavonoids, which were the most abundant (6 compounds) and represented 95.14% of the weight of polyphenols quantified, and phenolic acids (2 compounds) with 4.86%. The phenolic glycosides identified in our extract were flavonoic derivatives of kaempferol and quercetin. The PLLPE extract also contained unconjugated flavonoids such as catechin, gallocatechin, and myricetin. The dominant compound of PLLPE was a flavonol which is Kaempferol-3-O-glucoside with 1003.24 µg/mL, which corresponds to 31.52%, followed by 27.18% of quercetin-3-O-glucoside, 16.1% of luteolin- 7-O-rutinoside, 7.82% Gallocatechin, 7.25% catechin, 5.2% Myricetin, 2.46% galloylquinic acid and 2.39% protocatechuic acid.

These results are comparable to those of previous research which also identified *P. lentiscus* polyphenols from hydroalcoholic leaf extracts (Qabaha et al., 2016; Romani et al., 2002; Rodriguez-Pèrez et al., 2013; Mehenni et al., 2016).

Despite, the identification of other classes of polyphenols such as gallic acid and cinnamic acid in *P. lentiscus* leaf extracts by Mehenni et al. (2016), these compounds were, however, not recorded in PLLPE in this work. The polyphenol composition of plants varies significantly depending on different intrinsic and extrinsic factors, such as plant genetics and varieties, soil composition, phenological stages, and others (Farak et al., 2013).

The results of the present study reiterate the richness of *P. lentiscus* extract in Quercetin-3-Oglucoside, Kaempferol-3-O-glucoside, catechin, Myricetin; compounds with antioxidant and antimicrobial activities (Mehenni et al., 2016; Maruf et al., 2015, Vajic et al., 2018).

3.2. Evaluation of total phenols, flavonoids and antioxidant activity

The results of the total polyphenol (TPC) and flavonoid (TFC) contents are presented in Table 2. The richness of PLLPE in phenolic compounds (193.88 ± 3.95 mg EGA/g of extract) is similar to that reported by Djebari *et al.* (2021) (192.5 mg EGA/g) for a methanolic extract, and is superior than that recorded in the work of Missoun (2017) (114 mg EGA/g).

Table 2: Results of TPC and TFC

	TPC (mg EGA/g E)	TFC (mg EQ/g E)
<i>P. lentiscus</i> leaf phenolic extract (PLLPE)	193.88 ± 3.95	18.96 ± 0.52

$P < 0,05$. Total phenolic content (TPC); Total flavonoid content (TFC).

The flavonoid content found here (18.96 ± 0.52 mg EQ/g of extract) represents a higher rate than that determined in the ethanolic extract (5.18 mg EQ/g of extract) by Azib *et al.* (2019). These results for total polyphenol and flavonoid contents are consistent with those obtained by HPLC (Table 1).

The results reported in table 3 show antioxidant activity of PLLPE determined by different tests (DPPH, FRAP and β -carotene bleaching inhibition) in comparison with two standard antioxidants (i.e. ascorbic acid and quercetin). According to the ANOVA test, the effect of the antioxidants used (PLLPE, ascorbic acid, quercetin) in the three tests used is very highly significant ($p < 0.001$) on the resulting antioxidant activity. The Tukey test made it possible to determine the homogeneous groups, and therefore for the DPPH test, there is no significant difference between the activity recorded for the extract and that of quercetin. The latter has strong antioxidant activity (Cai *et al.*, 2004).

Table 3: In vitro antioxidant activity of *Pistacia lentiscus* leaf phenolic extract (PLLPE), ascorbic acid and quercetin

	DPPH scavenging activity (IC_{50} μ g/mL)	Inhibition of β -carotene bleaching (IC_{50} μ g/mL)	FRAP mmol Fe^{2+} /g
PLLPE	119.94 ± 1.24^b	298.07 ± 5.61^c	$2.18 \pm 0,99^b$
Quercetin	$121.54 \pm 1,15^b$	242.73 ± 2.35^b	1.54 ± 1.09^c
Ascorbic acid	80.19 ± 3.15^a	51.67 ± 1.9^a	6.8 ± 1.61^a

$P < 0,001$. Each value represents mean \pm S.D. Statistical analysis was performed using one-way ANOVA followed by Tukey's test.

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This test evaluates the inhibitory power which represents the antioxidant potential of the sample by measuring the amount of remaining β -carotene (Bamoniri *et al.*, 2010). The IC_{50} of PLLPE in this test ($298.07 \mu\text{g} / \text{mL}$) is 5.8 and 1.2 times higher than those of ascorbic acid ($51.67 \mu\text{g}/\text{mL}$) and quercetin ($242.73 \mu\text{g}/ \text{mL}$), respectively.

Moreover, the ferric reducing antioxidant power (FRAP) of the *P. lentiscus* extract is significantly higher than that of quercetin, but 3.1 times lower than that of ascorbic acid.

These results suggest that mastic pistachio leaf polyphenols could have therapeutic interest in the prevention and treatment of various diseases.

other work has also highlighted the antioxidant activity of phenolic extracts of mastic pistachio leaves (Bouakline et al., 2023; Mahmoudi et al., 2013; Salhi et al., 2014; Otmani et al., 2015).

3.3. Antimicrobial activity

Antimicrobial activity of PLLPE against several pathogens is reported as inhibition zone diameters (mm) in Table 5. The results of the MANOVA test reveal a significant difference between the antimicrobial activity of the sample (PLLPE) and the standards (i.e. ampicillin and miconazole) resulting in different levels of growth inhibition of bacteria and fungi tested. The Tukey test was undertaken to better appreciate the comparison between the efficacy of the extract and the standards used, and has shown that there is no significant difference between the antibacterial effect of the extract and ampicillin against *B. cereus*.

Table 4: Antimicrobial activity of *Pistacia lentiscus* leaf phenolic extract (PLLPE).

Strains	Phenolic extract (mm)	Ampicillin (mm)	miconazole (mm)	P value (MANOVA test)
<i>Staphylococcus aureus</i>	$35,12 \pm 1,2^a$	$37,1 \pm 0,86^b$	-	0,000
<i>Bacillus cereus</i>	$23,08 \pm 0,84^a$	$22,9 \pm 1,02^a$	-	0,001
<i>Escherichia coli</i>	$13,28 \pm 0,9^b$	$17,05 \pm 0,95^a$	-	0,001
<i>Proteus mirabilis</i>	$20,91 \pm 1,02^b$	$26,12 \pm 0,87^a$	-	0,005
<i>Klebsiella pneumoniae</i>	$15,02 \pm 1,12^b$	$16,84 \pm 0,72^a$	-	0,000
<i>Candida albicans</i>	$13,26 \pm 0,81^b$	-	$17,24 \pm 1,3^a$	0,001
<i>Aspergillus niger</i>	$24,18 \pm 1,01^a$	-	$21,65 \pm 0,5^b$	0,000
<i>Bifidobacterium animalis</i> subsp <i>lactis</i>	$7,2 \pm 0,9^b$	$12,2 \pm 1,09^a$	-	0,001
<i>Lactobacillus rhamnosus</i>	$5,13 \pm 0,8^b$	$8,7 \pm 0,18^a$	-	0,05

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In contrast, the growth of the two beneficial bacteria, namely *Bifidobacterium animalis*-subsp *lactis* and *Lactobacillus rhamnosus* was not significantly inhibited by the *P.lentiscus* leaf phenolic extract (less than 8 mm, which represents the limit near the halo limit). Such insensitivity of intestinal bacteria and generally probiotics to natural growth-inhibiting substances was also previously reported by other authors (Cueva *et al.*, 2010; Lee *et al.*, 2008). In revenge, ampicillin induced significantly higher zones of inhibition than PLLPE. This suggests that the extract studied does not have a negative impact on the bacteria of intestinal origin.

Gram-positive bacteria tested were sensitive to PLLPE with inhibition zone diameter of 23.08 mm for *B.cereus* and 35.12 mm for one of the most common bacteria responsible for food poisoning, *S. aureus* (Rauha et al., 2000).

Gram-negative bacteria were also sensitive to the extract with inhibition zone diameter ranging from 13.28 mm for *E. coli* to 20.91 mm for *P. mirabilis*.

C. albicans is responsible for most clinical mycoses; it was sensitive to the *P. lentiscus* leaf polyphenol extract with an inhibition zone diameter of 13.26 mm. The effect of miconazole was significantly lower than that of PLLPE against *A. niger*.

The antibacterial potential of the *P. lentiscus* leaf phenolic extract recorded in this work would be linked to its richness in flavonoids like kaempferol, quercetin and catechin which have antimicrobial activity against *S. aureus*, *E. coli*, *B. cereus* and *C. albicans* (Soni, 2013, Araruna et al., 2012); and of the phenolic acids which have broad-spectrum antibacterial activity against gram-negative and positive bacteria (Borges et al., 2013; Naz et al., 2006).

Minimal inhibitory concentration (MIC) was determined for bacteria in which zones of inhibition greater than 8 mm were recorded; the results are presented in Table 5. The lowest MICs were recorded with *A. niger* (1.25mg/mL), *S. aureus* (2.5mg/mL) and *B. cereus* (2.5mg/mL); while *E. coli* and *K. pneumoniae* are more resistant with MIC value above 20 mg/mL. Findings of the present work have shown that antimicrobial activity of PLLPE is bigger than that of whole methanolic extract of the leaf and of the fruit reported by Djebabri et al. (2021). In the work of Otmani and Slimani (2022), the phenolic extract of *P. lentiscus* leaves inhibited the growth of 14 bacterial strains, including *S. aureus* and *E. coli*, with MICs ranging from 0.25 to 1 mg/mL; this confirms once again the antimicrobial activity of phenolic extracts from *P. lentiscus* leaves.

Table 5: Minimum inhibitory concentration (CMI) of *Pistacia lentiscus* leaf phenolic extract (PLLPE).

Strains	MIC (mg.mL ⁻¹)
<i>Staphylococcus aureus</i>	2.5
<i>Bacillus cereus</i>	2.5

<i>Escherichia coli</i>	>20
<i>Proteus mirabilis</i>	5
<i>Klebsiella pneumoniae</i>	>20
<i>Candida albicans</i>	10
<i>Aspergillus niger</i>	1.25

4. Conclusion

In the present study, we evaluated the polyphenol composition, antioxidant and antimicrobial activity of polyphenols extracted from *P. lentiscus* leaves. The HPLC/MS analysis of the extract revealed the presence of 8 phenolic compounds, with a high content of flavonoids, mainly represented by kaempferol-3-O-glucoside and quercetin-3-O-glucoside; as well as by phenolics acids with the predominance of galloylquinic acid.

the antioxidant and antimicrobial activities de PLLPE was observed, highlight the potential of the leaves of *P. lentiscus* grown in Algeria to serve at a source of polyphenols that can be used in the agro-food and pharmaceutical fields.

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