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Extraction and Valorization of Phenolic Compounds of Leaves of Algerian *Pistacia lentiscus*

¹Asma Cherbal, ¹Mohamed Kebieche, ²Khodir Madani and ³Hala El-Adawi

¹Pharmacology and Phytochemistry Laboratory, Department of Molecular and Cell Biology, Faculty of Nature and Life Sciences, University of Jijel, Algeria

²Biochemistry and Biophysical Science Laboratory, Department of Food Sciences, Faculty of Nature and Life Sciences, University of Abderrahmane, Mira, Bejaia, Algeria

³Genetic Engineering and Biotechnology Institute, Department of Medical Biotechnology, Mubarak City for Scientific Research, Borg Elarab, Alexandria, Egypt

Abstract: *Pistacia lentiscus* is known since antiquity for its medicinal properties. Its leaves have anti-inflammatory, antibacterial, antifungal, antipyretic, hepatoprotective and anti-diarrheal effects. They are also used in the treatment of several other diseases. This present study aims to the valorization of the hydro-methanolic extract of *Pistacia lentiscus* leaves as an antioxidant. First, a hydro-methanolic extraction of the total phenolic compounds contained in the leaves of *Pistacia lentiscus* was measured and then quantitative analysis of the polyphenol, flavonoids and tannins contents were carried out. The antioxidant activity was evaluated by measuring the reduction of hydrogen peroxide and the scavenging ability with respect to a relatively stable free radical (DPPH). Our results revealed that, the leaves extract has a significant scavenging activity, where it reached up to 76.4% at 200 $\mu\text{g mL}^{-1}$ which is comparable with that of α -tocopherol. A moderate reducing power (37.04%) of H_2O_2 was recorded at 50 $\mu\text{g mL}^{-1}$. In addition, the *Pistacia lentiscus* leaves extract was rich in polyphenols which seem to have a comparable antioxidant capacity and even more significant than that of the standards.

Key words: *Pistacia lentiscus* leaves, phenolic compounds, hydro-methanolic extract, antioxidant activity

INTRODUCTION

Various studies have shown that many diseases are usually associated with an increased formation of free radicals and a decrease in antioxidant potential. Thus, the balance normally present in cells between radical formation and protection is disturbed. This leads to the oxidative damage of cell components such as proteins, lipids and nucleic acids (Naziroglu and Butterworth, 2005). Recently, there has been a considerable interest in finding natural antioxidants from plant materials to replace synthetic ones. Data from both scientific reports and laboratory studies show that plants contain a large variety of substances that possess antioxidant activities (Chanwitheesuk *et al.*, 2005). Plants often contain substantial amounts of antioxidants, including α -tocopherol (vitamin E), carotenoids, ascorbic acid (vitamin C), flavonoids and tannins (Larson, 1988) and it has been suggested that antioxidant action may be an important property of medicinal plants used in treating diseases.

Pistacia lentiscus L. which belongs to the family Anacardiaceae, is a dense bush with a strong characteristic aroma and green leaves which grows in many Mediterranean countries (Zrira *et al.*, 2003). The aerial part of *P. lentiscus* L. has traditionally been used in the treatment of hypertension and possesses stimulant and diuretic properties (Bentley and Trimen, 1980). Some researchers reported the chemical composition of the essential oil from leaves of *P. lentiscus* L. of diverse origins (Douissa *et al.*, 2005). In Algeria, the mastic tree is widespread in the forest belts. The use of this plant has been recommended in Algerian folk medicine in the treatment of many illnesses as eczema, oral infections, diarrhea, renal lithiasis, jaundice, headaches, ulcer, stomach ache, asthma and respiratory problems (Ali-Shtayeh *et al.*, 1998; Said *et al.*, 2002). However, there is only a small number of reports available in the literature, studying the antioxidant properties of the plant extract by DPPH (Baratto *et al.*, 2003) and reduction power (Benhammou *et al.*, 2008), as well as the total phenolic content estimated by the Folin-Ciocalteu assay (Stocker *et al.*, 2004).

Since the biological and pharmacological actions of *Pistacia lentiscus* leaves extract has not been well studied, this study attempted to evaluate the antioxidant activity and the total phenolic content with Folin-Ciocalteu of the hydro-methanolic leaves extract of this medicinal plant.

MATERIALS AND METHODS

Plant material: The leaves of *Pistacia lentiscus* were collected in April 2011 from El Kennar, Jijel, Algeria. The plant material was stored at room temperature in a dry place until used.

Extraction and concentration: Air-dried leaves were ground with the help of an electric grinder (Sayona, model: Sy-601) in order to get a fine powder (Awika *et al.*, 2005). The sifting was achieved with a sifter (Retche) of which the diameter of the stitches is 100 μm . The plant powder was then kept in small bottles of tinted glass to avoid the oxidization of their components. The extraction of polyphenols was carried out at ambient temperature for 72 h by maceration in methanol-water 80/20 (v/v) at a solid/liquid ratio of 1/10 (w/v) with continuous stirring.

The hydro-methanolic extract was filtrated with No. 1 Whatman millipore filter paper (0.45 μm Ref HAWP04700, bedford, MA, USA). The resultant hydro-methanolic filtrate was refluxed with hexane for de-fatting as described by Yu *et al.* (2005). Then the filtrate was concentrated at 40°C to dryness in a rotary evaporator (Heidolph, LABOROT 4003) to yield solid residue (Naczka and Shahidi, 2006). The extraction yield was calculated according to Stanojevic *et al.* (2009) and the dried extracts were kept in the dark at 4°C prior analysis.

Phenols content

Total phenolic contents: Total phenols content was determined by the Folin-Ciocalteu's method (Othman *et al.*, 2007). An aliquot 0.2 mL of the extract or standard solution was mixed with 1.5 mL of Folin-Ciocalteu's phenol reagent. Afterwards, 1.5 mL of 7.5% Na_2CO_3 solution was added to the mixture followed by incubation in the dark at ambient temperature for 90 min. The absorbance against a blank was measured at 750 nm. Gallic acid was used to prepare a standard curve (1-200 $\mu\text{g mL}^{-1}$; $Y = 0.008 X + 0.034$, $R^2 = 0.986$, where, Y is the absorbance and X is the standard concentration). The results were expressed as mg Gallic Acid Equivalents (GAE) per 100 g extract. The analyses were carried out 6 times and the mean value was calculated.

Total flavonoids content: Flavonoids content was determined according to the method of Djeridane *et al.* (2006). An aliquot 1.5 mL of the extract or standard solution was mixed with 1.5 mL of 2% AlCl_3 . Thirty minutes later, the absorbance against blank was determined at 430 nm. Quercetin was used for standard curve construction (1-400 $\mu\text{g mL}^{-1}$; $Y = 0.003 X + 0.248$, $R^2 = 0.939$, where, Y is the absorbance and X is the standard concentration). The results were expressed as mg Quercetin Equivalents (QE) per 100 g extract. The analyses were carried out 6 times and the mean value was calculated.

Tannins content: Tannins content was determined according to the method of Hagerman and Butler (1978). An aliquot 1 mL of the extract or standard solution was mixed with 2 mL of bovine serum albumin (prepared in 0.2 M acetate buffer, pH 5). After 24 h of incubation at 4°C, the solutions were centrifuged at 5000 rpm for 20 min. The precipitate was collected, re-dissolved in 4 mL of SDS/TEA (sodium dodecyl sulfate/triethanolamine) and then added with 1 mL of 0.01 M FeCl_3 (prepared in 0.01 N HCl). The well-mixed solution was incubated in the dark at ambient temperature for 15 min. The absorbance against blank was read at 510 nm. Tannic acid was used to make the standard curve (0.1-0.8 mg mL^{-1} , $Y = 1.507 X + 0.052$, $R^2 = 0.999$, where, Y is the absorbance and X is the standard concentration). The results were expressed as mg Tannic Acid Equivalents (TAE) per 100 g extract. The analyses were carried out 6 times and the mean value was calculated.

Antioxidant capacity

Scavenging ability for 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical: The DPPH scavenging ability was determined according to the method of Brand-Williams *et al.* (1995). A portion (100 μL) of extract samples (concentrations of 25, 50, 100 and 200 $\mu\text{g mL}^{-1}$) was added to 2.9 mL DPPH (0.025 g L^{-1} prepared in 95% methanol), followed by incubation in the dark at ambient temperature for 30 min immediately before monitoring the absorbance at 515 nm for the extract (A_{extract}) against the control (A_{control}). The absorbance of the control (distilled water) was also recorded after 30 min at the same wavelength. Then, the percentage of scavenging was calculated according to the following equation:

$$\text{Scavenging (\%)} = \frac{A_{\text{control}} - A_{\text{extract}}}{A_{\text{control}}} \times 100$$

The analyses were done in triplicate and the mean value was calculated.

Reducing power assay: The reducing power of the extract and the standards (gallic acid, tannic acid, α -tocopherol and the quercetin) is determined according to the method described by Ruch *et al.* (1989) and Sfahlan *et al.* (2009) with some modifications. An aliquot of 3 mL of the extract or standard solutions (concentrations of 25 and 50 $\mu\text{g mL}^{-1}$) was mixed with 50 μL of 40 mM H_2O_2 (prepared in 0.1 M phosphate buffer, pH 7.4). After 10 min of incubation in the dark at ambient temperature, the absorbance against a blank was measured at 230 nm. The percentage of hydrogen peroxide reducing was calculated according to the following equation:

$$\text{Reduction (\%)} = \frac{A_{\text{control}} - A_{\text{extract}}}{A_{\text{control}}} \times 100$$

The analyses were done in triplicate and the mean value was calculated.

RESULTS

Extraction yield: The extraction yield was 44.58 ± 1.76 g of dry extract/100 g of dry plant material.

Antioxidant activity: The results of the scavenging ability of the hydro-methanolic extract of *Pistacia lentiscus* leaves and the standards expressed in percentage are illustrated by Fig. 1. The extract of *Pistacia lentiscus* leaves was characterized by a high activity (76.4%) at 200 $\mu\text{g mL}^{-1}$ which is comparable with that of α -tocopherol.

Figure 2 illustrated the reducing power of two concentrations of the hydro-methanolic extract of *Pistacia lentiscus* leaves and the standards. The results in Fig. 2 indicated that, the plant extract as well as the standards possessed the capacity to decrease the concentration of H_2O_2 . The rate of inhibition of hydrogen peroxide was proportional to the concentration of the plant extract and the standards. However, the hydro-methanolic extract of *Pistacia lentiscus* leaves showed a moderate neutralization rate (37.04%) at 50 $\mu\text{g mL}^{-1}$.

Total phenols, flavonoids and tannins contents: In an attempt to establish a potential relationship with different activities, we have determined the amount of phenolic compounds in the hydro-methanolic extract of *Pistacia lentiscus* leaves. From the results summarized in Table 1, we can easily conclude that *Pistacia lentiscus* leaves are rich in tannins and poor in flavonoids.

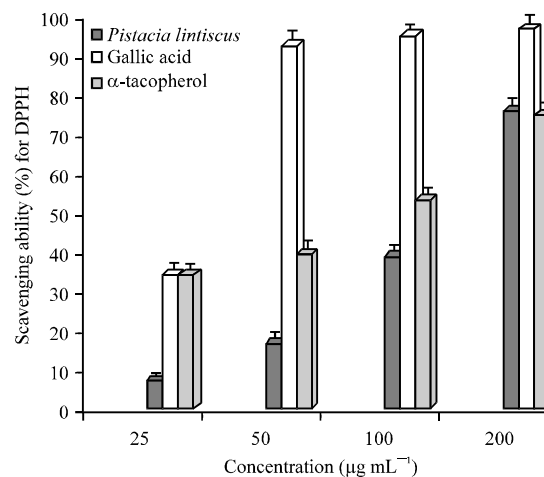


Fig. 1: Scavenging ability of the hydro-methanolic extract of *Pistacia lentiscus* leaves and the standards

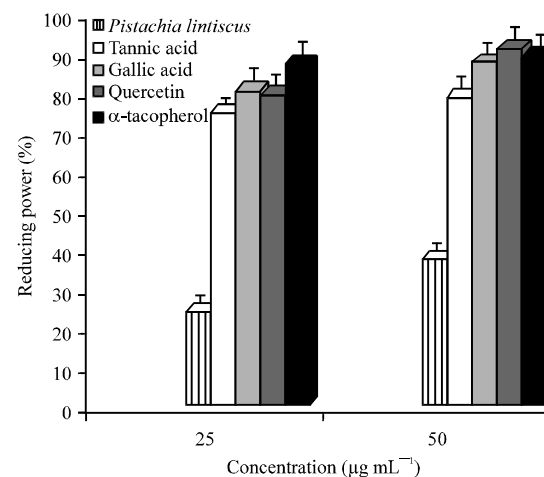


Fig. 2: The reducing power of the hydro-methanolic extract of *Pistacia lentiscus* leaves and the standards

Table 1: Total phenols, flavonoids and tannins contents of hydro-methanolic extract of *Pistacia lentiscus*

Total phenols, flavonoids and tannins contents	Amount
Total phenolic content (mg GAE/g extract)	632.9 \pm 1.35
Total flavonoids content (mg QE/g extract)	38.7 \pm 0.02
Tannins content (mg TAE/g extract)	175.3 \pm 1.07

Values are Means \pm SD

DISCUSSION

The extraction of phenolic compounds from plant material is influenced by their chemical structure, the method of extraction, the size of the particles forming the sample, the time and the conditions of storage as well as the presence of interferences (Naczki and Shahidi, 2004).

The solvent is one of the parameters that can affect the extraction of polyphenols (Trosznska *et al.*, 2002). The extraction may be carried out by several solvents as water, methanol, ethanol and acetone. Otherwise, the aqueous solvents give the best yields of extraction than the absolute solvents (Spigno *et al.*, 2007).

The reducing power and DPPH free radical scavenging ability of the extracts from the leaves of *Pistacia lentiscus* in solvents, such as ethanol, ethyl acetate, aqueous/ethyl acetate, hexane, aqueous/hexane, chloroform and aqueous chloroform has been studied *in vitro* (Atmani *et al.*, 2009). The method of the DPPH is widely used to evaluate the antioxidant activity of plant extracts (Hseu *et al.*, 2008). In the case of DPPH scavenging ability assay, it was found that all of the *Pistacia lentiscus* extracts, except for the chloroform extract, have a high radical scavenging ability equivalent to that of the standard, BHA. The ethanolic and aqueous fractions from the ethyl acetate extract have high scavenging activities with values of 78 and 90.29%, respectively. Overall, *Pistacia lentiscus* exhibited outstanding reducing power, good radical scavenging activity against DPPH and H₂O₂, slow inhibition of lipid peroxidation and richness in tannins; however, it also showed a lack of flavonoids (Atmani *et al.*, 2009).

Regarding the antiradical activity, several authors previously confirmed that gallic acid and α -tocopherol are powerful antioxidants, particularly with high antiradical activity (Gulcin *et al.*, 2004). According to the present work (Fig. 1), the hydro-methanolic extract of *Pistacia lentiscus* leaves showed a powerful antiradical activity, since it showed a high scavenging ability which was comparable to that of α -tocopherol. That activity might be due to the presence of flavonols glycosides (such as glucosides of quercetin and myricetin), gallic acid, gallic and quinic acids derivatives as previously discussed by Longo *et al.* (2007).

The H₂O₂ reducing power of *Pistacia lentiscus* leaves extract was moderate as shown in Fig. 2 (37.04%). Such reduction activity has been demonstrated before by Atmani *et al.* (2009), suggesting that, this activity is probably due to the presence of polyphenols having antioxidant properties (Vermerris and Nicholson, 2008).

The current work showed that the phenolic contents of *Pistacia lentiscus* leaves are rich in tannins and poor in flavonoids. This finding is in agreement with the work of Gardeli *et al.* (2008) which demonstrated that the greatest phenolic content in *Pistacia lentiscus* is 588 mg gallic acid/g of plant material. Those results illustrated that a strong correlation exists between the total phenolic content and the antioxidant properties of the investigated plant extracts, indicating that the phenol compounds play an important role in the beneficial effects of these medicinal plants. Several other authors noted that, the

antiradical activity is proportional to the concentration of the plant extract (Gulcin *et al.*, 2003; Kumaran and Karunakaran, 2007) which in agreement with our results (Fig. 1, 2).

However, further investigation is needed because the Folin-Ciocalteu assay does not differentiate between different phenolic compounds. Substances, such as sugars, aromatic amines, ascorbic acid, sulfur dioxide, iron and other compounds can interfere with the Folin-Ciocalteu assay and correction for interfering substances should be made to measure accurately the phenolic content of the samples (Teow, 2005). Inorganic substances may also interact with Folin-Ciocalteu reagent, giving an inaccurate result (Prior *et al.*, 2005).

The structural features of phenolic compounds are also another parameter that should be considered when the Folin-Ciocalteu assay is applied (Frankel *et al.*, 1995). The molar response of the Folin-Ciocalteu method is roughly proportional to the number of these structural differences are reported to be responsible for antioxidant activity, measurements of phenols in *P. lentiscus* L. extract may be related to their antioxidant properties.

CONCLUSION

Currently, the research of natural antioxidants like alternative sources of synthesis antioxidants was emerged and the exploitation of the various secondary metabolites of the plant was highlighted in recent years. Thus, the phenolic compounds in particular the flavonoids have drawn attention as a potential source of bioactive molecules.

Pistacia lentiscus is widely used in Algerian traditional medicine to treat a wide range of diseases. According to the results, it may be concluded that the extract of the phenolic compounds of *P. lentiscus* revealed the potential antioxidant capacity of hydro-methanolic leaves extract. The results indicated that *Pistacia lentiscus* leaves extract has a considerable antioxidant activity against DPPH and H₂O₂. The results of this study further support the view that *Pistacia lentiscus* is promising source of natural antioxidants. This plant showed potent antioxidant properties and contained significant amounts of phenolic compounds, as estimated by Folin-Ciocalteu method. These findings confirmed the potential uses of *Pistacia lentiscus* in traditional 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.

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