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Total phenolic content, antioxidant and antimicrobial activities of extracts from *Pistacia lentiscus* leaves

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ABSTRACT

The purpose of the current study was to investigate total phenolic (TPC), total flavonoids (TF) and flavonols (FV) contents, as well as antioxidant and antimicrobial activities of different extracts of *Pistacia lentiscus* leaves. TP compounds were quantified by Folin-Ciocalteu, while antioxidant properties were evaluated using 2,2-diphenyl-1-picrylhydrazyl (DPPH), radical scavenging assay (RSA), Ferric reducing activity power (FRAP) and β-carotene bleaching (βCB) assay. The antimicrobial activity was assessed against Gram-positive bacteria (*Micrococcus luteus*, *Bacillus subtilis* and *Listeria innocua*), Gram-negative bacteriaum (*Escherichia coli*) and fungi (*Candida pelliculosa* and *Fusarium oxysporum albidinis*). Overall, the ethanol fraction (EEPL) exhibited the highest TPC (70.4 ± 7.07 mg GAE g^{-1} powder), TF (32.06 ± 1.68 mg QE g^{-1} powder), FV (14.36 ± 1.68 mg QE g^{-1} powder) and RSA (IC_{50} = 5.34 ± 0.2 µg mL^{-1}). Whereas, methanol extract (EMPL) displayed the highest FRAP (1.20 ± 0.02) and dichloromethane extract (EDPL) showed a high βCB (90.32 ± 2.98%). Furthermore, the antioxidant activities of extracts exhibited strong positive correlation with TP, TF and flavonols. The extracts obtained showed good inhibitory effect against most of the bacterial and fungal strains tested, suggesting their possible use as antimicrobial agents in medical or agro-industry sectors.

Keywords: *Pistacia lentiscus*, Polyphenols, Antioxidant activity, Antimicrobial activity.

INTRODUCTION

Cellular oxidative stress has been proved to be correlated to the increase in free radicals, such as hydroxyls ($HO^{\bullet}$), super-oxides ($O_2^{\bullet}$), peroxyls ($ROO^{\bullet}$) and alkoxyls ($RO^{\bullet}$) (Maritim *et al*. 2003). These radicals play a fundamental role in damaging biomolecules of high interest in cellular metabolism, such as lipids, amino acids, proteins, carbohydrates and nucleic acids leading to several disorders in human beings, including pathogenesis of ageing, cancer, atherosclerosis, diabetes and Alzheimer's diseases (Maritim *et al.* 2003); Pietta, 2000; Apel & Hirt 2004; Takagi *et al*. 1992; 1993; 1994; 1996; Visioli *et al*. 1998; 1998). Recently, antioxidants have attracted a great deal of attention due to their ability to counter balance oxidative stress in cells by cellular antioxidant enzymes and other redox molecules (Apel & Hirt 2004). Therefore, scientists have focused for discovering natural antioxidants with weak or no side effects, to prevent or reduce the impact of oxidative stress on cells in preventive medicine and food industry (Dharmaraj *et al.* 2001; Corona-Bustamante *et al.* 2010). In this case, phenolic compounds are considered health-promoting phytochemicals (Duthie *et al.* 2000; Matilla *et al.* 2006). Numerous studies have

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suggested that biological and pharmaceutical activities of phenolic compounds are linked to their antioxidant activity. Generally, antioxidant activity is due to their ability to scavenge free radicals. Depending on their capacity to donate protons and electrons against to the effect of energetic oxidants, such as free radicals (Martins *et al.* 2004). *Pistacia lentiscus* is an evergreen slow growing 2-3 meters tall shrub excreting an unpleasant odor resin (More & white 2005), belonging to the large family of Anacardiaceae, which consists of over eleven species. This species is distinguished from two other wild species in Morocco, *Pistacia atlantica* Desf and *P. terebinthus* L, through its combined leaves paripinnate and persistent (Fennane *et al.* 2007). *P. lentiscus* is commonly found in the Mediterranean region of Europe, Asia, and Africa to the Canary Islands. In Morocco, *P. lentiscus* occurs in various regions, particularly in the center-west of the Rif Mountains (Barmout 1998). *Pistacia lentiscus* showed a great nutritional and industrial significance (Koutsoudaki *et al.* 2005), particularly in the pharmaceutical industry. Noteworthy, its aerial part is frequently used in treatment of hypertension, cough, sore throat and upset stomach. Previous studies have indicated that the essential oil of the aerial parts of *P. lentiscus* has significant biological properties, including the antimicrobial activity (Lamiri *et al*. 2001; Duru *et al*. 2003; Douissa *et al.* 2005). The aim of this work is to study the in-vitro antioxidant and antimicrobial activities of different extracts of P. lentiscus leaves.

MATERIALS AND METHODS

Chemicals

Folin-Ciocalteu's phenol reagent, aluminum (III) chloride ($AlCl_3$), potassium ethanoate, β-carotene, 2,2-diphenyl-1-picrylhydrazyl (DPPH), potassium ferricyanide, trichloroacetic acid, Tween 80, anhydrous sodium carbonate, iron (III) chloride ($FeCl_3$), gallic acid, quercetin, Butylated hydroxytanisole (BHA) and linoleic acid were purchased from Sigma-Aldrich (St Louis, MO, USA). The other chemicals and solvents used in the study were all of analytical grade.

Plant material

The leaves of *Pistacia lentiscus* were harvested in summer from the plants grown in the Al Hoceima National Park, northeast of Morocco (35°14'04.6"N and 3°58'45.0"W). A voucher specimen of the plant was deposited in the Herbarium of Faculty of Sciences, Oujda, Morocco. The aerial parts (leaves) of the fresh plant were dried in the shade for two weeks before the extraction.

Extraction method

Forty grams of powdered dried leaves underwent hot extraction with 200 mL of solvent by "Soxhlet" with a series of solvents of increasing polarity as follows: dichloromethane-ethyl acetate-ethanol-methanol-water. After evaporation under vacuum, the extracts obtained were named, EDPL, EAPL, EEPL, EMPL and EAqPL, respectively.

Determination of total phenolic, total flavonoids and flavonols contents of extracts

Folin-Ciocalteu reagent was employed to quantify the total phenol content, following the modified method described by Wong *et al*. 2006. Briefly, a mixture of 1 mL Folin-Ciocalteu reagent (10%) and 0.2 ml of samples was prepared, and kept in the dark for 4 min. Then, 0.8 ml Na_2CO_3 (75 g L^{-1}) was added to the mixture. The absorbance was measured at 765 nm after 30 min of incubation. Gallic acid was used as standard and the results were expressed as mg gallic acid equivalent (GAE)/g DM. The measure of the flavonoid content was performed according to the method of aluminum chloride, as described by Djeridane et al 2006. The analysis mixture was obtained with1 ml of each extract (1 mg mL^{-1}) and 1 mL $AlCl_3$ (2% in ethanol). The absorbance was measured at 430 nm and flavonoids concentrations were deduced against the calibration curve and calculated in mg quercetin equivalent (QE)/g DM. Flavonols content was assessed as described by Kosalec et al 2004. Initially, 0.5 mL the extract was mixed with 0.1 mL $AlCl_3$ and 0.1 mL CH_3CO_2K (1 M). The absorbance was read at 430 nm after 30 min. The flavonols content was expressed as mg quercetin (QE)/g P. lentiscus.

Determination of antioxidant activities

DPPH radical scavenging activity

The radical scavenging activity (RSA) of each extract was determined spectrophotometrically by the DPPH test (Moure *et al.* 2000). Different concentrations (5 to 200 µg mL^{-1}) of each sample and positive controls (BHA and

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ascorbic acid) have been prepared. Briefly, 0.1 mL of sample was mixed with 1.9 mL DPPH solution (in absolute ethanol). Thereafter, the absorbance was read against a blank at 517 nm using UV/Vis spectrophotometer. The scavenging activity DPPH radical was expressed as percentage of DPPH discoloration by the following formula: $\left[\left(A_{blank} - A_{sample}\right) / A_{blank}\right] \times 100$, where, A_{sample} is the absorbance of the sample, and A_{blank} is the absorbance of the DPPH solution.

Ferric reducing antioxidant power (FRAP) assay

The ferric reducing capacity of extracts and standards was determined according to the method of Oyaizu, 1986. This method assesses the ability to reduce ferric iron (Fe^{3+}) to ferrous iron (Fe^{2+}). 2.5 mL of aqueous samples (7.8-500 µg mL^{-1}) were mixed with 2.5 mL phosphate buffer (0.2 mol L^{-1}, pH 6.6) and 2.5 mL potassium hexacyanoferrate (1.0%). After the incubation for 20 min at 50 °C, 2.5 mL trichloroacetic acid (10%) was added to the mixture. Thereafter, 2.5 mL of the upper layer was mixed with 2.5 mL deionized water and 0.5 mL of Ferric chloride (0.1%), and the absorbance values were measured at 700 nm in UV/Vis spectrophotometer. A higher absorbance of the solution indicated greater reducing power. Ascorbic acid was used as positive control for comparison. Results are presented as absorbance values and mg of ascorbic acid equivalent per g of powder (mg AscAE/g powder).

β-carotene bleaching assay

The antioxidant capacity of the extracts was determined by measuring the inhibition of the oxidative degradation of β-carotene (discoloration) by the oxidation of linoleic acid according to the slightly modified method described by Kartal *et al.* 2007. The emulsion of β-carotene / linoleic acid was prepared by mixing 0.5 mg of β-carotene in chloroform (1 mL), 40 µL linoleic acid and 200 mg Tween-80 in a round bottom flask. The chloroform was completely evaporated at 50 ºC by vacuum evaporation, and the resulting mixture was topped up to 100 mL with oxygen-saturated distilled water and mixed well to make an emulsion. 200 µL of sample or reference antioxidants (BHA and acid ascorbic) dissolved in ethanol (2 mg mL^{-1}) were added to 1.8 mL of the above emulsion. The discoloration kinetics of the emulsion in the presence and absence of all extracts or standards (BHA and acid ascorbic) was followed at a wavelength of 470 nm at regular time intervals for 120 min. The antioxidant activity of the extracts was calculated according to the following equation: $\left(A_{St} / A_{S0}\right) \times 100\%$, where A_{S0} is the absorbance of sample at the beginning of incubation (0 min); A_{St} is the absorbance of sample after incubating for 120 min.

Antimicrobial activity evaluation

Preliminary antibacterial screening

The evaluation of the antimicrobial activity of different extracts was carried out using the disk diffusion method on Muller-Hinton Agar (MHA). The compounds were evaluated for their in vitro antibacterial activity against *Micrococcus luteus, Listeria innocua* and *Bacillus subtilis* for Gram-positive bacteria and *Escherichia coli* for Gram-negative bacteria, maintained in Muller-Hinton Broth (MHB) (DIFCO), and *Candida pelliculosa* maintained in Yeast Extract Glucose (YEG) (DIFCO) at – 20ºC. They were also evaluated for their in vitro antifungal potential against *Fusarium oxysporum* strains. For bacteria species, 200 µL of each stock-culture were added to 10 mL MH broth, while 10 mL YEG broth was used for *Candida pelliculosa*. The cultures were maintained at 37 ± 1ºC for 24 h for bacteria, and 30 ± 1ºC for 48 h for *C. pelliculosa*. The purity of cultures was checked after 8 h of incubation by their streaking on their corresponding culture media. Microbial suspensions in exponential growth phase were diluted with sterile physiological saline water solution to 10^5 CFU mL^{-1} (turbidity = McFarland barium sulfate standard). Test solutions were prepared at concentration of 1 mg mL^{-1} in anhydrous dimethyl sulfoxide (DMSO). Sterile Whatman paper discs (6 mm diameter) were impregnated with 20 µL (1 mg mL^{-1}) of each extract. 20 µL DMSO were used as negative control and 20 µL Tetracycline (1 mg mL^{-1}) as a positive control for the bacteria or 20 µL Cycloheximide (1 mg mL^{-1}) for fungi. The compounds tested were sterilized by filtration through millipore membrane 0.45µm (Durapore Membrane Filters).

These controls were deposited on the MHA previously inoculated, and the plates were incubated for 18 hours at 37°C for bacteria, and 30 ± 1ºC for 3-4 days for fungi. The results were recorded for each tested extract as the

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average of diameters of inhibition zones (IZ) of bacterial or fungal growth around the disks (mm). All essays were made in triplicate.

Minimum inhibitory concentration (MIC) measurements

The leaf extracts showing inhibition zone higher than or equal to 12 mm were selected for the determination of the MIC. The method used is based on liquid culture medium in tubes. The extracts which were prepared using DMSO as an emulsifier, were sterilized by filtration through millipore membrane 0.45µm (Durapore Membrane Filters), and serial dilutions from 50 to 450 µL were prepared in liquid culture medium, previously sterilized at 121°C/15 min, and inoculated with cultures. After 24 h incubation (at 37°C for bacteria, and 30 ± 1ºC for 3-4 days for fungi), the MIC of each sample was determined by measuring the optical density at 625 nm, using a spectrophotometer (Rayleigh-Model VIS-7220G/UV-9200), compared to a control containing the drug and not inoculated with tested bacteria. Each assay was repeated twice against each plant extract.

RESULTS AND DISCUSSION

Determination of total phenolic, total flavonoid and flavonos contents

Previous studies have shown that plant extracts possess bioactive compounds with antioxidant properties and have been declared reducing agents, hydrogen donors, singlet oxygen quenchers and metal chelating agents (Pietta 2000; Rice-Evans *et al.* 1997). In this case, the present work aimed to quantify the total phenolic content (TPC), total flavonoids (TF) and flavonols (FV) in order to establish the correlation between the antioxidant activity and these phytochemical compounds. The results listed below in Table 1 illustrate that, the TPC, TF and FV of the leaf extracts varied from 10.06 ± 0.06 to 70.4 ± 7.07 mg GAE g^{-1} DM, 5.81 ± 0.79 – 32.06 ± 1.68 mg QE g^{-1} powder and 2.79 ± 0.10–14.36 ± 1.68 mg QE g^{-1} powder, respectively. The TPC, TF and FV decreased in the order of EEPL > EAqPL > EMPL > EAPL > EDPL. The results obtained also indicate that amongst the five extracts, EEPL contained the highest amount. Our results are similar to previous results of Piluzza & Bullitta 2011) who reported that the TPC of the 30% aqueous acetone extract of Sardinian *P. lentiscus* were 2.0 times higher than the TPC of EEPL, Whereas TF of Sardinian *P. lentiscus* were similar to our results. Also, the earlier study of Dahmoune *et al.* 2014, who worked on *P. lentiscus* leaves as a source of phenolic compounds, reported that the TPC of ethanol extract were 2.0-fold higher than that of EEPL.

Table 1. Phenolic compounds of different extracts of *P. lentiscus.*

Extracts	TPC	TF	FV
EDPL	10.06 ± 0.06	5.81 ± 0.79	2.97 ± 0.10
EAPL	24.43 ± 2.72	12.63 ± 0.18	7.09 ± 0.29
EEPL	70.40 ± 7.07	32.06 ± 1.68	14.36 ± 1.68
EMPL	36.94 ± 1.35	15.12 ± 0.88	8.12 ± 0.88
EAqPL	62.90 ± 4.80	22.09 ± 0.76	11.61 ± 0.06

All the values are mean ± S.D. (n=3); TPC: total phenolic compounds (mg GAE g^{-1} powder), TF: total flavonoids (mg QE g^{-1} powder), FV: Flavonols (mg QE g^{-1} powder); EDPL: Dichloromethane extract; EAPL: Ethyl Acetate extract; EEPL: Ethanol extract; EMPL: Methanol extract; EAqPL: Aqueous extract..

Antioxidant activities

Scavenging activity of DPPH radical

Radical scavenging activity is frequently used to evaluate the ability to scavenge the ‘‘stable’’ free radical DPPH. It is an accurate and suitable method to neutralize free radicals (DPPH•) by natural or synthetic antioxidants. As shown in Table 2, the RSA values of the samples and standards ranged from 39.2 ± 2.8% to 94.4 ± 1.1% at 200 µg mL^{-1}. Besides, the IC_{50} values were calculated from the curves plotted using the regression analysis. The lower the IC_{50} value the higher the antioxidant capacity of the plant extract. Excepting the dichloromethane fraction (EDPL) showing the highest IC_{50} value [249.78 ± 2.9 µg mL^{-1}], all the other extracts exhibited strong reducing power and outstanding radical scavenging activity [EEPL: IC_{50} = 5.34 ± 0. 2 µg mL^{-1}], compared to the standards [BHA: IC_{50} = 5.55 ± 0.3 µg mL^{-1} and Ac. Asc: IC_{50} = 2.82 ± 0.1 µg mL^{-1}] (Fig. 1) (a). These findings are similar to those obtained in aqueous extracts issued from chloroform and hexane partitions of Algerian *P. lentiscus* [IC_{50} = 4.24 µg mL^{-1} and IC_{50} = 4.51 µg L^{-1}, respectively] (Atmani *et al.* 2009). Principally, the antioxidant activity of phenolic compounds is due to their redox property (Woidylo *et al.* 2007). Hence, the high phenolic and flavonoids

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contents in *P. lentiscus* may be responsible of its free radical scavenging activity. Furthermore, antiradical activity and TPC, TF and FV contents of extracts displayed strong correlation coefficients (r) (Table 3).

Table 2. Antioxidant activities of *P. lentiscus* extracts.

Samples	DPPH		RP		βCB
	$IC_{50\ (\mu g/mL)}$	% RSA	Abs	Ac. AscE $_{(mg/g)}$	% (2 g L^{-1})
EEPL[a]	05.44 ± 0.07	89.8 ± 0.8	1.07 ± 0.02	276.06 ± 0.12	84.43 ± 0.43
EMPL[a]	17.72 ± 1.23	87.7 ± 0.1	1.20 ± 0.02	309.60 ± 0.12	79.66 ± 0.43
EAqPL[a]	13.28 ± 0.56	85.4 ± 2.5	1.17 ± 0.03	301.86 ± 0.18	81.12 ± 0.34
EAPL[a]	38.70 ± 3.50	88.1 ± 0.4	0.27 ± 0.03	69.66 ± 0.18	87.39 ± 0.56
EDPL[a]	> 200	39.0 ± 3.5	0.12 ± 0.03	30.69 ± 0.18	90.30 ± 0.67
BHA	05.54 ± 0.09	90.2 ± 0.1	NT	NT	94.73 ± 1.00
Ac. Asc	2.82 ± 0.10SS	93.7 ± 0.3	1.29 ± 0.03	ND	81.44 ± 0.26

[a]See the note in Table 1 for the full names of extracts.
All the values are mean ± SD (n=3), DPPH: 1,1-diphenyl-2-picrylhydrazyl, RP: reducing power, βCB: β-carotene bleaching, RSA: radical scavenging activity, BHA: Butylated hydroxytanisole; Ac. Asc: acid ascorbic; Ac. AscE: acid ascorbic Equivalentt; NT: not tested; ND: not determined.

FRAP assay

In this study, the reducing power of different extracts of *P. lentiscus* and standards have been determined based on the redox reaction of ferric iron in presence of a reducer. It's expressed as absorbance value at 700 nm, wherein a greater absorbance corresponded to a higher reducing activity. As illustrated in Table 2, all extracts exhibited the reductive effects. Moreover, the reducing power was found to be gradually elevated by increasing extract concentration, Fig. 1 (b). Benhammou et al reported that the ethanol extract of *P. lentiscus* leaves from Algeria exhibited a reducing power of (2.011 ± 0.00) at 500 µg mL^{-1} (Benhammou *et al.* 2008), which is 1.87 times higher than EEPL (1.075 ± 0.025). Generally, extracts with a high phenolic content would display a high antioxidant capacity as well (Tavman *et al.* 2018). Furthermore, both reducing power and TPC of extracts exhibited the same order and a very strong positive correlation (r = 0.823): EEPL > EAqPL > EMPL > EAPL > EDPL.

β-carotene bleaching assays

In order to evaluate the antioxidant activity of the extracts, we used also the method of β-carotene bleaching. In this experiment, the presence of an antioxidant can inhibit the extent of β-carotene destruction by ''neutralizing" the linoleate free radical and any other free radicals formed within the system (Kamath & Rajini 2007). The results obtained showed that the absorbance of the mixture decreased to a lower value. However, this decline is less rapid compared to the reference solution (-) and becomes stable in a long time for all the extracts. The antioxidant activities of all samples (Fig. 1 c), were in the order of EDPL > EAPL > EEPL > EAqPL > EMPL. In this test, all samples exhibited remarkable antioxidant activities at 2 mg L^{-1}, which were close to that of BHA (94.73 ± 3.34%). In contrast to both previous methods, β-carotene bleaching activity and TPC, TF and FV were not in good agreement. This result was confirmed by negative value of the Pearson's coefficient (r) between β-carotene bleaching activity and TPC, TF and FV (Table 3). The high effect of both extracts EDPL and EAPL is probably attributed to their hydrophobic nature which allows them to interact with the hydrophobic reaction medium.

The Pearson correlation analysis

The Pearson correlation analysis was performed to establish the linear relation between all antioxidant activities. DPPH and FRAP showed the highest positive correlation coefficient (r = 0.940) (Table 3). This result indicates that both antioxidant activities DPPH and FRAP showed greater concordance in the polarity of the solvent. The higher the solvent polarity the higher the antioxidant ability of the plant extracts. On the one hand, this result is confirmed by the fact that the reaction mediums of both methods were polar. Furthermore, polar extracts such as ethanol, methanol and aqueous extracts were found to be rich in polar compounds, which are either hydrogen atoms donors or transfer agents of singlet electrons. In contrast, β-carotene bleaching exhibited a very strong negative relationship against DPPH and FRAP (r = -0.891, r = -0.966, respectively). These results can be probably confirmed by the polarity of solvent and the hydrophobic nature of the medium of reaction.

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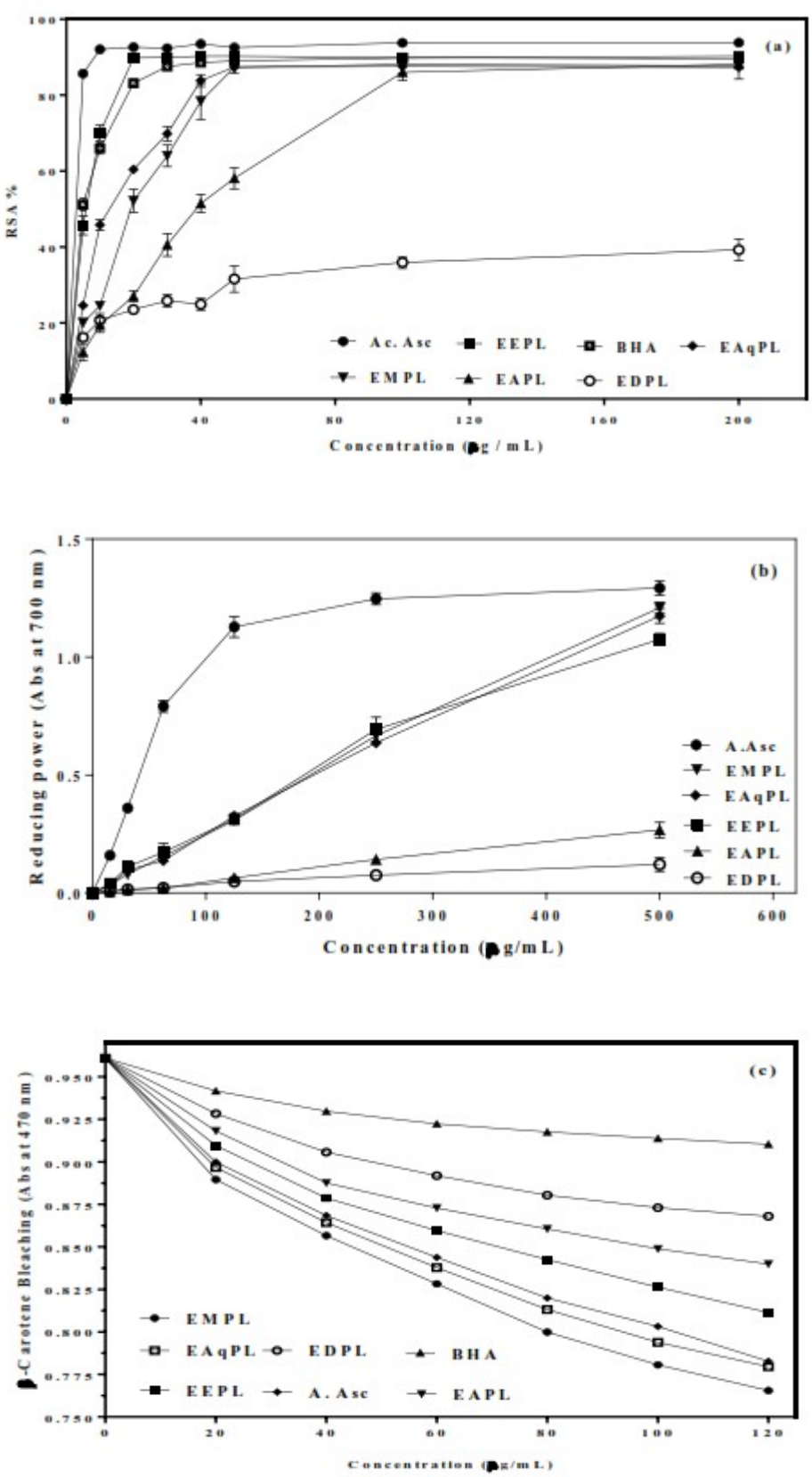

Fig. 1: Antioxidant activities of the examined extracts measured using: DPPH assay (a), reducing power (b) and β-carotene bleaching (c); EDPL: Dichloromethane extract of *P. lentiscus*; EAPL: Acetate ethyl extract of *P. lentiscus*; EEPL: Ethanol extract of *P. lentiscus*; EMPL: Methanol extract of *P. lentiscus*; EAqPL: Aqueous extract of *P. lentiscus*.

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Table 3. Correlation matrix between phenolic compounds and antioxidant activities.

	Correlation Coefficient (r)					
	RSA[a]	RP[a]	βCB[a]	TPC[b]	TF[b]	FV[b]
RSA[a]	1	0.940	-0.891	0.933	0.875	0.928
RP[a]	0.940	1	-0.966	0.823	0.722	0.775
βCB[a]	-0.891	-0.966	1	-0.696	-0.574	-0.659

[a] See the note in Table 2 for the full names of antioxidant methods.
[b] See the note in Table 1 for the full names of TPC, TF and FV.

Antimicrobial activity evaluation

Compared to the standard drugs, the antibacterial and antifungal activities showed variable results depending on the extraction solvent. For the antibacterial activity, the extracts exhibited generally higher inhibitory effect against *L. innocua and M. luteus* than *E. coli* and *B. subtilis*, whereas, the extracts EAPL, EEPL and EMPL extracts displayed an important antifungal activity against *C. pelliculosa* particularly EAPL. The growth inhibition zone measured ranged from 9 to 15 mm for all the sensitive bacteria (Table 4). Antimicrobial properties of medicinal plants are being increasingly reported from different parts of the world. The World Health Organization estimates that plant extracts or their active constituents are used as folk medicine in traditional therapies of 80% of the world's population. In the present work, the leaf extracts obtained from *P. lentiscus* exhibited good activity against most of the tested bacterial and fungal strains and the relative inhibition (RI) were also correlated with the results, indicating their possible use as antimicrobial agents in medical or agro-industrial sectors. Furthermore, the EDPL, EEPL and EMPL extracts displayed a strong inhibition (64%, 66% and 54% respectively) in growth of Fusarium Oxysporum Albidinis strain (Table 5).

Table 4. Minimal inhibitory concentrations (MIC, µL mL^{-1}) and inhibition zone (mm) of extracts.

	MIC (µL mL^{-1}) and Inhibition Zone (mm)									
	Bacteria								Fungi	
Extracts	Gram-positive						Gram-negative			
	B. subtilis	[c]RI(%)	*L. innocua*	RI(%)	*M. luteus*	RI(%)	*E. coli*	RI(%)	*C.*	RI(%)
EAPL[d]	12* ± 0.4(150**)	60	13± 0.1(150)	59.09	14 ± 0.3(100)	70	12± 0.7(250)	60	13±0.6	40.62
EEPL[d]	11 ± 0.2	55	13 ±0.2(150)	59.09	15 ± 0.3(100)	75	10 ± 0.6	50	12±0.4	37.5
EMPL[d]	10 ± 1.2	50	12 ±0.3(200)	54.54	13 ± 0.2(150)	65	10 ± 0.1	50		37.5
EAqPL[d]	9 ± 0.6	45	12 ±0.2(200)	54.54	14 ± 0.4(150)	55	11 ± 0.2	55	-	
EDPL[d]	[a]-		-		-		-		-	
Reference drugs										
Tetracycline	20	100	22	100	20	100	20	100	NT	NT
Cycloheximide	[b]NT	NT	NT	NT	NT	NT	NT	NT	32	100

*: Inhibition Zone (mm)
**: MIC (uL/ml)
[a] (-): totally inactive (no inhibition zone).
[b] NT: Not tested.
[c]RI : Relative inhibition(RI) (%) = (1-(d(control)-d(sample))/d(control))*100
[d]See the note in Table 1 for the full names of extracts.

Table 5. Screening Test of Antifungal activity of the extracts.

	Averages of the diameter of the growth of fungi (mm)					
	Control[a]	EAPL[b]	EDPL[b]	EEPL[b]	EAqPL[b]	EMPL[b]
Fusarium Oxysporum Albidinis	44	26	16	15	22	19

[a] Control was determined in absence of extracts.
[b] See the note in Table 1 for the full names of extracts.

All these findings showed that *P. lentiscus* leaves extracts were efficient against both Gram-positive (*M. luteus*, L. *innocua* and *B. subtilis*) and Gram-negative bacteria (*E. coli*). There is evidence in the literature that Gram-negative bacteria are more sensitive to plant extracts than Gram-positive, because of hydrophobic lipopolysaccharides in

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the outer membrane, which provides protection against different agents (Oyaizu 1986). On the other hand, these extracts did not have selective antibacterial activity because of the cell wall differences of bacteria. This observation is consistent with previous work carried out with several plants (Elgayyar *et al.* 2001; Adiguzel *et al.* 2007).

Conclusion

In this work, we tried to contribute to the valorization of a very used plant in Moroccan traditional medicine for its therapeutic properties by establishing a relationship between the chemical composition and biological activity. The results found showed that the polar extracts, which are rich in phenolic compounds, exhibited the highest antioxidant activity in both DPPH and FRAP assays, compared to nonpolar extracts, whereas, the nonpolar extracts (EDPL and EAPL) exhibited a strong β-carotene bleaching activity due to their hydrophobic nature. The results of the bioassays and the chemical profile of *P. lentiscus* extracts, support the possibility of using these extracts as potent natural preservatives to improve the hygienic quality of foodstuffs, and their preservation against microbial spoilages.

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ترکیبات فنولی کل، فعالیت ضداکسایشی و ضد میکروبی عصاره حاصل ازبرگ Pistacia lentiscus

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چکیده

هدف مطالعه حاضر، تحقیق در مورد ترکیبات فنولی کل، فلاوونوئیدهای کل، و فلاوونول‌ها و هم‌چنین، فعالیت‌های ضد اکسایشی و ضد میکروبی عصاره برگ *Pistacia lentiscus* است. ترکیبات فنولی کل توسط Folin-Ciocalteu اندازه‌گیری شد، در حالی که خواص ضد اکسایشی توسط 2,2-diphenyl-1-picrylhydrazyl (DPPH), و هم‌چنین سنجش خورندگی رادیکال‌های آزاد radical scavenging assay (RSA) به علاوه، قدرت کاهندگی فعالیت آهن فریک Ferric reducing activity power (FRAP) و هم‌چنین β-carotene bleaching (βCB) assay محاسبه شدند. فعالیت ضد میکروبی در مقابل باکتری‌های گرم مثبت (*Micrococcus luteus*, *Bacillus subtilis* and *Listeria innocua*)، باکتری‌های گرم منفی (*Escherichia coli*) و قارچها (*Candida pelliculosa* and *Fusarium oxysporum albidinis*) بررسی شد. به طور کلی عصاره اتانولی بیشترین ترکیبات فنولی (70.4 ± 7.07 mg GAE g^{-1}) ، فلاوونوئیدی $32.06 \pm$ (1.68 mg QE g^{-1}) و فلاوونولی (14.36 ± 1.68 mg QE g^{-1}) و هم‌چنین خاصیت اسکاونجری رادیکال‌های آزاد (IC_{50} = 5.34 ± 0.2 µg mL^{-1}). را به نمایش گذاشت، در حالی که عصاره متانولی بیشترین FRAP (1.20 ± 0.02) و عصاره دیکلرو متانی بیشترین βCB($90.32 \pm 2.98\%$) را نشان داد. به علاوه فعالیت‌های ضداکسایشی و عصاره‌ها ارتباط متقابل مثبتی با ترکیبات فنولی کل، فلاوونوئیدهای کل، و فلاوونول‌ها داشت. عصاره‌های حاصل اثرات ممانعت کنندگی خوبی در مقابل بیشتر سویه های باکتریایی و قارچی داشتند که امکان استفاده از آنها را به عنوان عوامل ضد میکروبی و بخش‌های صنایع کشاورزی پیشنهاد می‌کند.

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