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ANTIOXIDANT ACTIVITY OF *PISTACIA LENTISCUS* METHANOLIC EXTRACTS

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Abstract

Description of the subject: The use of synthetic antioxidant molecules is currently being a health issue because of their potential toxicological risks. Now, several researches on plants as new sources of natural antioxidants are being developed.

Objective: For this purpose we were interested to study the antioxidant activity of methanolic extracts of a plant from the Algerian flora: *Pistacia lentiscus*.

Methods: Methanolic extracts were prepared from both parts of the plant (leaves and/ or fruits). The quantitative estimation of the total phenols, flavonoids and tannins content present in the extracts was carried out by colorimetric methods. The antioxidant proprieties of the extracts were performed using two methods: DPPH (2, 2-diphenyl-1-picrylhydrazil) free radical scavenging activity and the ferric reducing power (FRAP).

Results: Colorimetric assays showed that the methanolic extracts of leaves and /or fruits contain high amounts of total phenols, tannins and less amounts of flavonoids. The antioxidant test revealed the presence of a strong reducing activity of both methanolic extracts, with a high percentage of reduction which were superior than 73% with an EC 50 (0.121mg / ml ± 0.001) for leaves and EC 50 (E: Extract, C: Concentration) (0,26 mg/ ml ± 0.0002) for fruits in the free radical DPPH test. On the other hand, the Ferric Reducing antioxidant power assay FRAP also showed a high reduction power proportionally to the concentration of the extract.

Conclusion: The study of the antioxidant activity of the methanolic extracts of *Pistacia lentiscus* has shown that they have a significant reducing activity. These extracts could therefore be a source of natural antioxidant molecules as an alternative to the use of synthetic antioxidants. It is very interesting to carry out further research to identify, isolate and purify these biomolecules.

Keywords: *Pistacia lentiscus* L. ; méthanolic extracts ; antioxydant activity ; polyphenols.

Activité antioxydante des extraits méthanoliques de *Pistacia lentiscus* L.

Résumé

Description du sujet : L'utilisation des molécules antioxydantes de synthèse est actuellement remise en cause en raison des risques toxicologiques potentiels. Désormais, de nouvelles sources végétales d'antioxydants naturels sont recherchées.

Objectives : Pour cela nous nous somme intéressé à l'étude de l'activité antioxydante des extraits méthanolique d'une plante de la flore Algérienne *Pistacia lentiscus*

Méthodes : Les extraits méthanoliques ont été préparés à partir des deux parties de la plante (feuilles et /ou fruits). L'estimation quantitative des phénols totaux, flavonoïdes et tannins présent dans les extraits a été effectué par des méthodes colorimétriques. L'évaluation du pouvoir antioxydant a été réalisée en utilisant deux méthodes : piégeage du radical libre DPPH (2,2-diphenyl-l-picrylhydrazil) et la méthode de réduction du fer FRAP.

Résultats : Les dosages colorimétrique on révélé que les extraits méthanolique des feuilles et ou fruits sont riches en phénols totaux et tannins et pauvre en flavonoïdes. Le test antioxydant a révélé la présence d'une forte activité réductrice de ces extraits, ces derniers possèdent un pourcentage de réduction du radical libre DPPH supérieur à 73% avec une (0.121 mg / ml ± 0.001) pour les feuilles et (0,26 mg / ml ± 0.0002) pour les fruits. D'autre part, le test FRAP a aussi montré une forte réduction du fer en fonction de la concentration de l'extrait.

Conclusion : L'étude de l'activité antioxydante des extraits méthanolique de *P. lentiscus* a montré que ces derniers possèdent une activité réductrice importante. Ces extraits pourraient donc être une source de molécules antioxydante naturelle comme alternative de l'utilisation des antioxydants synthétiques. Il est donc très intéressant de faire des recherches complémentaires pour identifier, isoler et purifier ces biomolécules.

Mots clés: *Pistacia lentiscus* L.; extraits méthanoliques; activité antioxydante; polyphénols.

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INTRODUCTION

Plants constitute an important source of active natural products which differ widely in terms of structure and biological properties. They have a remarkable role in the traditional medicine in different countries [1, 2].Many plant extracts have demonstrated potent cancer chemopreventive properties as observed in the last decade [3, 4]. Most of these extracts are known to exert their effects via antioxidant mechanisms either by quenching reactive oxygen species (ROS), inhibiting lipid peroxidation or by stimulating cellular antioxidant defenses [5, 6]**.**Synthetic antioxidants have been used in the food industry since the 1940s, but trends in many health-related industries tend to shift preferences to natural sources. Therefore, investigation of natural antioxidants has been a major research interest for the past two decades as many research groups and institutions have been screening plant materials for possible antioxidant properties [7].The protective effects of plants can be due to the presence of flavonoids [8], anthocyanins and other phenolic compounds [9].

Pistacia lentiscus L., commonly called lentisk pistachio, is an evergreen Mediterranean shrub belonging to the Anacardiaceae family [10]. It is a traditional medicinal plant of the Mediterranean area, commonly used by rural populations in Algeria. It grows wild in Algeria, also in Turkey, Morocco, France, Spain, Italy, and Greece [10] (In the folk medicine, the entire shrub is used, and some of its benefits were described as early as the 15th–16th centuries [10].The aerial part of *P. lentiscus* has traditionally been used in the treatment of hypertension and possesses stimulant and diuretic properties [11]**.** Some researchers reported the chemical composition of the essential oil from leaves of *P. lentiscus* of diverse geographic origins [12]. Indeed,this plant is known as a very rich source in secondary metabolites [13,14]. A recent study realized by Rodriguez-Perez *et al.* [15] allowed the identification of different compounds in *P. lentiscus* leaves where flavonoids, phenolic acids and their derivatives were the most abundant compounds. Therefore, the aim of this work is to study the antioxidant capacity of methanolic extracts from this plant using two tests: DPPH (2, 2- diphenyl-1 picrylhydrazyl) free radicalscavenging test and ferric reducing antioxidantpower assay (FRAP).

MATERIAL AND METHODS

1. Plant material

P. lentiscus leaves and fruits were collected from the chréa forest, at 600m in altitude (Blida, Algeria), in April 2013and identified in the Botanic laboratory of the National Superior Agronomic School (ENSA), El Harrach (Algeria). The harvested material was air-dried at room temperature (20–25°C) for 2 weeks and stored in cloth bags.

2. Preparation of themethanolic extracts

Methanolic extracts were obtained from the dried leaves and/or fruits which were initially defatted with n-hexane using Soxhlet apparatus and extracted exhaustively (6-hours extraction) with methanol. The solvent was removed from the sample using a rotary vacuum evaporator at 48◦C [16]. On complete evaporation of these solvents, dry extracts were stored and conserved at 4◦C in sterile glass jars and, sealed for use in further studies. The percentage yield was obtained using this formula:

$$(\%)\text{yield} = \frac{W2-W1}{W0} X100$$

W_2: weight of the extract and the container,
W_1: the weight of the container alone,
W_0: the weight of the initial dried sample.

All the expirments were carried out in the phisyco-chemical laboratory at SAIDAL Antibiocal grouop

3. Phytochemicals screening

3.1. Determination of total phenolic concentration

The total phenolic contents in methanolic extracts was determined by colorimetric method using ‘‘Folin-Ciocalteu’’ reagent assay [17]**.** A volume of 200 ml of the extract 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 [17]. After stirring and 30 min later, the absorbance was measured at 760 nm using a UV-Visible spectrophotometer (Perkin-Elmer Lambda 25). Gallic acid (GA) was used as a standard for the calibration curve. The total phenolic content was expressed as milligrams of gallic acid equivalent per gram of dry extract (mg GA. Eq/g extract). All the tests were carried out in triplicate.

3.2 .Determination of total flavonoid concentration

The total flavonoid content in the extracts was determined spec-trophotometrically using an aluminum chloride method involving the formation of a flavonoid–aluminum complex at 420 nm [18]**.** The concentration of the total flavonoid content was calculated by comparison with the absorbance of different concentrations of quercetin (QE), and the result was expressed as milligrams of QE equivalents per gram of dry extract (mg QE. Eq /g extract). Samples were prepared in triplicate for each analysis, and the mean value of absorbance was obtained.

3.3. Determination of total condensed tannins

The amounts of condensed tannins were estimated using the method of vanillin [19]. 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 [19]. Absorbance at 550 nm was measured against a blank. The concentration of tannins was estimated as milligrams catechin (CAT) equivalents per gram of dry extract from the calibration curve (mg CAT. Eq /g extract). All the tests were carried out in triplicate.

4. Antioxidant activity

The antioxidant activity was carried out using two different test systems for the extracts DPPH scavenging activity and the ferric reducing antioxydant power (FRAP).

4.1. DPPH free radical scavenging activity

The hydrogen atom donation ability of chemical compounds in MEL and MEF was measured on the basis to scavenge the 2, 2-diphenyl-1-picrylhydrazil free radical [20]. 50µl of various concentrations of the extracts in methanol was added to 1950 ml of a0.025 g/l methanol solution DPPH. 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 [20]:

$$\text{DPPHscavengingactivity (\%)} = (\text{Ablanc} \_ \text{Asample}) / \text{Ablancx } 100$$

Where A_{blanc} 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 (EC50) was calculated from the graph plotted of inhibition percentage against extract concentrations. Butylated hydroxyl toluene (BHT) methanol solution was used as positive control.

4.2. Measurement of ferric reducing antioxidant activity (FRAP)

The reduction power [21]was determined using various concentrations of methanolic extracts of *P.lentiscus* leaves and/or fruits in distilled water and mixed with a buffer phosphate solution (2.5 mL, 0.2 M; pH 6.6) and potassium ferricyanide (2.5 ml, 1% aqueous K3Fe(CN)6).

The resulting solutions were incubated at 50°C for 20 min. Afterwards, trichloracetic acid (2.5 mL, 10% in water) was added and centrifuged (3000 rpm) for 10 min. The supernatant phase (2.5 mL) was diluted with distilled water (2.5 ml) and FeCl3 (0.5 ml, 0.1% in water) was added [21]. The absorbance of the resulting solution was measured at 700 nm, using ascorbic acid (AA) as a positive control.

5. Statistical analysis

All the experiments were carried out three times using triplicate samples. The data were presented as the (mean ± standard error) and were processed by one-way analysis of variance (ANOVA). Multiple comparisons of means were carried out using tukey's and dunnett's tests. Differences between groups with P<0.05 were considered statistically significant [18]. Statistical analysis was performed by probit analysis method using XLStats 2016 software (Pros statistical software, Addinsoft, Paris, France).

RESULTS

1. Determination of total phenolics, flavonoids and tannins

The extraction yield of methanolic extracts was more important ($p< 0.001$) in *P.lentiscus* leaves compared to the fruits (36.03 %± 0.295 and 13.82 %± 0.061 respectively). In order to establish a relationship between the antioxidant activity of *P lentiscus* and phenolic compounds present in the methanolic extracts, the amounts of Total phenolics, flavonoids and condensed tannins of *P. lentiscus* leaves and fruits were determined. the results summarized in (Table 1) show that *P. lentiscus* leaves and fruits are rich in phenols and tannins and poor in flavonoids, however a significant difference ($p <0.05$) was observed in Total phenolic content in leaves (323.5± 0.28 mg GA. Eq./ g Extract) compared to those revealed in fruits (318.99± 1.02 mg GA. Eq/ g Extract), which showed less important contents. For the others polyphenolic classes; flavonoids and tannins, the highest concentrations of flavonoids and condensed tannins were also recorded in leaves (Flavonoids: 46.78±0.01 mg Q. Eq /g extract, Tannins: 359.83±2.12 mg Cat. Eq /g extract) with a hight significant difference ($p< 0.001$) compared to those in fruit (Flavonoids: 30.13± 0.04 mg Q. Eq /g extract, Tannins: 294.83± 1.02 mg Cat. Eq /gextract).

Table 1:Total phenolics, flavonoids, and condensed tannins contents in methanolic extracts from *P. lentiscus* leaves and fruits.

Extract	Extraction yield (%)	Total phenolics (mg GA.eq/ g extract)[a]	Flavonoïds (mg Q. Eq / g extract)[b]	Tannins (mg Cat. Eq/ g extract)[c]
MEL	36.03± 0.295***	323.5± 0,28*	46.78± 0.01***	359.83± 2.12***
MEF	13.82±0.061***	318.99± 1.02*	30.13± 0,04***	294± 1,02***

MEL: Methanolic extract of *P.lentiscus* leaves, MEF: Methanolic extract of *P. lentiscus* fruits
[a]: milligrams of gallic acid equivalents per gram of dry extract; [b]: milligrams of quercetine equivalents per gram of dry extract; [c]: milligrams of catechin equivalents per gram of dry extract. Values represent the (mean ± SE) of three separate experiments using triplicate samples. For each extract concentration: values with * are significant at ($p < 0.05$), values with ***are Significant at ($p < 0.001$).

2. Antioxydant activity

Several concentrations of the methanolic extract of *P. lentiscus* leaves and fruits were tested for antioxidant activity in two different *in-vitro* methods:

2.1. DPPH assay

Free radical scavenging activity of methanolic extracts of *P.lentiscus* leaves and fruits and the standard (BHT) are expressed in inhibition percentage (Fig. 1).

The obtained results show that *P. lentiscus* leaves and fruits have a strong antiradical activity to scavenge DPPH radical;
MEL has the highest scavenging activity (92.61 %± 0.15) at 0.4 mg/ ml followed by MEF (73.97± 0.06). The EC 50 concentrations were (0.121mg/ ml± 0.001) for the leaves and (0.261mg/ ml± 0.0002) for the fruits; the antioxidant capacity of all extracts was significantly (p< 0.001) lower than that of the control (BHT) (Table 2).

2.2. Ferric reducing antioxydant power (FRAP)

The results of the reducing power of *P.lentiscus* methanolic extracts are illustrated in (Fig. 2). FRAP assay also showed that *P.lentiscus* pocessess an important antioxydant activity but less than the controle (AA) with a significant diffrence (p< 0.001). The antioxydant capacity of *P lentiscus* methanolic extracts (MEL and MEF) was proportional to the concentration, the highest optical density was observed at 0.8 ml/mg for MEL, MEF and AA (1,818, 2,122, and 2, 45 respectively) (Fig.2) which means a high reducing capacity of the extracts.

Table 2: EC 50 of DPPH free radical scavenging activity and ferric reducing anioxydant power of *P. lentiscus* methanolic extracts.

Extract/ control	DPPH assay	FRAP assay
	EC 50 (mg/ml)	EC 50 (mg/ml)
MEL	0.121±0.001 ***	0.207 ±0.0002 ***
MEF	0.261± 0.0002 ***	0.163 ±00003 ***
BHT	0.078 ±0.0002	/
AA	/	0.036 ±0.001

MEL: Methanolic extract of *P.lentiscus* leaves, MEF: Methanolic extract of *P. lentiscus* fruits,
BHT: Butylated hydroxyl toluene, AA: Ascorbic acid..
Values represent the (mean ± SE) of three separate experiments using triplicate samples.
For each extract concentration: values with values with ***are Significant at ($p < 0.001$).

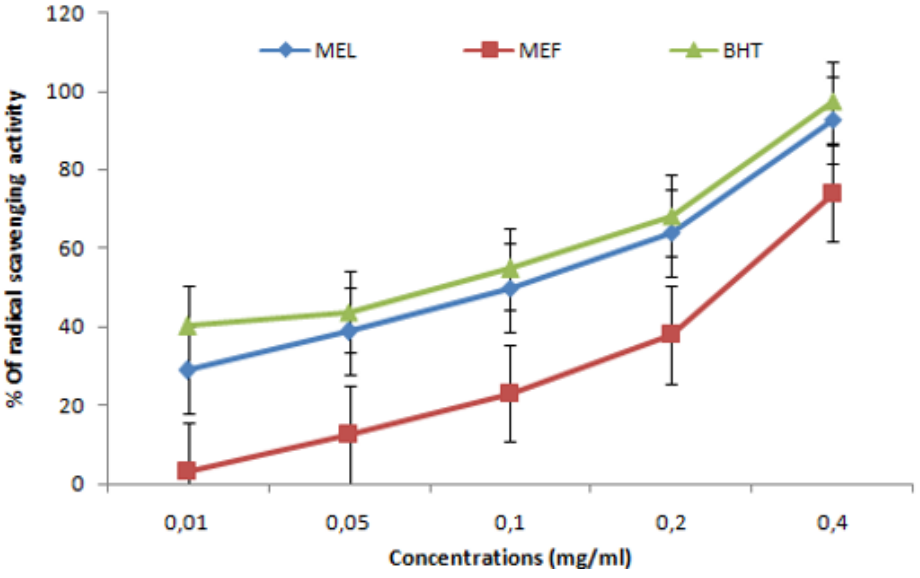

Figure 1: Effect of BHT (control) and *P. lentiscus* methanolic extractson DPPH radical inhibition *in-vitro* expressed as (means ± SE).

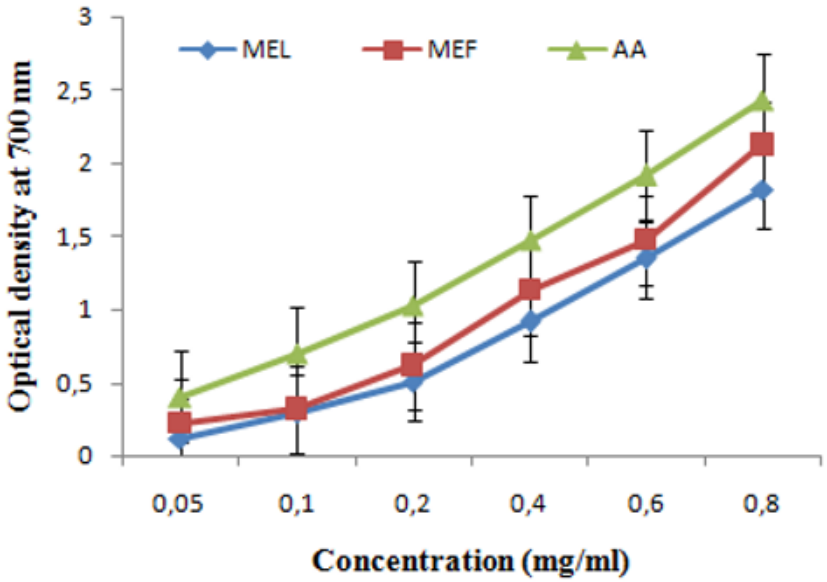

Figure 2: Ferric reducing power of control (AA) and methanolic extractsof *P lentiscus* expressed as (means ± SE)

DISCUSSION

Our results show that both parts of the plant (leaves and fruits) contain high concentrations of phenolic compounds. These data are correlatedto the data cited in the literature, a recent study by Zitouni *et al*..[22] showed a high level of phenolic compounds in leaves (216.289± 20.62 mg GAE/ g DM) and fruits (103.342± 2.317mg/ g). Cherbal *et al*. [23] and Atmani *et al*. [24] indicated that *P. lentiscus* leaves are poor in flavonoids (38.7± 0.02 mg QE Eq/ g extract and 12.93± 1.69 mg QE eq/ g extract, respectively), and rich on tannin (175.3± 1.07 mg AT Eq/ g extract and 909.4± 42.61 mg AT Eq/g extract respectively);similar results were reported by Djidal *et al*. [25], where the highest amount of phenolics (390± 0.05 mg GA Eq/g dry crud extract) were recorded in the methanolic extract of *P. lentiscus.* This was attributed to the fact that most of these compounds are soluble in the hydro-methanolic solution including hydrophilicand hydrophobic molecules (Phenolic acids, flavonoids, high molecular weight phenolics).

Oxidative stress has been implicated in the pathology of many diseases and conditions including diabetes, cardiovascular diseases, inflammatory conditions, cancer and ageing. Antioxidants may offer resistance against the oxidative stress by scavenging free radicals, inhibiting lipid peroxidation, and by many other mechanisms and thus preventing disease [26].

The DPPH radical scavenging assay measures the reduction of DPPH by antioxidants, which is recorded as a change in color [27] and a decrease in absorbance of DPPH with increase in extract concentration. According to the present study the methanolic extracts of *P. lentiscus* leaves and fruits showed high free radical scavenging activity (EC 50= 0.121 mg/ ml± 0.001 and 0,26mg/ ml± 0.0002 respectively) these data are similar to those reported by Zitouni *et al.* [22] (EC 50= 0.16mg/ lm for the leaves and EC 50= 0.77mg/ ml for the fruits. In the other hand the study of Atmani *et al*.[24] reported that the aqueous extracts issued from chloroform and hexane partitions exhibited the best reducing power also showed remarquable DPPH scavenging activity (EC 50 = 4.24ug/ ml and 4.51 ug/ ml, respectively), significantly lower than that of BHA (6.18ug/ ml);according to these results we can set up a relationship between the high scavenging activity of *P.lentiscus* extract and the high amount of phenolic compounds in the aerial parts of this plant.

Ferric reducing antioxidant power (FRAP) measures the ability of antioxidants to reduce ferric 2, 4, 6-triperidyl-s-triazine complex to intensively blue colored ferrous complex in acidic medium [28]. Atmani *et al*. [24] noticed that the best reducing power is obtained from the aqueous fractions issued from hexane chloroform of *Pistacia lentiscus*, significantly higher than that of the standards, BHA and a-tocopherol.

A recent study by Goncalves *et al*. [29]carried on ten Mediterranean medicinal plant species found out that the most potent infusions were those from *P.lentiscus* (at the lowest test concentration of 0.4 mg/ ml), which confirms our results. As reported by Alexieva *et al*. [30], the antioxidant activity of plants depends on the type, quality, part (leaves, flower, seeds) of the plant, location of habitat, climatic conditions, soil characteristics, etc. Extraction method and solvent agent (water, alcohol, etc.) are also important factors [30], considerably affecting plant antioxidants capacity.

CONCLUSION

According to the present results the methanolic extracts of *P. lentiscus* leaves and/or fruits have a considerable scavenging activity against the free radical DPPH and a high ferric reducing power; this could be related to the high content of this plant on secondary metabolites mainly phenols and tannins. These results support the large use of *P. lentiscus* in traditional medicine in Algeria. For further studies it is interesting to identify the chemical composition of the extracts and explore more biological activities of the plant.

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