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Phytochemical, organoleptic and ferric reducing properties of essential oil and ethanolic extract from *Pistacia lentiscus* (L.)

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ABSTRACT

Objective: To study the phytochemical composition and organoleptic properties of *Pistacia lentiscus* (L.) (*P. lentiscus*) from Algeria and to investigate the antioxidant activities of its essential oil and ethanolic extract.

Methods: Aerial parts of *P. lentiscus* were collected at Hammam Melouane (Blida), 50 km from Algiers. Different solvent extractions were made for the preliminary screening of phytochemicals. Additionally, the physicochemical and organoleptic properties of the Algerian variety have been characterized. Moreover, *P. lentiscus* essential oil and ethanolic extract were studied for their antioxidant potential by ferric reducing power test.

Results: Phytochemical screening of *P. lentiscus* revealed the presence of various biochemicals, including leuco-anthocyanins, condensed tannins, gallic tannins, saponoside, coumarins, and flavonoids, while others were absent (anthocyanins and alkaloids). Essential oil showed lower antioxidant potential compared to the ethanolic extract, reflecting the potential phenolic content responsible for this activity.

Conclusions: The present study reveals the presence of various phytochemical classes in *P. lentiscus*, and the antioxidant experiment shows a good bioactivity of the aerial parts of the Algerian *P. lentiscus*. The literature review of the essential oil composition shows also important variations due to geographic and environmental conditions. This preliminary investigation will help explore the bioactive compounds of *P. lentiscus*, and will bring data for a better physicochemical and organoleptic classification of the Algerian variety.

1. Introduction

The oxidation is one of the most important mechanisms for producing free radicals in food, drugs and even physiological systems[1,2]. Reactive oxygen species may be the causative factor involved in many human degenerative diseases, and antioxidant compounds are known to have some degrees of preventive and therapeutic effects on these disorders[3]. Antioxidant supplements

in foods may be used to help the human body reduce oxidative damage caused by free radicals[4]. It is well-known that oxidation damages various biological substances and subsequently causes many diseases. Accordingly, there are many research papers on the relationships between oxidative damages and various diseases including cancer[5], aging[6], inflammation[7], diabetes[8,9] and atherosclerosis[10]. Recently, the interest in natural antioxidants, in relation to their therapeutic properties, has increased dramatically. For these reasons, many extractions, identification and quantification methods have been developed[11,12].

The antioxidant activity of a compound is defined as the ability to scavenge or inhibit oxidation radicals. Major antioxidants are the ascorbic acid (vitamin C), tocopherol (vitamin E) as well as

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phenolic compounds. Indeed, most synthetic or naturally occurring antioxidants have phenolic hydroxyl groups in their structures, and antioxidant properties are attributed in part to the ability of these natural compounds to scavenge free radicals such as hydroxyl radicals (OH[•]) and superoxide (O₂^{•-}) [13,14].

Several methods are used to evaluate *in vitro* and *in vivo* antioxidant activity by scavenging different radicals by the oxygen radical absorbance capacity and total radical-trapping antioxidant parameter methods [15]; ferric ions by ferric ion reducing antioxidant parameter method [16]; or the method using radical ABTS^{•+} (ammonium salt of 2,2'-azinobis-3-ethylbenzothiazoline-6-sulfonic acid) [17] and the free radical DPPH[•] (diphenylpicrylhydrazyl) [18].

Given the complexity of diversified oxidation process and the nature of the antioxidants, with components both hydrophilic and hydrophobic, there is no universal method by which the antioxidant activity can be quantitatively measured in a precise manner. Most often it is necessary to combine the responses of different and complementary tests to get an indication of the antioxidant capacity of the test sample [19,20].

Pistacia is a particular genus of the Anacardiaceae family. *Pistacia lentiscus* (L.) (*P. lentiscus*) is a Mediterranean herb that develops on any soil type [21]. The essential oil of *P. lentiscus* is often used as a remedy to treat burns [22] and back pain [23]. The aim of this work was to evaluate the antioxidant properties of the essential oil and ethanolic extract of the Algerian *P. lentiscus*.

2. Materials and methods

2.1. Plant material

P. lentiscus (Figure 1) was collected by the end of February at Hammam Melouane (Blida), at 950 m of altitude, 50 km from Algiers (36°27' N and 2°52' E). The region is characterized by sub-humid climate and siliceous soil. The collection was carried out under the best possible conditions, avoiding dew, rain or excessive moisture. The freshly harvested plant was allowed to dry in the open air in the laboratory at an ambient temperature. After drying, the aerial part was ground with a mechanical mixer to obtain a fine powder in order to prepare the extracts. The plant material was identified at the Faculty of Natural Sciences and Life, University Saad Dahleb Blida 1, Algeria by Dr. Belkhoussa Samir.

2.2. Determination of water content

The loss rate during drying (water and the volatile matter content) was determined using 1 g of aliquot of the sample cut into small pieces in a porcelain dish, and dried in an oven set at a temperature of (103 ± 2) °C for 2 h up to a constant weight according to the European Pharmacopoeia [24]. The operation is repeated until a constant weight (by reducing the drying lasted 30 min) to prevent the caramelization.

The water content is determined using the following formula:

$$H (\%) = \frac{M1 - M2}{P} \times 100$$

where, H (%) is humidity percentage; M1 is the mass of the capsule and the fresh material before drying (g); M2 is the mass of the capsule and the fresh material after drying (g); P is the mass of sample tested (g).



Figure 1. *P. lentiscus*.

2.3. Determination of ash content

The *P. lentiscus* was cut into small pieces and then calcined at 550 °C in a muffle furnace until obtaining a constant weight of white ash [24].

In porcelain capsules, 2 g of *P. lentiscus* was weighed and cut into small pieces; then the capsules were placed in a muffle furnace set at (550 ± 15) °C for 5 h until a gray, clear or whitish matter was obtained, and the matter was put in the dryer to cool and then weighed.

The ash content was determined according to the following formula:

$$MO (\%) = \frac{M1 - M2}{P} \times 100$$

where, MO (%) means percentage of organic matter; M1 means a mass of the test portion + capsules; M2 means a mass of capsules + ashes; P means mass of the sample tested.

The ash content (Cd) was calculated by using the formula:

$$Cd = 100 - MO (\%)$$

2.4. Ethanolic extract preparation

The ethanolic extract was obtained by using the Soxhlet method, which enables a solid-liquid extraction with a high efficiency. The dried whole plant was crushed and reduced to a fine powder; 40 g of the powder obtained was mixed with 400 mL of a solvent (ethanol) in a reflux condenser for 6 h. After extraction, the solvent-rich substance extracted was recovered in a ball, and then passed to the rotary apparatus to evaporate the solvent then lyophilized. The extract thus obtained was placed in a desiccator, weighed and stored at 5 °C.

2.5. Essential oil preparation

The extraction of essential oil from the aerial part of *P. lentiscus*

was carried out by hydrodistillation using a modified Clevenger-type apparatus. This method is recommended by the European pharmacopoeia (2014)[24]. The essential oils were dried over anhydrous sodium sulphate and then stored in sealed glass vials at 5 °C prior to analysis.

2.6. Phytochemical screening

The ethanolic extract underwent phytochemical screening in order to detect the presence (or absence) of alkaloids (Dragendorff and Mayer’s reagent), anthocyanins (hydrochloric acid reaction), leuco-anthocyanins (hydrochloric acid and propanol reaction), saponosides (hydrochloric acid and sodium hydroxide reaction), coumarins (hydrochloric acid and potassium hydroxide reaction), flavonoids (cyanide reaction), tannins (iron chloride), and terpenoids (Liebermann-Burchard reaction)[25].

2.7. Antioxidant activity

The reducing power indicates the ability to reduce ferric iron (Fe³⁺) to ferrous iron (Fe²⁺), thus measuring the ability of an essential oil or extract to interact with the reactive species such as free radicals, as an electron donor. It should be recalled that 2,6-ditert-butyl-4-methyl-phenol (BHT) was taken as reference antioxidant. The ferric ions (Fe³⁺) reducing power was measured according to the method described by Oyaizu[26].

The method was based on the chemical reaction of Fe³⁺ to Fe²⁺. The working solution was prepared by mixing 0.125 mL of extracts with 2.5 mL phosphate buffer (0.2 mol/L, pH 6.6) and 2.5 mL of potassium hexacyanoferrate (N₆C₆FeK₃) (1%; m/v). The mixture was incubated at 50 °C for 20 min. The Fe²⁺ was monitored by measuring the absorbance of formation of the ferrous complex at 700 nm.

To explain the results, the most common way used by the majority of authors is to draw graphs of absorbance obtained for the different concentrations and used for the different plant extracts. The increase in absorbance corresponds to an increase of the reducing power of the tested fractions.

2.8. Statistical analysis

All the experiments were performed in three replicates. Data were expressed as mean ± SD (n = 3). The significance of differences was estimated using student’s paired t-test. The difference was considered statistically significant when the P value was less than 0.05. All statistical analyses were performed using STATISTICA software.

3. Results

3.1. Determination of water and ash content

The water content in the medicinal plant is one of the major indices that characterize the plant quality. Medicinal plants should not contain an amount of humidity that exceeds the standard value

(17%)[27]. The result of this analysis revealed a moisture content equal to 9.8% and a dry matter of 90.2%. The results obtained after calcination of the powder of the aerial part of *P. lentiscus* demonstrated an ash content of 6.25% and an organic matter of 93.75%.

3.2. Characterization of the essential oil of *P. lentiscus*

The organoleptic and physicochemical properties of the essential oil of *P. lentiscus* were demonstrated in Table 1. The essential oil of Algerian variety of *P. lentiscus* is characterized by a liquid and limpid appearance, a dark yellowish color, a strongly aromatic smell, and with spicy flavor.

Table 1

Physicochemical characters of essential oil of *P. lentiscus*.

Variety	Density at 20 °C	Index of ester	Index of acid	Refractive index	Optical rotation
Algeria	0.855	16.5	2.25	1.43	0.42°
Morocco	0.850–0.875	13.0–19.0	-	1.40–1.45	-1° – +2°

3.3. Phytochemical screening

Phytochemical screening was performed on the ethanolic extract of *P. lentiscus* (Table 2). Preliminary results have shown that *P. lentiscus* contains the majority of secondary metabolites such as condensed tannins, gallic tannins, saponosides, coumarins and flavonoids. The anthocyanins and alkaloids are absent in *P. lentiscus*.

Table 2

Phytochemical screening of *P. lentiscus* ethanolic extract.

Chemicals	Ethanolic extract of <i>P. lentiscus</i>
Anthocyanins	-
Leuco-anthocyanins	++
Condensed tannins	+++
Gallic tannins	+++
Saponosides	+++
Alkaloids	-
Coumarins	+++
Flavonoids	+++

+++ : Strongly positive; ++ : Low positive; - : Negative.

3.4. Antioxidant activity

The results of the evaluation of the reducing power of the essential oil of *P. lentiscus* are shown in Table 3 while those of the ethanolic extract are presented in Table 4.

Table 3

Reducing power of the essential oil of *P. lentiscus* compared to that of BHT.

Concentrations (µg/mL)	Absorbance of essential oil	Absorbance of BHT
100	0.120 ± 0.008	0.863 ± 0.011
200	0.052 ± 0.003	1.063 ± 0.024
400	0.200 ± 0.002	1.161 ± 0.008
600	0.307 ± 0.003	1.178 ± 0.036
800	0.331 ± 0.008	1.220 ± 0.036
1000	0.405 ± 0.029	1.303 ± 0.003
2000	0.500 ± 0.007	ND
4000	0.504 ± 0.004	ND

Values were expressed as mean ± SD. ND: Not determined.

Table 4

Reducing power of the ethanolic extract of *P. lentiscus* compared to that of BHT.

Concentrations (µg/mL)	Absorbance of ethanolic extract	Absorbance of BHT
2	0.003 ± 0.001	ND
5	0.015 ± 0.004	ND
10	0.024 ± 0.005	ND
20	0.107 ± 0.005	0.311 ± 0.006
50	0.425 ± 0.011	0.576 ± 0.001
100	0.816 ± 0.064	0.863 ± 0.011

Values were expressed as mean ± SD. ND: Not determined.

In general, the reducing power of the extracts of the plant depends on its concentration, while the reducing capacity of the extract is significantly stronger than that of the essential oil, and the reducing capacity of the latter compared to BHT is relatively very low. To this end, we noted that the reducing power of the extract was very weak at the concentrations of 2–10 µg/mL. At the concentration of 20 µg/mL, BHT reducing power was greater than that of the extract. Table 4 shows clearly that at 100 µg/L of concentration, the ethanol extract of *P. lentiscus* and BHT had the strongest reducing capacity, almost equivalent. The antioxidant activity of the essential oil can be attributed to its rich chemical composition (Table 5). However, the ethanolic extract shows more potent activity.

Table 5

Chemical composition of *P. lentiscus* from different geographic conditions[28-31].

Compounds	Morocco	Tunisia	United Kingdom	Greece
α-Phellandrene	0.6	2.8	n.d	1.2
α-Pinene	5.1	16.8	40.9	17.1
Camphene	1.1	4.0	1.0	1.1
Sabinene	3.5	5.7	0.3	1.0
β-Pinene	0.9	4.3	1.7	2.0
β-Myrcene	2.3	1.2	9.0	0.4
α-Terpinene	1.3	3.1	n.d	2.8
Limonene	8.1	n.d	0.8	13.9
γ-Terpinene	5.9	5.5	n.d	3.6
Terpinolene	1.1	1.5	n.d	1.3
Terpinen-4-ol	19.3	11.9	n.d	10.6
Bornylacetate	3.1	1.5	n.d	0.2
α-Copaene	1.4	0.4	n.d	0.4
β-Cubebene	1.0	n.d	n.d	n.d
β-Elemene	1.1	0.5	n.d	0.3
β-Caryophyllene	1.6	1.9	5.3	2.1
α-Humulene	0.2	0.7	0.1	0.8
δ-Cadinene	0.1	2.1	n.d	2.5
1,4-Cadinadiene	n.d	2.1	n.d	n.d
(Z,Z)-farnesol	n.d	n.d	11.9	n.d
Linalool	n.d	n.d	0.8	n.d
Perillene	n.d	n.d	0.8	n.d
Myrtenal	n.d	n.d	0.5	n.d
Verbanone	0.3	n.d	0.8	n.d

n.d: Not determined.

4. Discussion

The water content obtained indicates that our plant has been

well preserved. According to Abbott *et al.*[32], water is a source for polyphenols degradation by oxidation and also a troublesome component for calculating the extraction yield, hence its elimination is needed. The ash content of the aerial part of *P. lentiscus* is 6.25%, which is relatively low compared to that of *P. lentiscus* from Iran obtained by Mahmoudi *et al.* (10%)[33]. This large difference between the ash contents can be explained by the absence or presence of lesser silica in *P. lentiscus* of Algeria, because according to Krishnarao *et al.*[34], the presence of silica decreases the calcination generating a low ash content.

We noted that the physicochemical parameters of the essential oil of *P. lentiscus* are in agreement with those mentioned by Bellakhdar *et al.*[35]. The acid index (AI) shows the rate of free fatty acids in the essential oil. A higher value indicates essential oil degradation (hydrolysis of esters) during storage. Conversely, a lower AI than 2 is a good conservation index of the essential oil. The AI of *P. lentiscus* obtained in this study was 2.25; this value indicates that the essential oil is stable and does not cause oxidation (good conservation of the extracted essential oil). The low refractive indexes of essential oils (1.40 to 1.45) indicate their low refraction of light. This confirms our results, which could lead to their use in cosmetic products[36].

We can say that almost all of bioactive molecules are present, which prove their potent biological activities mainly exhibited by tannins, saponins and the coumarins; these results are consistent with those found by Dalila *et al.*[37], and these compounds found are responsible for the major activities including antioxidant[38] and healing activity[39].

According to Amarowicz *et al.*[40], FeCl₃/K₃Fe(CN)₆ system allows a “semi-quantitative” determination of concentrations of polyphenols involved in the redox reaction $Fe^{3+} + e^{-} \rightarrow Fe^{2+}$. A correlation between the reducing capacity of the extracts and their flavonoids and total phenols contents was observed. The reducing power is probably due to the presence of hydroxyl group in the phenolic compounds, which may serve as electron donors. Accordingly, antioxidants have been considered reducing and oxidizing inactivators[41] and on the other hand, Aidi Wannes *et al.*[42] showed that the reducing power of methanolic extracts of various parts of the mastic was higher than that of essential oils.

However Costa *et al.*[43] in the study on the relationship between phenolic content and antioxidant activity of infusions of Mediterranean medicinal plants indicated that aqueous extracts of different studied medicinal plants behave like electron donors with increasing activity in a dose-dependent manner. The most powerful aqueous extracts are those of *P. lentiscus* followed by *Citrus albidus*, *Lavanda viridis* and *Myrtus*. Recent studies on the essential oil of *P. lentiscus* in other countries have demonstrated its high amounts of compounds with antioxidant and anti-inflammatory activities with

prospects in pharmaceutical and cosmetic industries[28-31]. Other pharmacological activities have been studied in the literature showing that this plant contains other important therapeutic properties, for instance, anti-inflammatory and antiulcer[44], diuretic and antihypertensive[45], antibacterial[46], antifungal[47], and antiatherogenic activities[48].

P. lentiscus is a promising source of natural antioxidants. These findings confirm the potential uses of *P. lentiscus* in food technology and medicine. The present results encourage additional and more in-depth studies on the phenolic composition of the plant extracts and assessment of antioxidant activity of each compound separately. Some phenolic compounds remain to be identified and further biological tests should be conducted.

Conflict of interest statement

We declare that we have no conflict of interest.

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