Available online on www.ijppr.com
International Journal of Pharmacognosy and Phytochemical Research 2016; 8(4); 627-633

ISSN: 0975-4873

Research Article

Assessment of Phytochemical Composition and Antioxidant Properties of Extracts from the Leaf, Stem, Fruit and Root of *Pistacia lentiscus* L.

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Available Online : 31st March, 2016

ABSTRACT

Pistacia lentiscus belong to the family of Anacardiacea is widely used as medicinal plant in Algerian folk medicine for their powerful biological activities. The aim of this study is to quantify the different phenolic content from different parts of *P. lentiscus* (leaves, stems, fruits and roots), to evaluate their antioxidant activity, and to identify responsible molecules for this activity. The antioxidant activity of methanolic and selective extracts from all studied parts of *P. lentiscus* were evaluated by using DPPH free radical scavenging activity, reducing power, β-carotene bleaching assay and total antioxidant capacity (TAC). The phytochemical analysis of leaves, stems, fruits and roots extracts of *P. lentiscus* showed the presence of principal groups of secondary metabolites (flavonoids, tannins and anthocyanins). Leaves showed the highest value of phenolic compounds (216.289 ± 20.62 mg GAE/g DM), flavonoids (121.515± 9.171 mg CE/g DM) and condensed tannins (19.162 ± 0.436 mg CE/g DM). All extracts of leaves, stems and roots revealed a powerful total antioxidant capacity and high activities to scavenge DPPH radical, to reduce power and to inhibit the oxidation of β-carotene. Fruits exhibit the lowest values of antioxidant activity. Chemical composition realized by HPLC allowed the detection of gallic acid, ascorbic acid, ρ-coumaric acid, catechin and quercetin in the leaves of *P. lentiscus,* and for the first time: ferulic acid, vanillic acid and naringenin were also detected.

Keywords: *Pistacia lentiscus*, Phytochemical analysis, Antioxidant activity, Chemical composition, HPLC.

INTRODUCTION

Plants are considered to have a rich source of naturally occurring bioactive compounds with high medicinal activities against various diseases. Also the use of medicinal plants for local remedies is a traditional custom. In recent years, there has been increasing interest in natural plant-derived products as sources of active compounds with antimicrobial and antioxidant properties, because of their relatively low toxicity and strong bioactivities[1,2]. Considerable research works have been done on the pharmacological, phytochemical aspects, also on the antimicrobial and antioxidant properties. Besides, recent studies have shown that the ingestion of vegetables, fruit and herbs is associated with prevention of some bactericidal, anti-viral, analgesic, anti-inflammatory and anti carcinogenic disorders, due to their antioxidant activity[3,4]. These virtues are widely related in the presence of several substances, such as vitamin C, vitamin E, carotenes, tannins and phenolic compounds. These phenolic compounds are able to donate protons to free radicals, and are still capable to prevent the formation of reactive oxygen species. They also exhibit a wide range of biological activities; which can be attributed to their antioxidant properties[5]. *Pistacia lentiscus* L, commonly known as Mastic tree or lentisc, is a Mediterranean evergreen shrub of the family of Anarcadiaceae widely used in traditional medicine to treat such diseases as eczema, diarrhea, and throat infections. Furthermore, other properties are currently attributed to *P. lentiscus*, such as antioxidant capacity, hepatoprotective action, and anti-inflammatory effects[6]. This plant has been reported as very rich source in secondary metabolites[7-9]. Previous studies on *P. lentiscus* aerial parts have also reported an abundance of phenolic compounds[10,11]. A recent study realized by Rodriguez-Perez et al[6] reported that 46 different compounds were identified in leaves of *P. lentiscus*, 20 of which were tentatively characterized for the first time; Flavonoids, phenolic acids and their derivatives were the most abundant compounds. Therefore, the aim of the present study is to evaluate the polyphenolic profile of different parts of *P. lentiscus* (leaves, stems, fruits and roots); on the one hand to quantify the levels of phenolic compounds and to evaluate antioxidant activity, and on the other hand to highlight responsible molecules for this activity by HPLC analysis.

MATERIALS AND METHODS

Standards and reagents

All standards and chemicals were purchased from Sigma Chemical Co. (St. Louis, Mo, USA).

Plant material

The various parts (leaves, stems, fruits and roots) of *P.*

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Table 1: Yields of bioactive compounds from different parts of *P. lentiscus.*

Bioactive compounds	Yields %			
	Leaves	Stems	Fruits	Roots
Methanolic extract	28.50 ± 1.013	16.25 ± 1.060	13.369 ± 0.579	18.055 ± 0.55
Ethyl acetate fraction	6.831 ± 0.254	3.22 ± 0.023	2.631 ± 0.312	4.75 ± 0.25
Butanolic fraction	6.24 ± 0.587	2.25 ± 0.353	4.515 ± 0.536	3.25 ± 0.25
Tannins	3.35 ± 0.636	1.5 ± 0.027	5.267 ± 0.804	25.25 ±1.75
Anthocyanins	9.075 ± 0.813	8.56 ± 0.536	17.343 ± 0.895	17.055 ± 1

Table 2: Total phenolic, flavonoid, and condensed tannin contents in methanolic extracts from different parts of *P. lentiscus.*

Different parts	Total phenolics (mg GAE/g DM)[a]	Condensed tannins (mg CE/g DM)[b]	Flavonoids (mg CE/g DM)[b]
Leaves	216.289 ± 20.62	121.515 ± 9.171	19.162 ± 0.436
Stems	121.399 ± 3.354	80.215 ± 3.113	16.788 ± 0.733
Fruits	103.342 ± 2.317	7.893 ± 0.481	4.696 ± 0.329
Roots	30.188 ± 1.291	7.166 ± 0.818	4.287 ± 0.106

lentiscus were collected in December 2011 from Nedroma located near the Moroccan border, about 58 km northwest of Tlemcen. (Algeria). The samples collected were identified by Dr. Hassani F. and Stambouli H., Vegetable Ecological Laboratory. The voucher specimens have been deposited at the Herbarium of the Department of Biology, Tlemcen University, Algeria. The samples were air-dried in shadow at room temperature, and reduced to fine powder, just before extraction.

Preparation of methanolic extracts

The powder (1 g) of each part of *P. lentiscus* was placed in 20 mL of methanol 96.6° for 24 h. After filtration through filter paper Whatman nº 0.45, the resulting solution was evaporated under vacuum at 60° C by Buchi Rotavapor R-200 dry. The residue was then dissolved in 3 mL of methanol[12].

Ethyl acetate and butanolic fractions

The dry residue obtained by the same procedure for methanolic extract for each part, was treated with 10 mL of boiling water to dissolve the flavonoids. The filtrate aqueous solution was firstly extracted with 10 mL of ethyl acetate, then with 10 mL of butanol-1. The two fractions were evaporated and dissolved in 3 mL of methanol[13].

Tannins

The extraction of tannins from different parts of *P. lentiscus* was obtained according to the method of Zhang et al[14]. The powder of plant material (2.5 g) was extracted with 50 mL acetone-water (35/15, (v/v) and the mixture was stirred continuously for 72 h at room temperature. Then, the mixture was filtrated and evaporated under vacuum at 40 °C to remove acetone. The remaining solution was washed with 15 mL of dichloromethane to remove lipidsoluble substances. After that, the solution was further extracted with ethyl acetate at a ratio of 15/15 (v/v). The water layer was separated and extracted twice more similarly. Then resulting water layers were mixed, evaporated to dryness, and dissolved in methanol.

Anthocyanins

The extraction of anthocyanins was as described by Longo et al[7]. Amount of 2.5 g of berry powder was extracted twice with 12.5 mL of HCl/ methanol solution (v/v, 0.1 %) for 20 h at room temperature. After filtration and evaporation, the dry residue was dissolved in methanol.

Total phenolic content

The amounts of total phenolic compounds in methanolic extracts of leaves, stems, fruits and roots were determined by spectrometry method using Folin-Ciocalteu reagent[15]. A volume of 0.2 mL of the extracts was mixed with 1 mL of Folin- Ciocalteu reagent diluted 10 times with water and 0.8 mL of a 7.5% sodium carbonate solution in a test tube. After stirring and 30 min later, the absorbance was measured at 765 nm. Gallic acid was used as a standard for the calibration curve. The total phenolic content was expressed as milligrams of gallic acid equivalents per gram of dry matter (mg GAE/g DM). All the tests were carried out in triplicate.

Total flavonoid content

The flavonoid content was determined using the technique of Zhishen el al[16]. A volume of 500 µL of methanol solution at different concentrations of catechin or extract was added to 1500 µL distilled water. At time zero, 150 µL of sodium nitrite ($NaNO_2$) to 5% was added to the mixture. After 5 min, 150 µL of aluminium trichloride ($AlCl_3$) 10% (m/v) was added. After incubation for 6 min at room temperature, 500 µL of sodium hydroxide (NaOH) (1M) was added. Immediately, the mixture was thoroughly shaken to mix the contents. The absorbance of the solution of pinkish colour was determined at 510 nm against the blank. The total flavonoid content of extracts was expressed as milligrams catechin equivalents per gram of dry matter (mg CE/g DM). All the tests were carried out in triplicate.

Total condensed tannins

The amounts of condensed tannins were estimated using the method of vanillin[17]. A volume of 50 µL of the methanolic extract of each part was added to 1500 µL of vanillin/methanol solution (4%, m/v) and then mixed with the vortex. Then, 750 µL of concentrated hydrochloric acid (HCl) was added and allowed to react at room temperature for 20 min. Absorbance at 550 nm was measured against a blank. The concentration of tannins was estimated as milligrams catechin equivalents per gram of dry matter

Table 3: Reducing power, DPPH radical scavenging, β-carotene bleaching and total antioxidant capacity (TAC) of bioactive compounds from leaves, stems, fruits and roots of *P. lentiscus*. Values were the mean of two replicates ± SD. DM: dry matter; GAE: gallic acid equivalents; AAE: ascorbic acid equivalents.

Tests	Bioactive compounds	Leaves	Stems	Fruits	Roots
EC_{50} (mg/mL) Reducing power	Methanolic extract	0.185 ± 0.002	0.190 ± 0.001	0.666 ± 0.035	0.236 ± 0.001
	Ethyl acetate fraction	0.187 ± 0.001	0.416 ± 0.004	0.134 ± 0.006	0.213 ± 0.002
	Butanolic fraction	0.161 ± 0.001	0.193 ± 0.000	0.369 ± 0.002	0.102 ± 0.001
	Tannins	0.075 ± 0.001	0.148 ± 0.003	0.132 ± 0.015	0.061 ± 0.001
	Anthocyanins	0.088 ± 0.000	0.119 ± 0.001	0.122 ± 0.000	0.191 ± 0.000
	Gallic acid	0.060 ± 0.000			
EC_{50} (mg/mL) DPPH assay	Methanolic extract	0.166 ± 0.001	0.076 ± 0.001	0.772 ± 0.043	0.370 ± 0.002
	Ethyl acetate fraction	0.171 ± 0.000	0.402 ± 0.015	0.080 ± 0.003	0.347 ± 0.006
	Butanolic fraction	0.127 ± 0.000	0.221 ± 0.002	0.484 ± 0.042	0.216 ± 0.000
	Tannins	0.069 ± 0.001	0.057 ± 0.001	0.099 ± 0.019	0.090 ± 0.011
	Anthocyanins	0.102 ± 0.005	0.163 ± 0.000	0.380 ± 0.030	0.245 ± 0.004
	Ascorbic acid	0.090 ± 0.002			
EC_{50} (mg/mL) β-carotene assay	Methanolic extract	0.252± 0.002	0.321 ± 0.033	0.317 ± 0.009	0.316 ± 0.038
	Ethyl acetate fraction	0.165 ± 0.013	0.521 ± 0.010	0.499 ± 0.018	0.052 ± 0.006
	Butanolic fraction	0.141 ± 0.011	0.151 ± 0.012	0.768 ± 0.001	0.058 ± 0.018
	Tannins	0.252 ± 0.000	0.373 ± 0.027	0.376 ± 0.007	0.213 ± 0.006
	Anthocyanins	0.603 ± 0.122	0.477 ± 0.001	0.104 ± 0.005	0.283 ± 0.007
	Gallic acid	3.220 ± 0.020			
	BHT	0.010 ± 0.000			
TAC (mg AAE/g DM)	Methanolic extract	69.959 ± 2.609	44.546± 0.343	25.414 ± 0.208	9.606 ± 1.269
	Ethyl acetate fraction	24.952 ± 0.619	5.676 ± 0.236	17.051 ± 0.918	5.073 ± 0.453
	Butanolic fraction	63.714 ± 3.972	7.372 ± 0.170	9.927 ± 0.141	0.824 ± 0.192
	Tannins	16.952 ± 0.484	8.124 ± 2.455	17.971 ± 0.488	7.376 ± 0.338
	Anthocyanins	33.532 ± 2.491	2.748 ±1.594	18.061 ± 0.394	10.675 ± 0.573
TAC (mg GAE/g DM)	Methanolic extract	43.967 ± 1.639	27.996 ± 0.215	15.977 ± 0.137	6.032 ± 0.791
	Ethyl acetate fraction	15.681 ± 0.389	3.567 ± 0.148	10.718 ± 0.575	3.186 ± 0.287
	Butanolic fraction	40.042 ± 2.496	4.633 ± 0.107	6.237 ± 0.081	0.519 ± 0.129
	Tannins	10.654 ± 0.304	5.106 ± 1.543	11.299 ± 0.302	11.584 ± 0.536
	Anthocyanins	21.074 ± 1.565	16.810± 1.002	11.352 ± 0.241	0.908

from the calibration curve (mg CE/g DM). All the tests were carried out in triplicate.

Reducing power assay

The reducing power of the extracts was determined according to the method of Oyaizu[18]. Various concentrations of the extracts (mg/ mL) in distilled water were mixed with phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and 1% of potassium ferricyanide water solution (2.5 mL, $K_3[Fe(CN)_6]$). The mixtures were incubated at 50°C for 20 min. Aliquots of trichloracetic acid (2.5 mL, 10% aqueous solution) were added to the mixtures which were then centrifuged at 3000 rpm for 10 min. The supernatants (2.5 mL) were mixed with distilled water (2.5 mL) and freshly prepared $FeCl_3$ solution (0.5 mL, 0.1%). The absorbance was measured at 700 nm. Gallic acid was used as a positive control. In this method, a higher absorbance indicates higher reducing power. EC_{50} value (mg/ mL) is the effective concentration giving an absorbance of 0.5 for reducing power and was obtained from linear regression analysis.

DPPH scavenging assay

The hydrogen atom donation ability of different extracts of all parts of *P. lentiscus* was measured by 2, 2-diphenyl-1-picrylhydrazil (DPPH) free radical scavenging assay[19]. A volume of fifty microliters of various concentrations of the extracts in methanol was added to 1950 µL of DPPH methanol solution (0.025 g/L). After a 30 min incubation period at room temperature, the absorbance was read against a blank at 515 nm. DPPH free radical scavenging activity in percentage (%) was calculated using the following formula:

DPPH scavenging activity (%) = $(A_{blank} - A_{sample} / A_{blank}) \times 100$

Where A_{blank} is the absorbance of the control reaction (containing all reagents except the test compound), A_{sample} is the absorbance of the test compound. Extract concentration providing 50% inhibition (EC_{50}) was calculated from the graph plotted of inhibition percentages against extract concentrations. The ascorbic acid methanol solution was used as positive control.

β-carotene bleaching assay

The antioxidant activity of extracts from all parts of *P. lentiscus* was evaluated using β-carotene-linoleic model system, as described by Moure et al[20]. β- Carotene (2 mg) was dissolved in 10 mL of chloroform and 1 mL of β-carotene solution was mixed with 20 mg of purified linoleic acid and 200 mg of Tween 40 emulsifier. Chloroform was then evaporated; the resulting mixture was immediately diluted with 100 mL of distilled water. To an aliquot of 4 mL of this emulsion, 0.2 mL of different

Table 4: Quantitative analysis of phenolic content (µg/g DM) of ethyl acetate and butanolic fractions of the leaves of *P. lentiscus*. (1) tannic acid; (2) rutin; (3) gallic acid; (4) ascorbic acid; (5) vanillic acid; (6) ρ-coumaric acid; (7) catechin; (8) syringic acid; (9) ferulic acid; (10) Quercetin; (11) naringenin. N.D.: not determined. r.t: retention time.

Standards	1	2	3	4	5	6	7	8	9	10	11
r.t (min)	1.58	1.85	3.50	4.60	15.20	15.48	18.48	21.50	24.80	31.68	34.00
Ethyl acetate fraction	N.D.	N.D.	15.44	118.41	N.D.	131.90	41.06	N.D.	91.23	1945.45	1652.04
Butanolic fraction	N.D.	N.D.	N.D.	93.77	32.67	N.D	N.D.	N.D.	44.08	139.45	N.D.

concentrations of *P. lentiscus* extracts or the reference antioxidants was added and mixed well. The absorbance at 470 nm, which was regarded as t_0, was measured, immediately, against a blank consisting of the emulsion without β-carotene. The capped tubes were placed in a water bath at 50 °C for 120 min. For the positive control, sample was replaced with gallic acid or BHA. A negative control consisted of 0.2 mL distilled water or solvent instead of extract or reference antioxidants. The antioxidant activity (AA) was measured in terms of successful bleaching of β-carotene by using the following equation:

$AA= ((A_{A(120)} - C_{C(120)} /(C_{C(0)} - C_{C(120)}) \times 100$

$A_{A\ (120):}$ is the absorbance in the presence of the extract at 120 min; $C_{C\ (120):}$ is the absorbance of control at 120 min; $C_{C\ (0)}$: is the absorbance of control at 0 min.

Total antioxidant capacity

Total antioxidant capacity (TAC) of all extracts was determined according to the method of Prieto et al[21]. Briefly, 0.3 mL of sample

was mixed with 3 mL of standard reagent (0.6 M sulfuric acid, 28 mM sodium phosphate, 4 mM ammonium molybdate). Then, reaction mixture was incubated at 95°C for 90 min. After, the mixture had cooled to room temperature and the absorbance was measured at 695 nm. The antioxidant activity of the samples was expressed as milligrams of ascorbic acid equivalent (mg AAE/g DM) and gallic acid equivalent (mg GAE/g DM) per gram of dry matter. The sample was analyzed in triplicate.

High performance liquid chromatography analysis (HPLC) analyses

Hydrolysis of extracts

For HPLC analysis, we selected 2 from 20 studied extracts (ethyl acetate and butanolic fractions of the leaves) which are the most active. The selected extracts were hydrolyzed with 1.2 M HCl by refluxing in a water bath for 1 h. All samples were filtered through a 0.45 µm pore size syringe-driven filter before injection[22].

Chromatographic separation of phenolic compounds by HPLC

A 20 µL aliquot of sample solution was separated using high performance liquid chromatography system (YL 9100 HPLC system, Korea) equipped with UV–Vis detector and C-18 column (25 cm × 4.6 cm; 5 µm). The mobile phase consisted of solvent A (water/formic acid 0.4%) and solvent B (acetonitrile). Solvent gradient was used as followed: 0–2 min, 1% B; 2–15 min, 7% B; 15–25 min, 20% B; 25-35 min, 40% B; 35-46 min, 100% B; 46-47 min, 100% B; 47-48 min, 1% B; 48-55 min, 1% B. The flow rate was 1.2 mL /min. UV-detection was performed at 280 nm and identified phenolic compounds were quantified by comparison with retention time and peak area of each pure commercial compounds. Phenol standards including tannic acid, rutin, gallic acid, ascorbic acid, vanillic acid, ρ-coumaric acid, catechin, syringic acid, ferulic acid, quercetin and naringenin are prepared freshly and immediately injected to HPLC column.

Statistical analysis

Data were expressed as means ± standard derivation (SD) using Excel programme and Microcal Origin 6. For the quantification of phenolic compounds and total antioxidant capacity, all experiences were repeated in triplicates.

RESULTS

Yields of extraction

Extraction results of different parts (leaves, stems, fruits and roots) of *P. lentiscus* are shown in table 1.

The four parts of plant were characterized by the presence of different groups of secondary metabolites, where the higher yields were found in leaves for ethyl acetate (6.831 ± 0.254 %) and butanolic (6.24 ± 0.587 %) fractions, in roots for tannins (25.25 ±1.75 %) and in fruits (17.343 ± 0.895 %) and roots (17.055 ± 1 %) for anthocyanins.

Determination of total phenolics, flavonoids and condensed tannins

Total phenolic, flavonoid and condensed tannin contents of the leaves, stems, fruits and roots of *P. lentiscus* are shown in Table 2. Total phenolic content in leaves (216. 289 ± 20. 62 mg GAE/g DM) was significantly higher than those revealed in stems (121.399 ± 3.354 m g/g) and fruits (103.342 ± 2.317 mg/g), which showed less important contents. Roots extract had comparatively lower total phenolic content of 30.188 ± 1.291 mg/g. For the others polyphenolic classes, the highest concentrations of flavonoids and condensed tannins were recorded in leaves and stems of *P. lentiscus*

[a]milligrams of gallic acid equivalents per gram of dry matter; [b]milligrams of catechin equivalents per gram of dry matter.

Reducing power

The reducing power of all extracts of *P. lentiscus* is shown in Table 3. All extracts showed high activity to reduce power. The highest capacity was observed in roots tannins (EC_{50} = 0.061± 0.001 mg/mL) followed by leaves tannins (EC_{50} = 0.075 ± 0.001), which is comparable with gallic acid, indicating a good activity. Methanolic extract of fruits (EC_{50} = 0.666 ± 0.035 mg/mL) and ethyl acetate fraction of stems (0.416 ± 0.004 mg/mL) exhibited the lowest activity to reduce power. For the others extracts, the EC_{50} ranged between 0.088 ± 0.000 and 0.369 ± 0.002 mg/mL.

DPPH radical scavenging activity

From Table 3, all extracts of *P. lentiscus* exhibited a better scavenging efficiency toward DPPH radical. The tannins of all parts possess the powerful antiradical activity to scavenge DPPH radical. The EC_{50} concentrations were 0.069 ± 0.001 mg/mL for leaves and 0.057 ± 0.001 mg/mL for stems; these values are better than that of the ascorbic acid (0.090 ± 0.002 mg/mL)

Tannins roots and fruits exhibited also high activity with EC_{50} of 0.090 ± 0.011 mg/mL and 0.099 ± 0.019 mg/mL, respectively. These values are similar to that of ascorbic acid indicating a very good activity.

β -Carotene–linoleic acid assay

Capacities of inhibiting the bleaching of β-carotene by scavenging linoleate-derived free radicals are shown in Table 3. The highest antioxidant activity was recorded in ethyl acetate fraction (0.052 ± 0.006 mg/mL) followed by butanolic fraction (0.058 ± 0.018 mg/mL) of roots. For the others extracts, the range of this activity was varied between 0.104 ± 0.005 and 0.768 ± 0.001 mg/mL.

Total antioxidant capacity

The result of total antioxidant capacity was shown in Table 3 and expressed in gallic acid and ascorbic acid equivalents. The results indicate that all extracts have a high antioxidant capacity. The leaves methanolic extract (69.959 ± 2.609 mg AAE/g DM; 43.967 ± 1.639 GAE/g DM) and butanolic fraction (63.714 ± 3.972 mg AAE/g DM; 40.042 ± 2.496 GAE/g DM) revealed a highest antioxidant capacity compared to others extracts. The lowest antioxidant capacity was found in roots butanolic fraction (0.824 ± 0.192 mg AAE/g DM; 0.519 ± 0.129 GAE/g DM).

Chromatographic separation of phenolic compounds by HPLC

The results of quantitative analysis (µg/g DW) of each identified phenolic compound are shown in Table 4. In ethyl acetate fraction, the amount of identified compounds varied widely, from 1945.45 µg/g DW of quercetin to 15.44 µg/g DW of gallic acid. However, tannic acid, rutin, vanillic acid and syringic acid were not found. In butanolic fraction, the highest and lowest amount of identified phenolic compounds were quercetin 139.45 µg/g DW and vanillic acid 32.67 µg/g DW respectively. Ascorbic acid and ferulic acid were also identified in this fraction.

DISCUSSION

This study provides support to traditional and alternative use of *P. lentiscus* against various diseases. Phytochemical analysis showed that the major constituents of the extracts were flavonoids, tannins, and anthocyanins in leaves, stems, fruits and roots. High levels of total phenolic content, flavonoids and condensed tannins were found in all parts of *P. lentiscus.* Our results correlate with some authors[23,7,8] who found the anthocyanins, the flavonoids and triterpenes respectively in this plant. Similarly, the review of phytochemical screening realized by Hamad et al[9] revealed the presence of unsaturated sterols and/ or triterpenes, carbohydrates, flavonoids and tannins in *P. lentiscus*. Another recent study by Arab et al[10] reported an abundance of phenolic compounds in leaves (116.49 %) and fruits (61.34 %)

In comparison with others authors, Gardeli et al[24] found that the total phenolic content was 588 mg GAE/g in leaves of *P. lentiscus.* This wealth indicated in our result was confirmed by the literature[9-11]. Others studies reported by Djeridane et al[25] (23.5 mg GAE/ g DM), Benhammou et al[26] (0.90 ± 0.05 mg pyrocatechol equivalent/ g dry extract) and Atmani et al[8]. (136.25 ± 18.9 mg CE/ g DM) showed low levels of phenolic contents compared to our result. Comparing to others reports, the flavonoid contents in leaves were of 12.93 ± 1.69 mg/g and 8.21 ± 0.09 mg/g, respectively found by Atmani et al[8] and Krimat et al[27]. About condensed tannins, Atmani et al[8] reported that leaves showed a high content of 909.4 ± 42.61 mg tannic acid equivalents/ g dry extract. Antioxidants are known to affect cardiovascular health, atherosclerosis, cancer and aging processes, drawing a broad spectrum of attention from a variety of biomedical fields. Antioxidant compounds found in plants have free radical scavenging effects and provide an important function in maintaining oxidative resistance[28]. The test of reducing power can be used as a significant indicator of potential antioxidant activity of a compound. It is based on the ability of the phenolic compounds to reduce the ferric iron (Fe^{3+}) to ferrous iron (Fe^{2+})[29]. Our results are in agreement with those of Atmani et al[8] and Atmani et al[30] who found that *P. lentiscus* exhibited a great reducing power. The DPPH assay has been largely used as a quick, reliable and reproducible parameter to search for the in vitro antioxidant activity of pure compounds as well as plant extracts [31]. The radical scavenging activity of the extract was observed from the decrease in absorbance of DPPH with increase in extract concentration. According to Gardeli et al[26]. the methanolic extract of aerial part of this specie showed highest free radical scavenging activity (EC_{50} = 0.050 mg/mL), this value is similar to tannins stems extract in our study. Similarly, the result of Atmani et al[8]. and Krimat et al[27] showed that leaves of *P. lentiscus* exhibited a strong antioxidant activity to neutralize the DPPH radical (EC_{50} = 0.004 ± 0.000 mg/mL). These data are higher than those found in our study. In β-carotene test, the decrease in absorbance of β-carotene in the presence of different extracts due to the oxidation of β-carotene and linoleic acid. The results indicate that all tested extracts possessed this antioxidant property. Our results correlate with those found by Gardeli et al[24] who showed that leaves of *P. lentiscus* had a high antioxidant capacity (131 mmol/L). Both ethyl acetate and butanolic fractions of leaves prepared in the current study were analyzed by HPLC and identified by comparing their retention times to those of eleven reference standards (tannic acid, rutin, gallic acid, ascorbic acid, vanillic acid, ρ-coumaric acid, catechin, syringic acid, ferulic acid, quercetin and naringenin). The results found in our investigation are in a close agreement with those reported by Rodriguez-Perez et al [6] who identified different phenolic compounds in leaves, 3 of which were gallic acid, catechin and quercetin. Some phenolic compounds identified in our study were tentatively characterized for the first time, such as ferulic

acid in both fractions, naringenin in ethyl acetate fraction and vanillic acid in butanolic fraction of the leaves. These results suggest that the antioxidant activity of the leaves extracts is in correlation with their composition of polyphenols. These findings may be useful in establishing a relationship between the chemical composition and the highest antioxidant activity of the leaf extract of *P. lentiscus*, showing the interest in studying this part of this plant.

CONFLICT OF INTERESTS

The author declares that there is no conflict of interests in this study.

ACKNOWLEDGMENTS

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

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