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Phenolic contents and antiradical capacity of vegetable oil from *Pistacia lentiscus* (L)

A. Bouyahya[1,2*], N. Dakka[2], A. Talbaoui[1], N. El Moussaoui[2], J. Abrini[2], Y. Bakri[1]

[1] *Biochemistry-Immunology Laboratory, Department of Biology, Faculty of Sciences, Mohammed V University, Rabat, Morocco*
[2] *Biology and health Laboratory, Department of Biology, Faculty of Science, Abdelmalek Essaadi University, Tetouan, Morocco*

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boyahyaa-90@hotmail.fr
Phone: +212601350878

Abstract

Oxidative stress has been shown to be involved in the development of different pathologies. Antioxidant can be used for preventing or delaying oxidation. Natural products are rich in molecules capable of such activity. In the present work, the antiradical capacity of vegetable oil of *Pistacia lentiscus* L. (VOPL) was compared to the activity obtained with synthetic antioxidants. We first determined total phenolic content in VOPL by the Folin-Ciocalteau's method, total flavonoid content was assessed by a colorimetric method using quercetin as standard flavonoid, whereas antioxidant activity of the extracts was determined spectrophotometrically using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and evaluated following the kinetics of the DPPH reduction. The total phenol content was 122.72±1.16 mg GAE/g of extract and the flavonoid content was 18.41±0.43 mg QE/g. The saponification and acid index of PLVO are also determined in the selected vegetable oil using potassium hydroxide (KOH). VOPL had higher antioxidant activity (IC_{50}=37.38 µg/ml) than ascorbic acid (IC_{50}=27.20 µg/ml). The parameter IC_{50} and its time equivalent T_{IC50} have an influence on the antiradical capacity of vegetable oil. The antiradical efficiency (AE) of Pistacia vegetable oil and ascorbic acid was respectively 7.23 and 20.40 mg/ml.mn. This parameter was reported to be a powerful parameter to select antioxidants compared to IC_{50}. Acid and saponification index were respectively 5.5 and 140 giving an acidity value of 4% for VOPL. This study has revealed that *P. lentiscus* is a considerable source of natural antioxidants.

1. Introduction

Medicinal plants take an important place for health human system. They have used for fight against different illnesses in traditional knowledge and numerous of them have proved *in vitro* and *in vivo* biological effects [1-5]. In morocco, medicinal plant products have been investigated for several biological properties such as antibacterial [6,7, 8, 9], antioxidant [5,10], antitumor [5,11] and antiparasitic [12].

Pistacia lentiscus L. (Family- Anacardiaceae) is widely distributed in Mediterranean Europe, Morocco, Iberian Peninsula, in the west through southern France, Turkey, Iraq and Iran. The resin part of this plant known as mastic resin and plant called as mastic tree [13]. It has been used in traditional medicine for treatment of several diseases [14,15]. Its various parts contain a variety of chemical constituents which are medicinally important such as resin, essential oil, gallic acid, anthocyanins and flavonol glycosides, nortriterpenoids, α-tocopherol and arabinogalactan proteins [16]. It has been reported that this plant has antidiabetic [17], antimicrobial [18], antioxidant [19,20], lipid lowering effect [21], antiarthritic and antigout [22,23], antifungal [24], hepatoprotective [25] and anticancer [26,27].

Oxidation is the transfer of electrons from one atom to another and represents an essential part of both aerobic life and our metabolism, since oxygen is the ultimate electron acceptor in the electron flow system that produces energy in the form of ATP. However, problems may arise when the electron flow becomes uncoupled (*i.e.* transfer of unpaired single electrons), generating then free radicals [28]. Antioxidants are important in living organisms as well as in food because they may delay or stop formation of free radical by giving hydrogen atoms or scavenging them. Oxidative stress is involved in the pathology of cancer, atherosclerosis, malaria and rheumatoid arthritis. An antioxidant can be defined as any molecule capable of preventing or delaying oxidation of biological substrates such as lipids, proteins or nucleic acids [29]. As far as we know, no available data on

VOPL antioxidant activity have been reported. In this study we evaluated this antioxidant activity and compared it to Ascorbic acid.

2. Material and Methods

2.1 Vegetable oil of Pistacia lentiscus

The VOPL tested in the present study was kindly provided from local producers in the Zarka area (North Morocco: 34°47'50"N and 5°34'56"W). It was extracted from the berry of *P. lentiscus* by pressing extraction and stored at 4°C before use. Breiftly, vegetable oils are mechanically pressed from the botanical material at high pressure to obtain maximum yield. The separation of the oils was carried out using the difference between the density and water.

2.2. Determination of Total Phenolic Content (TPC)

The concentration of the phenolic compounds in the plant extracts was determined using the Folin Ciocalteu assay [30], with some modifications. In brief, the extract was diluted to the concentration of 1mg /ml, and aliquots of 100 µl or a standard solution of gallic acid (20, 40, 60, 80 and 100 mg/l) were mixed with 500 µl of Folin Ciocalteu reagent (previously diluted 10-fold with distilled water) and 400 µl of Na_2CO_3 (7%). After 40 min of incubation at room temperature (23 ± 2°C), the absorbance was measured at 760 nm using a Spectrophotometer (J.P. Selecta, sa 4120007, Barcelona, Spain) against a blank sample [10]. The total phenolic content was calculated using a calibration curve for gallic acid (R^2= 0.998) (**Figure 1**). The results were expressed as the gallic acid equivalent per gram of VOPL (mg GAE/g of VOPL). Alls samples were analyzed in triplicate.

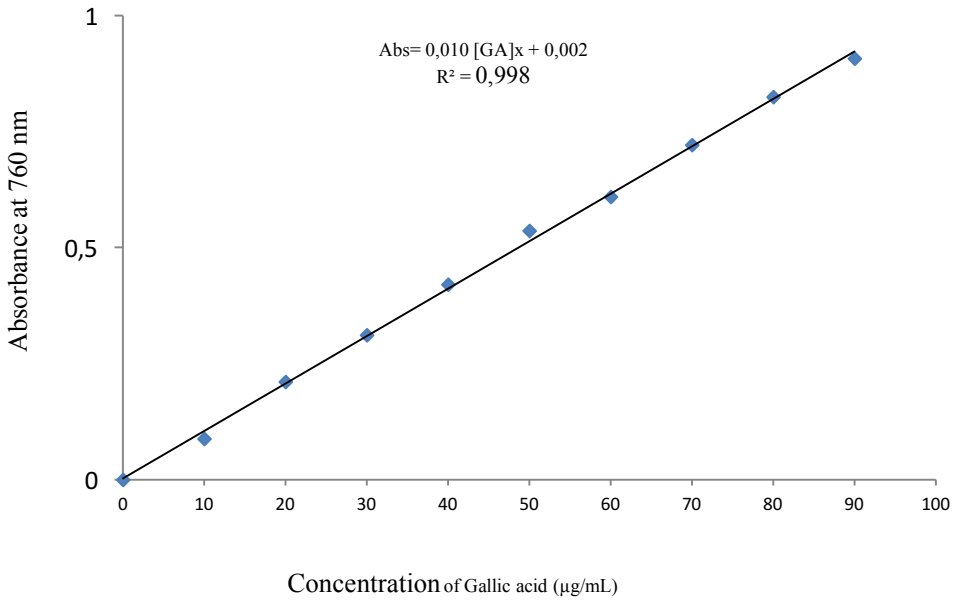

Figure 1: Calibration curve of Gallic acid. Concentrations used are: 0, 20, 40, 60, 80 and 100 µg/mL and the absorbance measured at 760 nm. The exponential equation was obtained by modeling the absorbance values at 760 nm versus the concentration of gallic acid.

2.3. Determination of Total Flavonoid Content (TFC)

The total flavonoid content of the extracts was determined using the aluminum chloride ($AlCl_3$) colorimetric method described by [31]. Briefly, 1 ml of the extract (1 mg/ ml in methanol) or a standard solution of quercetin (20, 40, 60, 80 and 100 mg/l) were mixed with 1 ml $AlCl_3$ (2% w/v) in methanol. After 40 min of staying at room temperature (23 ± 2 °C), the absorbance against blank was measured at 430 nm using a Spectrophotometer (J.P. Selecta, sa 4120007, Barcelona, Spain) [32]. The total flavonoid content was calculated using a calibration curve for quercetin (R^2= 0.985) (**Figure 2**). The results were expressed as the quercetin equivalent per gram of VOPL (mg of QE/g of VOPL). All samples were analyzed in triplicate.

2.4. Determination of acid index of PLVO

The acid number is the number of mg of potassium hydroxide (KOH) required to neutralize the free fatty acids (FFA) contained in the g fat. It is expressed in mg / g. A test sample solution is put in a solvent mixture (ethanol / diethyl ether) and then as the fatty acids present with a hydroxyl potassium solution in the presence of phenolphthalein as a color indicator. The principle for determining the acidity of oil consisting of an acid-base assay corresponding to the neutralization of the free fatty acids according to the following reaction.

R - COOH + KOH----R-COOK+H20
Fatty acid + potash ---------- Salt + Water
The index of acidity is calculated by the following formula

$$AI = \frac{(VW - VT) \times CHCL \times MKOH}{P}$$

Where :
IS : Acid index
VT : Volume paid to witness
VE : Volume testing
CHCL : Concentration hydrochloric acid solution (mol/l)
MKOH : Molar mass du KOH (g/mol)
P : Past of weighed oil (g)

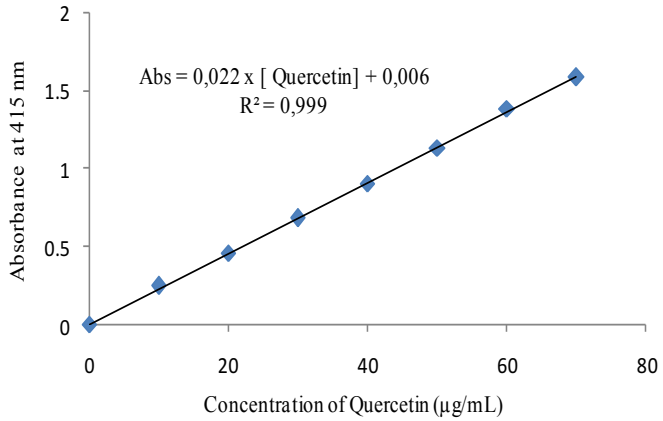

Figure 2: Calibration curve of Quercetin. *Concentrations used are: 0, 20, 40, 60 and 80 µg/mL and the absorbance measured at 760 nm. The exponential equation was obtained by modeling the absorbance values at 760 nm versus the concentration of gallic acid.*

2.5. Determination of saponification index
The saponification value is the mass of potassium hydroxide (KOH) (mg) necessary to saponifie the fatty acid esterified and neutralizes non-esterified fatty acids in one gram of fat/fatty substance. The amount of potassium hydroxide KOH used varies with the molecular weight of fatty acids. The higher the molecular weight, the higher the saponification number is low saponification value is an indirect measurement of molecular weight fatty acids.

$$IS = \frac{(VW - VT) \times CHCL \times MKOH}{P}$$

IS : Index of saponification
VT : Volume paid to witness
VE : Volume testing
C_{HCL}: concentration hydrochloric acid solution (mol/l)
M_{KOH} : Molar mass du KOH (g/mol)
P: Past of weighed oil (g)

2.6. Antioxidant activity
2.6.1. Calibration curve of the DPPH
The antiradicular activity of VOPL is measured by using the test 2,2-diphenyl-1- picrylhydrazyl (DPPH). It is reduced to the form of hydrazine (no radicular) by accepting a hydrogen atom. Briefly, we first established the calibration curve of the DPPH solution before starting the antioxidant activity assays [32]. The stability and linearity interval of solutions of DPPH were evaluated. The concentration of DPPH radical (g/l) in the reaction medium was calculated starting from a calibration curve using six of DPPH radical concentrations [0, 0.01, 0.02, 0.03, 0.04, 0.05 g/l] by a linear regression. A_{517} nm = a × [DPPH] + b, where $[DPPH]_t$ was expressed as g/l. It is observed that there is no significant difference in the absorbance between 0 and 0.05 g/l for the concentrations tested and a very good linearity of the absorbance in terms of the concentration.

The equation obtained from a calibration curve is Absorbance = 0.01 × [DPPH] + 0.002, with $R^2 = 0.998$.

Determination of inhibition percentage

The effect of VOPL on the DPPH is measured by the procedure described by [33]. In brief, Aliquots (0.2 ml) of various concentrations (30-480 µg/ml) of the plant extracts samples were added to 1.8 ml of a 0.004% methanolic solution of DPPH prepared daily. The inhibition percentage of DPPH by the oil was calculated using the formula:

$$\% \text{ Inhibition} = [(A_{blanc} - A_{sample})/A_{blanc}] \times 100$$

Where Abs (blank) is the absorbance of the control with $_{t=0}$ and $Abs\ (Sample)_t$ represents that in the presence of antioxidants at a time t, which varies according to the concentrations. Ascorbic acid was used as positive control.

The antioxidant capacity (IC_{50}) is defined as the concentration providing 50% inhibition. It was calculated from the graph of inhibition percentage plotted against the extract concentration.

Determination of the Equilibrium Time T_{IC50}

The parameter T_{IC50} is defined as the time necessary to reach the steady state at a concentration corresponding to IC_{50}. It was calculated from the graph of times at steady state conditions versus the concentrations for each extract.

Determination of Antiradicular Efficiency (EA)

The two factors IC_{50} and T_{IC50} can be combined in order to obtain the efficiency antiradicular parameter. It was calculated using the flowing formula**:**

$$AE = 1 / [\ IC_{50} \times T_{IC50}]$$

3. Results and discussion

3.1. Total phenol and flavonoids content

The estimation of total phenolic content (TPC) and total flavonoids content of VOPL is presented in **table 1**. The amount of the total phenolic content and total flavonoid content is 22.61 ± 1.42 gallic acid equivalents (GAE mg/g) and 13.76 ± 0.58 quercetin equivalents (QE mg/g) of VOPL, respectively. Three major classes of the secondary metabolites were described for *P. lentiscus*: gallic acid and its derivatives with glucose and quinic acid; flavonol glycosides (myricetin and quercetin glycolsides), and anthocyanins (delphinidin 3-O-glycoside and cyaniding 3-O-glucoside) [34]. The abundance of the polyphenols and flavonoids was also noted in the aerial parts of *P. lentiscus* [35].

Table 1: Total phenolic content (TPC) and total flavonoid content (TFC) of VOPL

TPC (mg GAE[a]/g) of extract	TFC (mg QE[b]/g) of extract
22.61 ± 1.42	13.76 ± 0.58

TPC and TFC values are mean ± standard deviation of three separate experiments.

[a] gallic acid equivalents

[b] Quercetin equivalents

3.2. Acid and saponification index

Results of acidity and saponification of PLVO are shown in **table 3.** The values of acid index and saponification index are respectively 5.6 and 140.we note that the acidity of our oil (4%) is high and exceeds the limits established standards, which are between 1 and 3.3%. This highest value of acidity is caused from the acidity index (5.6mg / g). It's may be due to the late harvest fruit and storage for a long time, the damage will be more pronounced as the storage will be long and made in bad conditions. This causes heating of seeds and triggers the fermentation process increasing acidity rate (4%). The free fatty acids resulting from the action of lipases on triglycerides, or other hydrolytic activities [36]. However, a high level of acidity can also be attributed to the advanced state of maturity of fruit. Indeed, Dugo et al., have shown that early harvesting fruit product oils whose acidity is less than 1% [37]. A good quality oil should present no or low acidity, cannot be allowed as it exceeds 2g% in oleic acid, corresponding to an acid value of about 4 mg of potassium hydroxide / g.

3.3. Antiradical activity

3.3.1. The Kinetics Study of the Antiradicular Reaction

The antioxidant activity of the VOPL against DPPH radicular was evaluated spectrophotometrically by following its reduction which is accompanied by its passage from the purple color to the yellow color measured at 517 nm. **Figure 3** shows the kinetics of DPPH reduction in terms of time for each concentration of ascorbic acid and VOPL.

Table 2: Acid and saponification index of PLVO

Acid index (mg/g)	Saponification index (mg/g)	Acidity (%)
5.6	140	4

Recalling that the extracts are added to the DPPH radical in methanol and the absorbance at 517 nm (absorption maximum of the DPPH) is raised to different time t (min). The hydrogen transfer reaction of the antioxidant to the DPPH is monitored by visible spectroscopy by recording the decrease of the absorption band of the DPPH to 517 nm. The results showed in **figure 3** represent two phases of the reaction of *Pistacia* oil and DPPH, a rapid decrease in absorbance value is explained by the antiradicular reaction of vegetable oil. This one is drawn after the first 20 seconds for the ascorbic acid and 120 seconds for the *Pistacia* oil. After that, a slow decrease explained by a low kinetic trapping of the radical followed by a plateau, it is observed after the first 20 seconds with ascorbic acid and 240 seconds with the oil especially in the low concentrations (0.25 and 0.5 µg/mL). The antiradical activity of VOPL has proved to be weak and very slow compared to the antioxidants tested. Antiradical reaction is carried out by transferring a hydrogen atom or an electron from an antioxidant toward the DPPH radical giving the not radicular form stable DPPH-H. The yellow color obtained at the end of reaction means the antioxidants hydrogen is exhausted. Many studies have been reported on the antioxidant property of *P. lentiscus* but not for vegetable oil [19, 38, 39, 40].

3.3.2. Determination of the Percentage of Inhibition

The different optical densities were used to calculate the percentages of inhibition for *P. lentiscus* oil and ascorbic acid using the formula in materials and methods. The results obtained are presented in **figure 3**. Graphically in terms of the concentrations tested of VO and ascorbic acid, we note the existence of a proportional relationship between the percentage reduction of free radical and the concentration of the extract in the solution. This result represents the percentage of the radicular inhibition in terms of different concentrations of the ascorbic acid and VOPL.

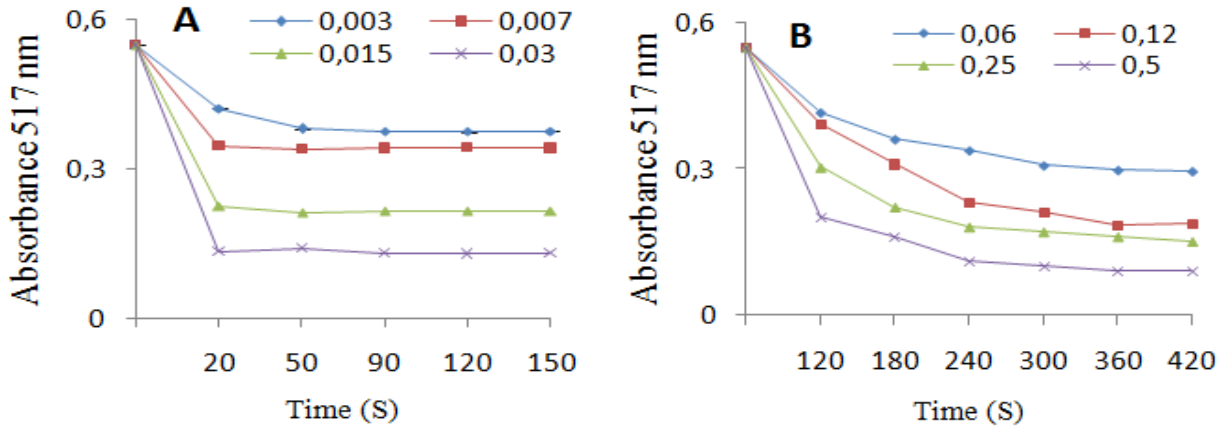

Figure 3: Kinetics of DPPH reduction obtained with the ascorbic acid (A) and VOPL (B*). Free radical scavenging activity of VOPL at concentration of 0.06, 0.12, 0.25 and 0.5 mg/mL, and positive controls (ascorbic acid) at the concentration of 0.003, 0.007, 0.015, and 0.03 mg/ml. Kinetics of DPPH reduction obtained with the ascorbic acid (A) and VOPL (B) is measured by time function.*

3.3.3. Determination of IC_{50}

The antioxidant capacity of VOPL was determined from the IC_{50}. It is the antioxidant concentration necessary to reduce 50% of the radical DPPH. The IC_{50} and the antioxidant activity of the oils are generally inversely proportional [41]. The IC_{50} values (**Table 3**) were calculated from the percentages inhibition of DPPH plotted versus the concentrations of the VO and ascorbic acid using an exponential equation (**Figure 3A**). The values of IC_{50} calculated are presented in the **table 3**. According to the results obtained, VOPL have a significant antioxidant power compared (IC_{50}=37.38 mg/mL) with ascorbic acid (IC_{50}=27.20 mg/mL). Many studies have experimentally determined the capacity of natural extracts to trap free radicals, the terpeneoides, polyphenols, flavonoids, alkaloids and tannins of the oils are considered as potential antioxidant substances [42]. Chemical analysis of the VOPL shows in fact a predominance of polyphenols and flavonoids, these compounds are probably responsible for the antioxidant activity of this oil.

3.3.4. Determination of T_{IC50}

T_{IC50} is the necessary time to reach the steady state at a concentration corresponding to IC_{50}. It was obtained by the modeling of times at steady state conditions versus the concentrations of VOPL. The values of T_{IC50} are generally different between standard antioxidant and vegetable oil (**table 3**). T_{IC50} recorded are 1.8 min for ascorbic acid and 3.7 min for VOPL. For the ascorbic acid, our T_{IC50} results are comparable to those of [19, 43]. We found no data for oils and our study is the first one recording T_{IC50} for VOPL.

3.3.5. Antiradical efficiency

The AE combines two previous parameters; IC_{50} and T_{IC50}. The calculation of EA allows to classify the antioxidants tested according to the classification proposed by Sanchez-Moreno et al. [43]; AE is low for values below 1.10^{-3}, intermediate between 1.10^{-3} and 5.10^{-3}, high between 5.10^{-3} and 10.10^{-3}, and very high for AE above 10.10^{-3}.The parameters for calculating the antioxidant activity are summarized in **table 3**. Low IC_{50} values combined with low T_{IC50} resulted in high AE values. The difference in T_{IC50} between these antioxidants indicates that IC_{50} values do not show time dependence (**Table 3**). From these results, it appears that the oil which contains the phenols has very significant efficiency compared to the antioxidant of reference ascorbic acid with EA equal to 7.23 ml/mg.min. Several studies have been shown that the antioxidant capacity of VOPL is due mainly to phenol content. The AE of ascorbic acid is higher (20.40×10^{-3}) than VOPL (7.23×10^{-3}). This shows that AE remains a powerful parameter for evaluating the antioxidant activity, compared to IC_{50}. The obtained results indicate that ascorbic acid has a higher antioxidant power for scavenging of DPPH compared to results obtained by Sanchez-Moreno et al. [43] (11.44×10^{-3}) and lowest than obtained by Benhammou et al. [19] (41.74×10^{-3}).

The oxidative stress is implicated in several human pathologies such as diabetes, cancer and cardiovascular diseases [44]. Therefore, various neutraceutical products have been developed from medicinal plants to treat these diseases [44]. In addition, oxidative stress is also a serious problem in food safety and the search on natural alternatives for food preservation have take more attention today [45]. The funding of this study showed clearly that *P. lentiscus*, species widely used by Moroccan population, offers interested antiradical efficacy, and thus this vegetable oil could be used as neutraceutical and additive product.

Table 3: The parameters for calculating the antioxidant activity of VOPL and ascorbic acid

	IC_{50} (µg/mL)	R^2	T_{IC50} (mn)	R^2	AE (mL/mg.mn)$\times 10^{-3}$	Classification
Vegetable oil	37.38	97.46	3.7	98.73	7.23	high
Ascorbic acid	27.20	98.37	1.8	97.91	20.40	very high

Conclusion

This work was conducted to study of the antioxidant activity of vegetable oil extracted from *P. lentiscus*. The study of the antioxidant activity according to the method of trapping free radical DPPH showed that the *Pistacia* oil is a best hydrogen or electron donors. It shows a significant efficiency than that of ascorbic acid. However, it would be difficult to believe that the antioxidant activity of this oil is limited only to some of its major constituents; it could also be due to some minor constituents or to a synergistic effect of several constituents. This study helps to orient the food, cosmetic and pharmaceutical industries toward alternatives to some synthetic additives. However, further investigations for identification of the main compounds of VOPL are necessary. Moreover, other investigations on the antioxydant activities in food systems and *in vivo* biological models are strongly recommended

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