Full Length Research Paper

Antioxidant and antimicrobial activities of the *Pistacia lentiscus* and *Pistacia atlantica* extracts

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The antimicrobial activity and antioxidant properties of *Pistacia lentiscus* L. and *Pistacia atlantica* Desf (Anacardiaceae) leaves extracts were studied against eight bacteria, five moulds and yeast. A strong antifungal activity and a weak antibacterial activity were observed. They showed also a high reducing power capacity and a weak scavenging activity for superoxide anions (14.16 % for *P. lentiscus* and 19.3 % for *P. atlantica*).

Key words: *Pistacia lentiscus*, *Pistacia atlantica*, antimicrobial activity, antioxidant activity.

INTRODUCTION

Food safety and organoleptic preservation properties is a high topic for researcher and consumer. In a conventional way, chemical food additives, like preservatives and antioxidants, can be used. However, in the light of recent works, some of them as t-butylhydroxytoluene (BHT) or 4-hydroxyanisole (BHA) used to prevent oxidative rancidity of fats, are cause of concern (Safer et al., 1999). Several researches reported the extensive use of these additives in agro alimentary industry. In 12 foods of different categories, 133 food samples reveal the presence of BHT and TBHQ, The estimated daily intake (EDIs) of this antioxidant and BHA for average consumers ranged from 6.00 to 14.42% of the acceptable daily intake (ADI) of each antioxidant in Korea (Suh et al., 2005). In parallel, the increased consumption of the additives in software drinks to benzoates intake, nuts and canned juices to sulphites intake, bread and biscuits to BHA intake and chewing gum to BHT intake was also exposed by the Lebanese population. The ADI could be exceeded for sulphites and BHT by the children of 9 - 13 years old (Soubra et al., 2007). Restrictions on the use of these compounds are being imposed because of their carcinogenicity (Mahdavi and Salunkhe, 1995).

Currently, the research of natural antioxidants like alter-

native 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. Their flavan nucleus structure is linked to the antioxidant capacity. These substances are able to reduce free radicals like superoxide, peroxyle, alkoxyle and hydroxyl. The reduction occurs by hydrogen transfer reaction to the reactive oxygen species (ROS), (Jovanovic et al., 1994). Beside that direct scavenging effect, the mechanisms of action can include the inhibition of the enzymes and/or the chelation of the trace metal that catalyze the formation of ROS (Halliwell, 1994).

The resiniferous pistachio tree belongs to *Pistacia*, a genus of eleven species in the Anacardiaceae family distributed in the Mediterranean area. The leaves are alternate, pinnately compound, and can be either evergreen or deciduous depending on species (Quezel and Santa., 1963).

Pistacia lentiscus L (Mastic) is an evergreen shrub or small tree growing to 1 – 8 m tall (Iauk et al., 1996) with a long tradition in folk medicine since the ancients Greeks (Palevitch and Yaniv, 2000). The aerial part has traditionally been used as a stimulant, for its diuretic properties, and to treat hypertension, coughs, sore throats, eczema, stomach aches, kidney stones and jaundice (Bentley and Trimen, 1980; Palevitch and Yaniv, 2000).

The chemical composition of the essential oil of this

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plant reveals the presence of several main compounds: myrcene (19 - 25%), (Calabro and Curro, 1974; Boelens and Jimenez, 1991); α-pinene (16%), (Buil and Garnero, 1975); terpinen-4-ol (22%), (Picci et al., 1987); δ-3-carene (65%),(De Pooter et al., 1991), myrcene, limonene, terpinen-4-ol, α-pinene, β −pinene, α -phellandrene, sabinene, *para*-cymene and γ-terpinene (Castola et al., 2000).

The aerial parts seem to have no or only a weak antimicrobial activity against the Gram (-) and Gram (+) bacteria (Bonsignore et al., 1998, Tassou and Nychas., 1995). The antifungal activity appears to be much more interesting against the clinical yeast (Iauk et al., 1996) and the pathogenic agricultural fungi (Kordali et al., 2003). The essential oil of the resin proved to be very active against micro-organisms and fungi, whereas the oils from the leaves and the twigs showed a moderate activity against the bacteria and was completely inactive against the fungi (Magiatis et al., 1999).

The antioxidant properties of the leaves phenolic compounds were reported: they act as a scavenger of the 1, 1-diphenyl-2-picrylhydrazyl (DPPH) (Baratto et al., 2003; Benhammou et al., 2007; Gardeli et al., 2007). It was also shown that the presence of gallic acid and its derivative, the 1, 2, 3, 4, 6-pentagalloylglucose in the fruits, play a protecting role against lipid peroxidation induced by H_2O_2 in K562 cell line (Abdelwahed et al., 2007). The Chios mastic gum (CMG) is also known to contain compounds that inhibit the proliferation and induce the death of HCT116 human colon cancer cells in vitro (Balan et al., 2007). The iron-induced lipid peroxidation in rat liver homogenates was suppressed by aqueous extracts, without affecting mitochondrial respiration in cultured HepG2 and PC12. This extract administered daily for 5 weeks to rats was shown to contain compound causing hepathotoxic effect (Ljubuncic et al., 2005 a, b).

In Algeria, the *Pistacia atlantica* (Betoum) is a tree which can reach 25 m in height. It is the most characteristic plant species of the pre-Saharian regions of the country (Yousfi et al., 2002). This plant has also been used for the treatment of peptic ulcer and as mouth freshener (Delazar et al., 2004). A reported hypoglycemic activity is probably in relation with its ability to inhibit the α -amylase activity (Hamdan and Afifi, 2004).

Various compounds were characterized: for instance, α− pinene in the essential oil has been recently reported (Delazar et al*.,* 2004), as well as monoterpenes and oxygenated sesquiterpenes as terpinen-4-ol (21.7 %) or elemol (20%) (Barrero et al., 2005). On an ethnobotanical point of view, the oil from the fruit is used as an antidiarrheal (Yousfi et al., 2002). This oil has good nutritive quality because of its content in unsaturated fatty acids (oleic + linoleic = 73%) and saturated fatty acids (palmitic + stearic = 25.8%) (Mensier, 1957; Yousfi et al., 2002). In comparison with *P. lentiscus*, there are few reports in the literature about the antioxidant properties of

P. atlantica for the DPPH (Benhammou et al., 2007)*.*

The aim of this work was to evaluate the antimicrobial and antioxidant activities of the ethanolic leaves extracts of *P. lentiscus* and *P. atlantica* from the region of Tlemcen (Algeria), in relation with the structure of their phenollic compounds.

MATERIALS AND METHODS

Plant material

The leaves of *P. lentiscus* (mastic tree) and *P. atlantica* (pistachio tree of the Atlas) were collected from the Oum El Alou and Ain Fezza stations, respectively, region (Algeria), in 2005. The leaves were air dried in a shadowy place, then ground into powder.

Preparation of the ethanolic extracts of *P. lentiscus* (EEPL) and *P. atlantica* (EEPA)

50 g of each plant material were macerated with 200 ml of 96.6% ethanol for 15 days. After filtration, the ethanolic solution was evaporated under reduced pressure at 70°C (Rotary evaporator-4000-efficient Laborota) to afford dark green, nearly odourless viscous material. The extraction yields were 13.53% (*P. atlanticus* from Ain Fezza) and 12.64% (*P. lentiscus* from Oun El Alou), on the dry powder basis, and close to values (11.32 and 16.59%) reported elsewhere (Kordali et al., 2003). The residues were dissolved in 20 ml of methanol to give the ethanolic extract solutions of *Pistacia lentiscus* (EEPL) and of *Pistacia atlantica* (EEPA). Because the ethanolic extracts of *Pistacia vera*, *Pistacia terebinthus* and *P. lentiscus* leaves are known to be rich in phenolic compounds and it must be the same for our EEPL and EEPA.

Preparation of the fractions of extracts

For each plant, 50 g of the dried powder of the leaves was macerated with 150 ml of absolute ethanol for 6 days at room temperature. After filtration, the ethanolic extract was evaporated under reduced pressure at 70°C. The dry residue was treated with 50 ml of boiling water to dissolve the flavonoids. Further filtration through filter paper (Wattman N°1, Ø 13 cm, SELECTA, Germany), afforded the aqueous solution that was firstly extracted with 50 ml of ethyl acetate (Fraction 1), then with 50 ml of n-butanol (Fraction 2) (Bekkara et al., 1998). The various extracts were evaporated and each residue dissolved in 3 ml of methanol.

Chromatographic material

The various extracts from ethyl acetate and n-butanol were analyzed by thin layer chromatography (TLC). The analysis were performed either on silica gel (Silica Gel GF$_{254}$, Merck), with Chloroform / Methanol / Water / Acetic acid: (100 / 15 / 0.5 / 0.3) as eluent (Harbone and Williams, 1983), or polyamide (Polyamide-DC 6 UV$_{254}$, Macherey and Nagel) and n-butanol / Acetic acid / Water: (4 / 1 / 5) (Markham, 1982). Rutin, vanillic acid, *para*-coumaric acid, ferulic acid, dihydroquercetin, syringic acid, myricetin, resorcinol, hydroquinone, phloroglucinol, quercetagetin, gallic acid, pyrocatechol, were from Sarsyntex, (France).

Antimicrobial activity of the extracts

Strains tested

Gram negative: *Escherichia coli*: 5044172; *Klebsiella Pneumo*

Table 1. *In vitro* antibacterial activity of the extracts of *P. lentiscus* and *P. atlantica.*

Strains	Inhibition zone(mm)					
	EE of *P. lentiscus*		EE of *P. atlantica*		Control[*]	
	5µl	10µl	5µl	10µl	5µl	10µl
Staphylococcus aureus	11.5	21.5	13.6	16.5	6.5	9
Listeria monocytogenes	11	10	8	9	0	7
Klebsiella pneumoneae	0	0	0	0	7	9
Pseudomonas aeruginosa	10.5	14.5	8.5	10.5	8	9.5
Salmonella typhi	11	25.5	10	21.5	7	13.3
Proteus mirabilis	9	13	8	10	7.5	9
Escherichia coli	0	0	0	9.5	7	7.5
Enterobacter cloacea	0	14.6	8	12	8	19
Candida albicans	12	23.5	15	23.5	0	11

[*]Control was determined in absence of ethanolic extract.

neae: 5215773; *Pseudomonas aeruginosa*: 22212004; *Salmonella typhi*: 4404540; *Enterobacter cloacea*: 1305573; *Proteus mirabilis*: 0536040.

Gram positive: *Listeria monocytogenes*: ATCC 19111; *Staphylococcus aureus*: ATCC 601 were from the Hospital of Tlemcen (Algeria).

Yeast: *Candida albicans*: 444.

Moulds: *Aspergillus flavus*; *Rhizopus stolonifer*; *Trichoderma sp*; *Fusarium sp*; *Aspergillus flavus,* reference 994294 [Museum National d'Histoire Naturelle (MNHN) (France)]. Isolation from samples of cereals and identification were performed by the Laboratory of mycology, Department of biology, Tlemcen (Algeria).

Evaluation of the antibacterial activity

The disc diffusion method was used for the determination of the antibacterial activity (Gulluce et al., 2003). Sterile Discs, 6 mm in diameter (Wattman paper N°1 - SELECTA, Germany), impregnated with 5 and 10 µL of EEPL (0.316 g/mL) and EEPA (0.338 g/mL), were placed in Petri dishes on Mueller-Hinton agar, which had been surface spread with 1 mL of logarithmic phase bacteria adjusted to a 10^8 UFC/mL fixed by the optical density (OD = 0.08 and 0.1) (Greisiele et al., 2003). The Petri dishes were then incubated for 18 h at 37°C. The diameter of the inhibition zone was measured to compare the in vitro antibacterial activity (Table 1).

Evaluation of the antifungal activity

The antifungal activity was determined following the method described by Chang et al. (1999, 2000). Various volumes (50, 100, 500 µL and 1 ml) of EEPL (0.316 g/mL) and EEPA (0.338 g/mL) were added to 20 mL of a solution of sterilized potato dextrose agar (PDA). The mixtures were cast on the Petri dish. Thereafter, a mycelial disc of approximately 5 mm in diameter, cut from the periphery of a 7 days old culture, was inoculated in the centre of each Petri dish, and then incubated at 25 ± 2°C. The diameters of growth of the hypha were recorded after 48 h and 96 h, respectively, for *Rhizopus stolonifer* and for the other moulds. The antifungal index was determined as follows:

$$Antifungal\ index\ (I) = \left(1 - \frac{D_a}{D_b}\right) \times 100$$

Where: D_a and D_b are the diameter of the growth zone in test and in control Petri dish (mm).

Antioxidant activity of the extracts

Reduction power of the ethanolic extracts

The reduction power (Oyaizu, 1986) was determined using various concentrations of EEPL and EEPA in distilled water (0.25; 0.5; 0.75; 1; 2; 3 mg/mL) and mixed with of a buffer phosphate solution (2.5 mL, 0.2 M; pH 6.6) and potassium ferricyanide (2.5 ml, 1% aqueous $K_3Fe(CN)_6$). 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 $FeCl_3$ (0.5 ml, 0.1% in water) was added. The absorbance of the resulting solution was measured at 700 nm, using ascorbic acid as a positive control.

Superoxide anion scavenging activity

The method of Markulund (1974) modified by Ekanayake et al., (2004) was used in this test. The method is based on the inhibition of the autoxidation of pyrogallol by phenolic compounds. To the assay mixture composed of a phosphate buffer solution (2.6 ml, 50 mM in water, pH 8.22 ± 0.03) with the analytical sample extract (0.3 ml) was added a freshly prepared solution of pyrogallol (0.1 mL of a 3 mM solution of pyrogallol in 0.010 M HCl (CHEMINOVA, Madrid, Spain; 37.5 %). The autoxidation reaction rate of pyrogallol was determined at 325 nm by monitoring the absorbance every 30 s for a total period of 10 min, corresponding to the end of the reaction. The scavenging activity of the superoxide anion (O_2^-) was calculated by the following formula (Sun et al., 2001):

$$S = \frac{(K_0 - K_1)}{K_0} \times 100$$

Where K_0 and K_1 are autoxidation rates of the pyrogallol without and with the leave extract, respectively.

Statistical analysis

The statistical analysis was performed with Origin 6 Professional program for superoxide anion scavenging essay, and with Microsoft

Table 2. Antifungal activity of the extracts of the leaves of *P. lentiscus* and *P. atlantica*

Extract	Quantity (µl)	R. stolonifer		A. flavus		A. flavus of reference		Trichoderma sp		Fusarium sp	
		G	I	G	I	G	I	G	I	G	I
		(mm)	(%)	(mm)	(%)	(mm)	(%)	(mm)	(%)	(mm)	(%)
P. lentiscus	50	68.5	13.07	16.5	61.17	23	31.95	85	0	50	8.25
	100	63	14.63	19	59.66	17.5	40.27	85	0	49	10.09
	500	20.5	-64	16	71.53	16	45.20	12.5	84.70	34	24.44
	1000	9	-20	20	33.33	12	-71.42	9	48.57	21	28.81
P. atlantica	50	85	-7.86	52	-22.35	48.5	-43.5	85	0	55	-1
	100	85	-15.17	56	-18.9	32.5	-10.92	78.5	7.64	54	1
	500	21	-68	60	-6.76	49.5	-69.52	72	11.87	36	20
	1000	10	-33.3	33	-10	12.5	-78.57	27	-54.28	20	32.2
Control	50	78.8		42.5		33.8		85		54.5	
	100	73.8		47.1		29.3		85		54.5	
	500	12.5		56.2		29.2		29.2		45	
	1000	7.5		30		7		17.5		29.5	

G: Growth; I: antifungal index.
Control was determined in absence of ethanolic extract.

Office Excel 2003 for the other tests (ethanolic yield, antimicrobial activity and reduction power). The data obtained are means of two replicates along with standard deviations (SD).

RESULTS AND DISCUSSION

Identified compounds by the TLC

The antioxidant activity was studied on the ethanolic extract of leaves. The use of ethanol like solvent allows the extraction of phenolic compounds from the leaves of *Pistacia vera*, *Pistacia terebinthus* and *P. lentiscus.* The TLC analysis UV light, allowed the identification of a pattern of flavonols (yellow colours), phenolic acids (blue fluorescent), flavones (purple chestnut), anthocyans (red colour) and gallic acid and the *para*-coumaric acid.

Few studies mention the analysis of phenolic compounds in *P. atlantica* and *P. lentiscus*. It has been shown that the Anacardiaceae family is characterized by the occurrence of both gallic acid and myricetin derivatives (Umadevi et al., 1988). 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) by Romani et al. (2002). The abundance of the flavonoids glycosides was also noted in the aerial parts of *P. lentiscus, P. atlantica, Pistacia vera*, *Pistacia chinensi* and *Pistacia khinjuk* (Kawashty et al., 2000). *Para*-coumaric acid was identified in the ethanolic leave extract of *P. lentiscus* and *P. atlantica*.

Antimicrobial activity

The antibacterial activity of the ethanolic extracts is shown in the Table 1. In the present study, *Klebsiella pneumoneae* and *Escherichia coli* are not sensitive to the extracts of *P. lentiscus* and *P. atlantica*. The strains *Candida albicans, Staphylococcus aureus* and *Salmonella typhi* showed a sensitizing effect at the 5 µl level and a very significant effect at 10µl level.

The antifungal activity is reported in Table 2. The EEPL generally shows an inhibiting activity on all the tested strains, except for *R. stolonifer* and for *Aspergillus flavus* of reference at 1ml of extract. In parallel, no inhibiting activity was observed for EEPA. This result is in agreement with previously reported results (Iauk et al., 1996; Ali-Shtayeh and Abu Ghdeib, 1999).

It is worth to note that the flavonic extracts of *P. lentiscus* present an antifungal activity against yeasts and moulds and no antibacterial activity. The inhibition of growth of *Fusarium sp* varies between 8.25 to 28.81% for *P. lentiscus* and 20 to 32.20% for *P. atlantica* depending of the concentrations, as compared with *Fusarium sambucinum* (–175.6%) (Kordali et al., 2003).
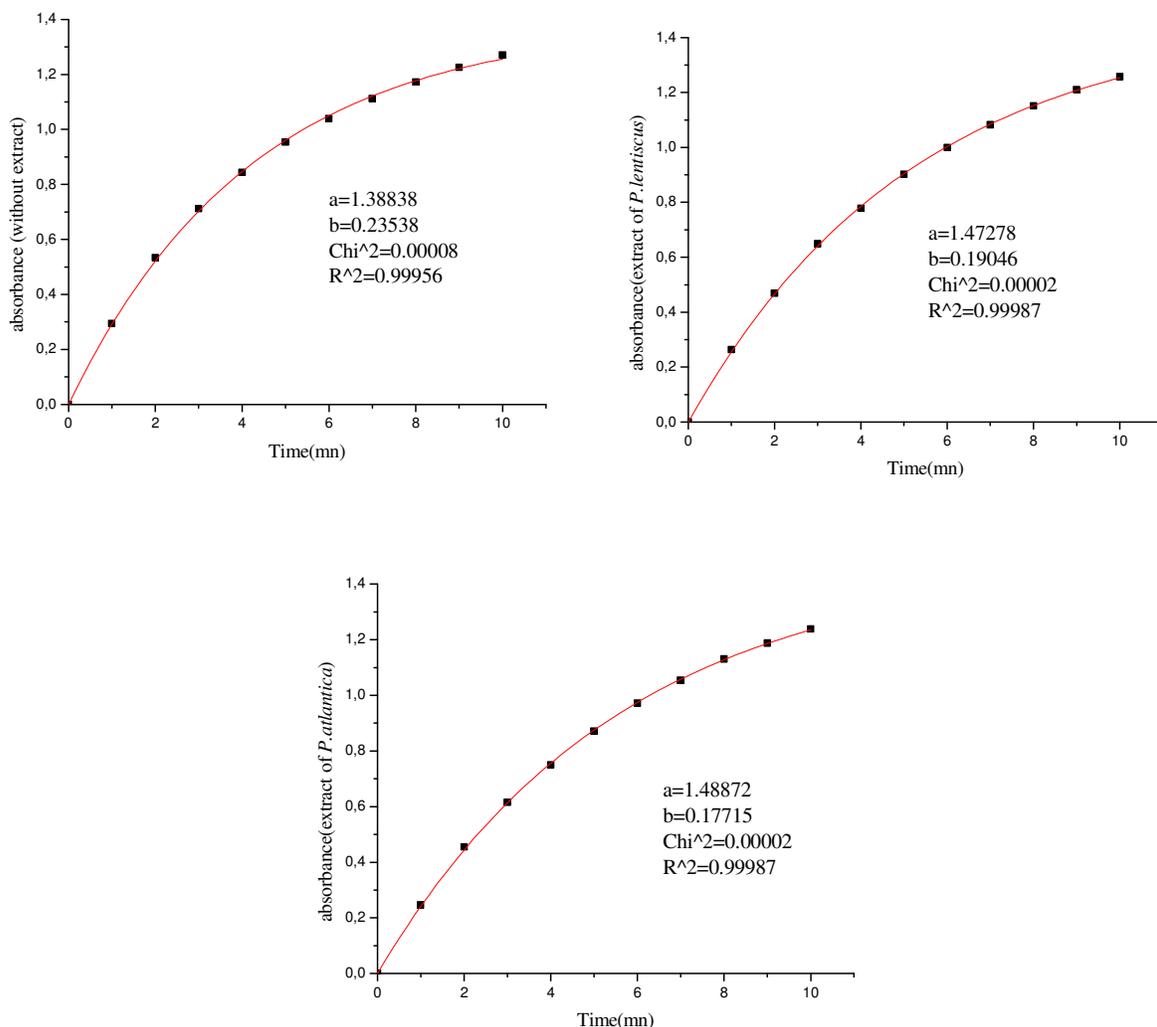
Antioxidant activity

Reduction power

Several methods have been developed to measure the efficiency of dietary antioxidants. These methods are based on different kinds of defence systems: scavenging reactive oxygen species (ROS), hydroxyl radicals, reduction of lipid peroxyl radicals, inhibition of the lipid peroxidation and chelating of the metal ions (Zhu et al., 2002). The reducing power based on the transformation of Fe^{3+} into Fe^{2+} in the presence of the extracts of *P. lentiscus* and *P. atlantica* is illustrated in Table 3.

Table 3. Reduction power of the extracts of the phenolic compounds of *P. lentiscus* and *P. atlantica*

Concentrations (mg/ml)	Absorbance (700 nm)		
	EE of *P. atlantica*	EE of *P. lentiscus* acid	Ascorbic acid
0.25	1.089 ± 0.06	1.086 ± 0.11	1.405 ± 0.24
0.5	2.234 ± 0.12	2.011± 0.00	2.674 ± 0.003
0.75	2.418 ± 0.04	2.647± 0.00	2.705 ± 0.01
1	2.499 ± 0.00	2.715 ± 0.00	2.733 ± 0.009
2	2.513 ± 0.01	2.804 ± 0.00	2.781 ± 0.01
3	2.551 ± 0.001	2.862 ± 0.009	2.824 ± 0.02

Each value represents mean ± S.D (two repetitions).

a=1.38838
b=0.23538
Chi^2=0.00008
R^2=0.99956

a=1.47278
b=0.19046
Chi^2=0.00002
R^2=0.99987

a=1.48872
b=0.17715
Chi^2=0.00002
R^2=0.99987

Figure 1. Absorbance of superoxide anion scavenging by EEPL and EEPA versus time.

The reduction activity of EEPL and EEPA is generally proportional to the concentration (Gow-Chin and Pin-Der, 1993; Gow-Chin and Hui-Yin, 1995; Hadafi et al., 1998). In this work, the both extracts were characterized by a high reduction power. The *P. lentiscus* show a potential reduction power than that of *P. atlantica*. At a concentration of 3 mg/mL, the reduction power of EEPL expressed an absorbance is close to values observed ascor- for

Table 4. Estimate of the parameters of the model of Wallach et al. (1996).

Samples	Rate of oxidation	Correlation Coefficient (r^2)	Scavenging ability (S)(%)
Without extract	0.3267	0.9995	
EE of *P. lentiscus*	0.2805	0.9998	14.16
EE of *P. atlantica*	0.2637	0.9998	19.3

bic acid: 2.862 and 2.8245, respectively. The reduction power decreases inversely to the polarity of extraction solvent (Hadafi et al., 1998) and the capacity of reduction of a compound may serve as a significant indicator of its antioxidant potential (Mier et al., 1995).

Superoxide anion scavenging activity

Dioxygen form superoxide anions O_2^- by a single electron transfer during the pyrogallol autoxidation in basic solutions. The superoxide anions are scavenged by antioxidants and consequently, decrease the rate of pyrogallol autoxidation or even inhibit it. The ability of the phenolic compounds from the ethanolic extract to scavenge the superoxide anion was carried out using this pyrogallol autoxidation method. The results are reported in Figure 1. From these experiments, the both extracts are effective superoxide anions scavenger at a concentration as low as 0.0625 mg/ml.

Table 4 are reported the superoxide anion scavenger activities of EEPL and EEPA using the model of Wallach et al. (1996). The higher scavenging activity was observed for the EEPA (19.3%) as compared with EEPL (14.2%). This effect is probably related to the concentration and chemical structures of the phenolic compounds extracted, particularly for the flavonoids. It was reported that the galloyl quinic derivatives isolated from *P. lentiscus* L. leaves such as gallic acid, 5-*O*-galloyl, 3,5-*O*-digalloyl, 3,4,5-*O*-trigalloyl quinic acid derivatives is responsible for the scavenger activities against 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical, superoxide anion (O_2) and hydroxyl ($OH^.$) radical. The scavenging activity is known to increases with the number of galloyl groups on the quinic acid skeleton (Baratto et al., 2003).

Conclusion

According to the results, it may be concluded that the extracts of the phenolic compounds of *P. lentiscus* and *P. atlantica* revealed considerable antimicrobial activity in particular antifungal activity. Furthermore, the examined extracts have a high reducing activity and scavenging activity for the superoxide anion in vitro. These activities were found are probably in relationship with the structure of the phenolic compounds. In this study, gallic acid and *para*-coumaric acid were identified by TLC. Therefore, further work should be performed to fully evaluate on the pharmacological activity of these extracts against the

lesions of arteriosclerosis, the coronary disease inducing by the oxidation of the LDL and why not cancer while exploiting on animal models.

ACKNOWLEGDEMENTS

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