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Total phenols content and antioxidant activity of plant parts extracts from *Pistacia lentiscus*

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Abstract
The mastic tree is a widespread plant in the Mediterranean Basin. This plant is known for its therapeutic qualities and its diverse biological properties. The aim of this study was to determine *in vitro* antioxidant activity and the total phenolic content of six different extracts from plant parts (leaves, shoots and roots) of *Pistacia lentiscus* L. Solvents used for extracts preparation were water and methanol. Antioxidant activity was analyzed *in vitro* using DPPH reagent. The total phenolic content in the extracts was determined using Folin-Ciocalteu reagent. The IC_{50} obtained ranged from 0.05 to 0.5 mg/mL. The total phenolic amounts ranged between 1.31 to 5.35 mg GA/g. Methanol extracts showed the most important antioxidant activity and the lowest IC_{50} values. A high correlation was determined between the antioxidant activity and the total phenols of the extracts. Based on these results of investigation, it could be concluded that *P. lentiscus* is a natural source of phenolic compounds as antioxidans of high value.

Keywords: leaves, roots, shoots, *Pistacia lentiscus*, phenols, antioxidant activity

Introduction
Mastic tree, *Pistacia lentiscus* L., belongs to the family *Anacardiaceae*. This is an evergreen shrub growing in dry and rocky areas. This dioecious species can reach 3-4 m in height and grows in the Mediterranean countries. The most important component of *P. lentiscus* is resin. It has a great medicinal value and has already been used in traditional system of medicines for the treatment of some stomach diseases and as antiseptic for respiratory system (ICMR, 1986). The essential oil extracted from mastic is used to soothe rheumatism and stomach pains and to shrink tumors cells (Teyssou, 2007) [17]. The essential oil of leaves of *P. lentiscus* has been reported to have high antioxidant property. It is commonly used as a decongestant and for varicose veins problems (Ansel, 2002) [2].
In some countries, especially in Tunisia and Algeria, the oil extracted from mature fruits is commonly used in traditional medicine as an anti-ulcer, wound healing and antiseptic (Rejeb *et al*., 2006; Mezghani, 1992) [14, 11].
The aerial part of *P. lentiscus* has traditionally been used in the treatment of hypertension and possesses stimulant and diuretic properties (Bentley and Trimen, 1980) [3]. In Tunisian folk medicine, decoction of roots is used for the treatment of stomach ulcers and gastric and intestinal problems.
The aim of the present study, which is carried out for the first time for *P. lentiscus* grown in Tunisa, is to analyze the antioxidant activity and the total phenol content of extracts from different parts of mastic tree.

Material and methods
1. Plant material
Leaves, shoots and roots of *Pistacia lentiscus* L. were collected in Nefza region located in the North West of Tunisia (N 37° 0'43.70"; E 8°54'6.11"). *Pistacia lentiscus* was identified by Dr A. Khaldi from I.N.R.G.R.E.F-Tunisia and certified specimens (VS1-PL2009) were deposited at the Herbarium run by I.N.R.G.R.E.F.

2. Preparation of aqueous and methanol extracts
The leaves, shoots and roots were dried and then differently blended into a fine powder.
20 g of dried powder was soaked in 200 ml of solvent (water or methanol) for 24 hours with intermittent shaking. The plant extracts were filtered through Whatman filter paper into pill vials. The filtrates were dried until a constant dry weight of each extract was obtained. The residues were suspended in 50 ml of solvent and used for the experiments.

3. Free radical scavenging activity
The effect of the different extracts on DPPH radical was studied, employing the method described by Brand-Williams *et al*. (1995) [4]. Briefly, 5 ml of DPPH solution (0.004%, in Methanol) was incubated with varying

concentrations of the extracts (0.1–0.8 mg/L). The reaction mixture was shaken well and incubated for 30 min at room temperature and the absorbance of the resulting solution was read at 517 nm against a blank. The radical scavenging activity was measured as a decrease in the absorbance of DPPH and was calculated using the following equation:

$$\text{Scavenging effect (\%)} = \left[1 - \frac{A_{\text{Sample (517 nm)}}}{A_{\text{Control (517 nm)}}}\right] \text{x } 100$$

IC_{50} values denote the concentration of sample, which is required to scavenge 50% of DPPH free radicals.

4. Total phenols determination

Total phenols were determined by Folin Ciocalteu reagent (Singleton and Rossi, 1965) [16]. A dilute extract of each plant extract (0.03-0.5 g/L) or Gallic acid (standard phenolic compound) was mixed with Folin Ciocalteu reagent (500 µl, 1:10 diluted with distilled water) and aqueous Na_2CO3 (2 ml, 2%). The mixtures were allowed to stand for 30 min and the total phenols were determined by colorimetry at 755 nm. The standard curve was prepared using 0, 0.03, 0.06, 0.12, 0.25, 0.5 g/L solutions of Gallic acid in water. Total phenol values are expressed in terms of Gallic acid equivalent (mg/g of dry mass), which is a common reference compound.

5. Statistical analysis

The statistical significance between antioxidant activity values of the extracts was evaluated with the GLM procedure (General Linear Models) of the SAS (9.0) program. P values less than 0.05 were considered to be statistically significant. All values are the mean of three replications.

Results and discussion

1. Extraction yield

The extraction yield of the *P. lentiscus* extracts is shown in Table 1. Methanol could solublilize the most important rate of the materials from *P. lentiscus*. This implied that most of the soluble components in lentisk were high in polarity. There was significant difference ($P<0.05$) in the extraction yield between the extracts of *Pistacia lentiscus*.

Table 1: Yield of *Pistacia lentiscus* extracts by different solvents (% dry plant material)

Plant material	Aqueous extract	Methanol extract
Roots	0.11 ± 0.01	10.18 ± 2.52
Shoots	0.1 ± 0.34	7.38 ± 0.30
Leaves	0.18 ± 0.04	13.69 ± 2.55

2. Antioxidant activity and total polyphenol content

The IC_{50} values of the aqueous and methanol extracts from leaves, shoots and roots of *Pistacia lentiscus* (mg/ml) are summarized in Table 2.

Significant DPPH radical scavenging activity was evident at all the tested concentrations of different extracts. The scavenging effect increased with increasing extract concentration.

Statistical analyses showed high significant differences between the different extracts ($P < 0.001$).

The antioxidant activity of different parts of *P. lentiscus* may be due to the reduction of hydro peroxides, chelation of metal ions, inactivation of free radicals or combinations thereof. This antioxidant property suggests that the analyzed extracts contain a high amount of antioxidant agents (Zainol *et al*., 2003) [19]. Numerous factors could affect the polyphenol content of plants; these include degree of ripeness at the time of harvest, processing, environmental factors and storage (Manach *et al*., 2004) [10].

When compared with aqueous extracts, methanol extracts showed the most important antioxidant activity and the lowest IC_{50} values. The highest antioxidant activity was reached by the methanol extract of roots (IC_{50}= 0.05 ± 0.002 mg/ml). The aqueous extract of roots exhibited the lowest antioxidant activity (IC_{50}= 0.5 ± 0.03 mg/ml). The higher inhibition percentage in the methanol extracts of roots was probably due to their higher phenolic contents. Several studies have demonstrated that phenolic compounds, and particularly tannins, are mainly concentrated in roots (Hemingway and Laks, 1992; Atawodi *et al*., 2010) [7, 1].

The results of the total polyphenol content of the studied samples, expressed as Gallic acid equivalent (GAE; mg/g plant material), are presented in Table 3.

Significant variation was observed between the plant material (shoots, leaves and roots) and the solvent used for extract preparation. The highest polyphenol content was found in the methanol extract of roots, 5.35 ± 0.11 mg GAE/g plant material. The lowest rate was reached by the aqueous extract of roots (1.31 ± 0.06 mg GAE/g plant material).

It is well known that plant polyphenols are widely distributed in the plant kingdom and that they are sometimes present in high concentrations (Harborne, 1993) [6]. Polyphenols are the most abundant antioxidants in plants. The antioxidant properties of polyphenols have been widely studied, and it has become clear that the mechanisms of action of polyphenols go beyond the modulation of oxidative stress. Several studies demonstrated

the benefits of polyphenols on human health. There are increasing evidences that as antioxidants, polyphenols may protect cell constituents against oxidative damage and, therefore, limit the risk of various degenerative diseases associated with oxidative stress (Hollman *et al*., 1997; Spencer *et al*., 1999) [8, 15].

In the literature, it had been reported that the antioxidant activity of plant materials are well correlated with the content of their phenolic compounds (Velioglu *et al*., 1998) [18]. In this study, a high correlation was demonstrated between the total phenol content and antioxidant capacities. Pearson's correlation coefficient (r^2) was 0.972 (Figure). These results are in agreement with other reports in the literature (Bendini *et al*., 2006; Paixao *et al*., 2007; Krishnaiah *et al*., 2011) [5, 12, 9].

Table 2: The IC_{50} values of the aqueous and methanol extracts of *Pistacia lentiscus* (mg/ml)

Plant material	Aqueous extract	Methanol extract
Roots	0.5±0.03	0.05±0.002
Shoots	0.3±0.02	0.061±0.004
Leaves	0.11±0.001	0.067±0.001

Table 3: Total phenol contents in the aqueous and methanol extracts of *Pistacia lentiscus* (mg GAE/g plant material)

Plant material	Aqueous extract	Methanol extract
Roots	1.31 ± 0.06	5.35 ± 0.11
Shoots	2.84 ± 0.14	4.66 ± 0.78
Leaves	3.02 ± 0.13	1.39 ± 0.35

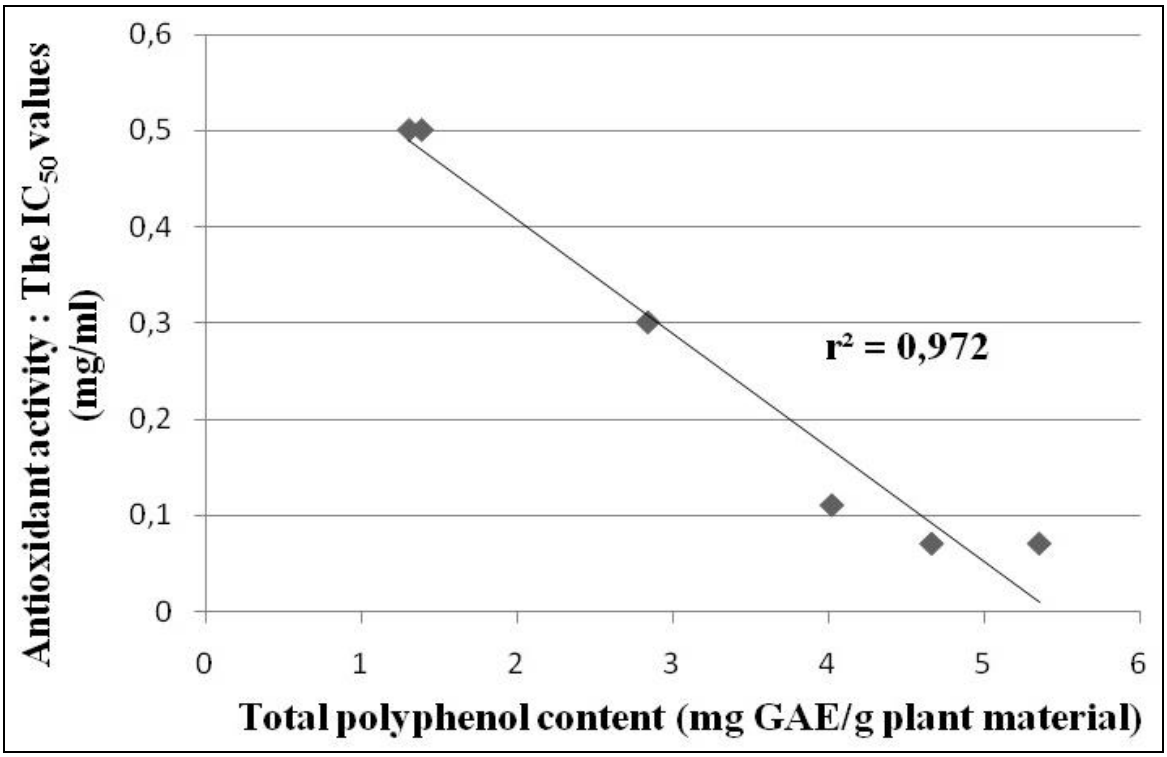

Fig 1: Correlation between the antioxidant activity and the total polyphenol content of the aqueous and methanol extracts from *P. lentiscus*

Conclusions

In conclusion, the different parts from *P. lentiscus* exhibited a high content of phenolic compounds and a good antioxidant activity; there fore they can be used to treat several diseases in which there is an increase in free radical production. In this case, further studies are needed to identify which phenolic compounds are responsible for the antioxidant activity of the species, and assess the way in which the phenolic substances contribute to this activity.

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