Journal of Medicinal Plants Research Vol. 6(39), pp. 5266-5271, 10 October, 2012
Available online at http://www.academicjournals.org/JMPR
DOI: 10.5897/JMPR12.473
ISSN 1996-0875 ©2010 Academic Journals

Full Length Research Paper

Fatty acid composition, antioxidant and antibacterial activities of *Pistacia lentiscus* L. fruit oils

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Accepted 15 May, 2012

This study was performed on oil extracted from mature fruits of *Pistacia lentiscus* L. harvested from Northern and North Western Tunisia. Extraction was done by two methods: Traditional method practiced by women in forest areas and pressing method that was proposed to improve the yield and the quality of oil. Fatty acid composition was determined by gas chromatography-mass spectrometry (GC/MS). Oleic acid was the main fatty acid with more than 56%, followed by palmitic with 27%. Antioxidant activity of these oils was improved by pressing method; the percentage of inhibition of DPPH was increased from 21 to 43% for Nefza provenance and from 19 to 29% for Bizerte one. Similarly, the highest values of trolox equivalent antioxidant capacity (TEAC) were reached by oils extracted by pressing method. Antibacterial activity was tested against three strains: *Escherichia coli, Salmonella typhimurium* and *Clostridium perfringens*. The results showed the existence of a significant bactericidal effect in the case of *C. perfringens* for oils from Bizerte with an inhibition diameter of about 13 mm for the oil extracted by pressing method and 8 mm for that extracted by traditional one. The effect was not significant in the case of *E. coli* and *S. typhimurium*.

Key words: *Pistacia lentiscus* L., fixed oil, fatty acids, antioxidant activity, 2,2-diphenyl-1-picrylhydrazyl (DPPH), trolox equivalent antioxidant capacity (TEAC), antibacterial activity.

INTRODUCTION

Pistacia lentiscus L. is a dioecious evergreen shrub that grows up to 3 to 4 m in height and widely distributed throughout the Mediterranean region. It is an aromatic and medicinal plant of the Anacardiaceae family. In Tunisia, *P. lentiscus* L. grows in various regions, particularly in the North. The aerial part of this species has traditionally been used in the treatment of hypertension and possesses stimulant and diuretic properties (Bentley and Trimen, 1980). The essential oil extracted from leaves is commonly used as a decongestant and for varicose veins problems (Ansel, 2002). Mastic gum from *P. lentiscus* has been used by traditional healers for the relief of upper abdominal discomfort, stomachaches and peptic ulcer (Al-Habbal et al., 1984). Oil extracted from mastic is used to soothe rheumatism and stomach pains and to shrink tumors cells (Teyssou, 2007). The fixed oil extracted from mature fruits is commonly used in Tunisian traditional medicine

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Abbreviations: DPPH, 2, 2-Diphenyl-1-picrylhydrazyl; **TEAC,** trolox equivalent antioxidant capacity; **TCS,** trypto-casein soy medium; **TGY,** thioglycholate yeast medium; **CFU,** colony forming unit.

as an anti-ulcer, wound healing and antiseptic (Rejeb et al., 2006; Mezghani, 1992). Some researchers have reported the chemical composition of this oil (Tej Yaakoubi and Dhaou, 2007; Trabelsi et al., 2012). To our knowledge, at least in Tunisia, researches concerning the biochemical and particularly biologicalproprieties of mastic tree fixed oil are scarce. In view of its wide use, it is important to gather information about this non-wood forest product and to contribute to the certification of its traditional medicinal uses. This study aims, therefore, to valorize *P. lentiscus* fixed oil through the determination of its antioxidant and antibacterial properties. This kind of valorization can allow a better chance to conserve this natural resource by the local population.

MATERIALS AND METHODS

The mature drupes of *P. lentiscus* L. were harvested during December 2009 from two different sites: Bizerte and Nefza, located respectively in Northern and North Western Tunisia. The geographic coordinates of Nefza and Bizerte sites are respectively (37°12' 24.23" N, 9° 59' 39.79" E) and (37°02' 35.43" N, 9°02' 16.38" E).

Oil extraction

Two methods used to extract the fixed oil are:

1. Traditional method: This method is practiced by rural women in forest areas. After the harvest, the fruits are ground using stone grinder, the paste is then well mixed by hands or feet and then let stand overnight. The next day, cold water is added to this paste and the upper part is removed and placed on fire to heat up until it starts boiling. Subsequently, the liquid phase is separated from oil meal using a tissue, and then placed on the fire until all water evaporates. Finally, the oil is then filtered to be sold or used. This method was considered as a reference; we extracted the oil, jointly with rural women, in order to compare its yield and quality with the pressing method.
2. Pressing method: By this method, drupes are ground using an ordinary meat chopper. The paste is then mixed during a half hour by a mixer, and then left standing for one night. The next day, the paste is introduced into a hydraulic press (Pressa Oleodinamica, Albrigi Luigi s.r.l.) to separate the liquid phase from oil meals. Finally, a centrifugation was performed to recover floating oil.

Fatty acid composition

Fatty acids methylation was performed by the saponification and etherification procedure described by Metcalfe et al. (1966). The dosage of methyl esters was achieved using gas chromatography coupled to the spectrometry of mass (GC/MS) equipped with a capillary column of type HP5 MS, 30 m of length and 250 µM of internal diameter; the thickness of the film was of 0.250 µM. The temperature of the injector was 250°C. The vector gas is helium, well stocked to a debit of 0.8 ml / min, the fashion of the injection is the fashion Split 50: 1 and the program of temperature is of 150°C (0 min) to 5°C / 240°C.

DPPH radical scavenging activity

Antioxidant activity of oil samples was determined as the percentage of inhibition of DPPH (2, 2-diphenyl-1-picrylhydrazyl). For assessing the DPPH radical scavenging activity, the method described by Brand-Williams et al. (1995) was adopted. Briefly, 50 µl of oil in ethanol (50 µl/950 µl) was added to 1 ml of ethanolic DPPH solution (60 µM) and absorbance was read at 517 nm after one hour of incubation at ambient temperature. The radical scavenging activity was expressed as the inhibition percentage (IR) and monitored using the following equation:

$$IR = [(AC - AS) / AC] \times 100$$

where AC is the absorbance of control and AS is the absorbance of sample solution. The DPPH solution without sample was used as control.

Trolox equivalent antioxidant capacity

The trolox equivalent antioxidant capacity (TEAC) assays were first carried out with different concentrations of trolox (1; 0.25; 0.125; 0.06; 0.03 and 0.01 mg/ml), as a standard reference compound. A linear relationship was established for absorbance decrease versus trolox concentration. The percentage of DPPH inhibition was determined and then reported to trolox equivalent values using this linear relationship (Barton et al., 2005).

Antibacterial activity

Bacterial strains

The bactericidal effect was tested against two aerobic strains: *Salmonella typhimurium* (NCTC 6017) and *Escherichia coli* (ATCC 8739) and an anaerobic one: *Clostridium perfringens* (MG 109). S. typhimurium and *Escherichia coli* culture was performed on a trypto-casein soy medium (TCS), while *C. perfringens* was kept under anaerobic conditions in thioglycholate yeast medium (TGY). All bacterial strains are provided by the Laboratory of Immunotechnology and Production of Biological Materials located in Pasteur Institute of Tunis, Tunisia.

Determination of antibacterial effect

The disc diffusion method was used according to Sacchetti et al. (2005). First, medium was distributed into sterilized Petri dishes with a diameter of 9 cm (15 ml), and then 100 µl of suspension of the tested microorganisms containing 5×10^5 CFU/ml of bacterial strains was poured in the medium. The filter paper discs (6 mm in diameter) were individually impregnated with 10 µL of studied oils and then placed onto the agar plates. Before incubation, all Petri dishes were kept in the refrigerator (4°C) for 2 h and then incubated at 37°C for 24 h for bacteria growth. After incubation, the diameters (mm) of the inhibition zones were measured. The Cefotaxime antibiotic was used as a positive control to compare inhibition zones with the tested oil samples.

Statistical analyses

Data were processed by the general linear models of SAS program.

Table 1. Fatty acid composition of *Pistacia lentiscus* fruit oils.

Fatty acid	Nefza		Bizerte	
	Pressing method (%)	Traditional method (%)	Pressing method (%)	Traditional method (%)
Oleic ($C_{18:1}$)	54.23 ± 0.85	54.45 ± 2.11	56.26 ± 3.98	54.45 ± 3.08
Palmitic ($C_{16:0}$)	27.21 ± 0.90	26.94 ± 0.80	25.05 ± 0.60	27.79 ± 0.80
Linoleic ($C_{18:2}$)	15.82 ± 2.50	16.03 ± 1.50	15.98 ± 1.90	15.47 ± 3.10
Palmitoleic ($C_{16:1}$)	1.13 ± 0.69	1.41 ± 0.08	1.05 ± 0.08	0.66 ± 0.34
Stearic ($C_{18:0}$)	1.58 ± 0.02	1.15 ± 0.10	1.66 ± 0.30	1.60 ± 0.50
Saturated	28.79	28.09	26.71	29.39
Unsaturated	71.18	71.89	73.29	71.52
Saturated/ unsaturated	0.4	0.39	0.36	0.41

Values represent mean ± standard deviation of experiments in duplicate.

Table 2. DPPH radical scavenging activity of *P. lentiscus* fruit oils extracted by traditional and pressing methods.

Sites	Extraction method	IR (%)
Nefza	Traditional	21 ± 5.04[a]
	Pressing	43 ± 0.59[b]
Bizerte	Traditional	19 ± 2.05[c]
	Pressing	29 ± 1.59[d]

Values followed by the same letter are not significantly different (P>0.05). Values represent mean ± standard deviation of experiments in duplicate.

Differences were considered to be statistically significant when P<0.05.

RESULTS

Fatty acid composition

Results showed that the mastic tree oil contains five major fatty acids: oleic, palmitic, linoleic, palmitoleic and stearic acids. The differences between fatty acid compositions of oils extracted by the two methods and from the two sites were not significant. The main fatty acid was oleic acid with more than 56% of total fatty acids, followed by the palmitic and the linoleic acids with respective rates of 27 and 16% (Table 1). Unsaturated fatty acids represented more than 70% of the total fatty acids and they correspond to the oleic, the linoleic and the palmitoleic acids. The saturated /unsaturated fatty acids ratio was almost 0.4.

DPPH radical scavenging activity

There was a significant variation on the DPPH radical scavenging activity according to the site of harvest and the extracting process. The values of DPPH inhibition percentage of different oil samples are shown in Table 2. For both Nefza and Bizerte sites, oil extracted by the pressing method showed the highest DPPH radical scavenging activity. In the case of Nefza oil, the DPPH inhibition percentage was increased from 21% for the traditional method to 43% for the pressing one. Similarly, for Bizerte oils, this percentage was increased from 19 to 29% respectively. Therefore, in using the two extracting methods, Nefza oil had the greatest DPPH radical scavenging activity (43%).

Trolox equivalent antioxidant capacity

The linear relationship of absorbance versus Trolox concentration is presented in Figure 1. The highest TEAC was reached by the oil extracted using the pressing method. Nefza oil showed the most important TEAC with about 87 µg of Trolox/g of oil, while Bizerte oil had 20 µg of Trolox/g of oil as a TEAC value (Table 3). The pressing method allowed the improvement of the antioxidant activity of both Nefza and Bizerte oils. TEAC was increased from 43 to 87 µg of Trolox/g for Nefza oil and from 20 to 30 µg of Trolox/g of oil for Bizerte one.

Antibacterial activity

The *in vitro* antibacterial activity of the studied oils estimated by the diameter of inhibition, varied significantly according to the extracting process, the harvest site and the bacteria strains (Table 4). The most important bactericidal effect was reached by oil extracted using the pressing method. For both traditional and pressing process, Nefza oils had a slight activity contrary

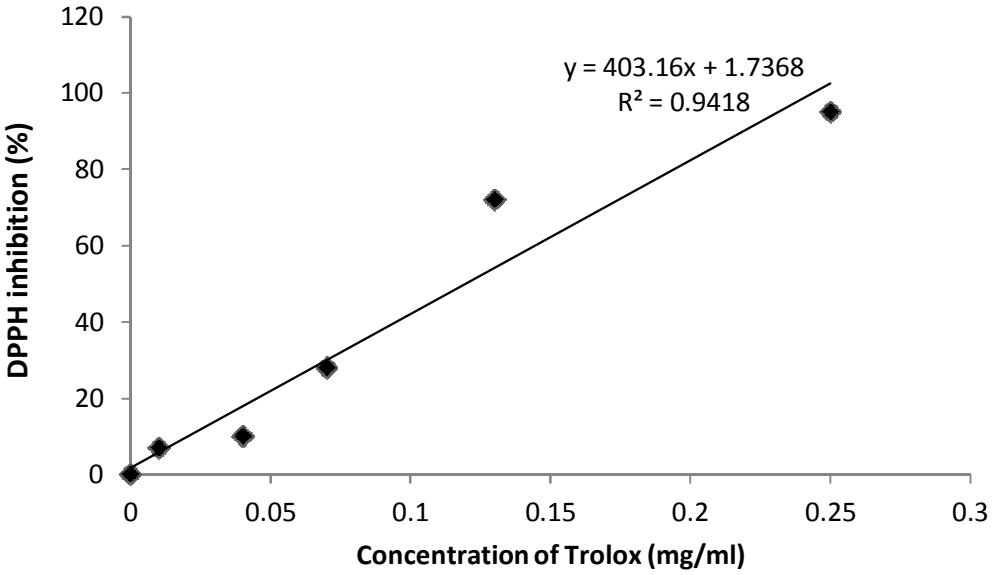

Figure 1. Linear relationship of absorbance decrease versus trolox concentration.

Table 3. Trolox equivalent antioxidant capacity of *P. lentiscus* fixed oils extracted by the traditional and the pressing methods.

Sites	Extraction method	TEAC(µg Trolox/g oil)
Nefza	Traditional	43 ± 1.06[a]
	Pressing	87.4 ± 0.58[b]
Bizerte	Traditional	20.7 ± 0.18[c]
	Pressing	30.9 ± 0.16[d]

Values followed by the same letter are not significantly different (P>0.05). Values represent mean ± standard deviation of experiments in duplicate.

Table 4. Antibacterial activity estimated by diameter of inhibition of *Pistacia lentiscus* fixed oil.

Sites	Extraction method	*C. perfringens*	*S. typhimurium*	*E. coli*
Nefza	Traditional	NA	NA	NA
	Pressing	NA	NA	NA
Bizerte	Traditional	8 ± 00[a]	NA	NA
	Pressing	13.33 ± 0.66[b]	NA	NA
Cefotaxime		15 - 21 mm		

NA, No antibacterial activity. Values followed by the same letter are not significantly different (P>0.05). Values represent mean ± standard deviation of experiments in triplicate.

to Bizerte oils. The highest activity was observed against *C. perfringens* with about 13 mm of inhibition zone recorded with Bizerte oil extracted by the pressing method. The inhibition diameter reached 8 mm for that extracted by the traditional one. The pressing method allowed a significant improvement of the bactericidal effect by increasing the inhibition diameter from 8 to 13 mm. This bactericidal effect is slightly lower than the minimal inhibition diameter determined for the Cefotaxime (15 mm). Meanwhile, against *S. typhimurium* and *E. coli,* oil samples showed a slight and non-significant bactericidal effect.

DISCUSSION

Fatty acid composition of the studied oils is consistent with that determined by Tej Yaakoubi and Dhaou (2007); the mainly fatty acid is the oleic one presenting more than 50% of the total fatty acid composition. This was also studied by Trabelsi et al. (2011), who showed the same profile for oleic and palmitic acids and a highest percentage of the linoleic one. This could be related to the difference in the method used for oil extraction or to the year of fruits harvest. The low saturated/unsaturated fatty acids ratio (0.4) indicates that this oil have a high content in unsaturated fatty acids which may make it more attractive for the consumer and gave it nutritional and dietetic properties. The profile of the fatty acid composition of *P. lentiscus* fixed oil is similar to that determined by Chahed et al. (2006) for *Pistacia vera* seed oil and to that considered by Ghalem and BenHassaini (2007) for *Pistacia atlantica*. The main fatty acid was the oleic acid for mastic tree, *P. vera* and *P. atlantica* oils with respective percentage of 56, 49 and 54%. *P. lentiscus* oil present a highest palmitic rate and this could explain its rapidly congealing at room temperature (Frénot and Vierling, 2002).

The DPPH radical scavenging activity of the studied oil showed an important antioxidant activity with about 43% compared to that determined for *P. vera* seed oil with 8% (Perez-Jimenez et al., 2008). The highest antioxidant activity was observed for oils extracted by the pressing method. This could be explained partially by an important amount of natural antioxidants compounds contained in this fixed oil, such as phenols (Stellman and Dufresne, 2000; Cioffi et al., 2010; Samaniego Sanchez et al., 2007). The difference between the oil extracted by pressing method and that extracted by traditional one is probably due to repeated exposure of oil on fire during the traditional extraction (Benhayoun and Lazzeri, 2007). This warming causes the thermal degradation of phenols and therefore the decrease of antioxidant activity (Larrauri et al., 1997).

The inhibitory effect of fatty acids on various microorganisms has long been known (Kabara, 1979). Various studies reported that linoleic and oleic acids, which are important constituents of *P. lentiscus* fixed oil, have potential antibacterial properties and are attributable to its unsaturated long-chain (McGaw et al., 2002). According to results found during this study, the differences between fatty acid compositions of studied oils were not significant. By this way, it's impossible to explain this bactericidal effect by the presence of fatty acids. On the other hand, many researches on other species attributed the bactericidal effect to the presence of phenols (Meccia et al., 2007; Rojas et al., 2007). In this study, Nefza oil, presenting the highest antioxidant activity and supposed to contain the highest rate of phenols, had the slightest antibacterial effect. That can be related to the variability of bactericidal effect according to the nature of phenols (Karou et al., 2005). Indeed, the molecular weight of polyphenols is an important factor controlling their antimicrobial activity (Field and Lettinga, 1992).

The results indicated that mastic tree fruit oil showed antibacterial activity only against the Gram-positive bacterium (*C. perfringens*). This activity was slight for Gram-negative strains. The Gram-positive and Gram-negative microorganisms differ in the structure of their cellular walls. The presence of lipoproteins and lipopolysaccharides in Gram-negative bacteria present a barrier to hydrophobic compounds such as mastic tree fixed oil (Zhao et al., 2001; Mazutti et al., 2008). This can explain the absence of bactericidal effect in the case of the two Gram-negative strains; *S. typhimurium* and *E. coli.*

In conclusion, we found that *P. lentiscus* fixed oil has a fatty acid composition similar to that determined for *P. atlantica* and *P. vera* oils and an antioxidant activity more important to that determined for the latest one. Both antioxidant and antibacterial activities were improved using the pressing method. This extraction process is more convenient and gave better quality of oil. These results suggest that *P. lentiscus* fruit oil is beneficial to human health, having the potential to be used for nutritional purposes as an interesting source of omega 6 and omega 9.

ACKNOWLEDGEMENTS

This study was financially supported by IRDC–Canada (subvention no. 105568-006) and WWF-Tunisia.

REFERENCES

Al-Habbal MJ, Al-Habbal Z and Huwez FU (1984). A double-blind controlled clinical trial of mastic and placebo in the treatment of duodenal ulcer. J. Clin. Exp. Pharmacol. Physiol. 11:541-544.

Ansel J (2002). Les arbres guérisseurs. Eyrolles, Paris, France. pp. 90-93.

Bentley RY, Trimen H (1980). Medicinal plants. J. and A Churchill, London. p. 276.

Brand-Williams W, Cuvelier ME, Berset C (1995). Use of a free radical method to evaluate antioxidant activity. Food Sci. Technol. 28:25-30.

Barton H, Folta M, Zachwieja Z (2005). Application of FRAP and DPPH methods to estimation of antioxydant activity of food products. Now. Lek. 74:510-513.

Benhayoun G, Lazzeri Y (2007). L'olivier en Méditerranée : du symbole à l'économie. Harmattan. France. p. 78.

Cioffi G, Pesca MS, De Caprariis P, Braca A, Severino L, De Tommasi N (2010). Phenolic compounds in olive oil and olive pomace from Cilento (Campania, Italy) and their antioxidant activity. Food Chem. 121:105-111.

Chahed T, Hamrouni I, Dhifi W, Msaada K, Kchouk ME, Marzouk B

(2006). Lipid evaluation during the development of Pistachio seed from the region of Kairouan (middle of Tunisia). J. Food Lipids 13:375-389.

Frénot M, Vierling E (2002). Biochimie des aliments: Diététique du sujet bien portant. Editions Doin, 2ème édition, Bordeaux. p. 224.

Field JA, Lettinga G (1992). Toxicity of tannic compounds to microorganisms. Plants Polyphenols: Synthesis, Properties, Significance. Basic Life Sci. 59:673-692.

Ghalem BR, Benhassaini H (2007). Etude des phytostérols et des acides gras de *Pistachia atlantica*. Afr. Sci. 03(3):405-412.

Kabara JJ (1979). Toxicological, bactericidal and fungicidal properties of fatty acids and some derivates. J. Am. Soc. Oil. Chem. 56:757-760.

Karou D, Dicko MH, Simpore J, Yameogo S, Sanon S, Traore AS (2005). Activités antioxydantes et antibactériennes des polyphénols extraits de plantes médicinales de la pharmacopee traditionnelle du burkina faso. Maitrise des procédés en vue d'améliorer la qualité et la sécurité des aliments, utilisation des OGM, analyse des risques en agroalimentaire.ouagadougou. pp. 8-11.

Larrauri JA, Rupérez P, Saura-Calixto F (1997). Effect of drying temperature on the stability of polyphenols and antioxidant activity of red grape Pomace peels. J. Agric. Food Chem. 45:1390-1393.

Mezghani S (1992). L'exploitation traditionnelle du maquis au nord de la Tunisie : possibilité d'une meilleure utilisation. Office de l'élevage et des pâturages. Tunis. pp. 99-158.

Metcalfe LD, Schimitz AA, Pelka JR (1966). Rapid preparation of fatty acid esters from lipids for gas chromatographic analysis. Anal. Chem. 38:514-517

McGaw LJ, Jäger AK, Staden van J (2002). Antibacterial effects of fatty acids and related compounds from plants, South Afr. J. Bot. 68:417-423.

Meccia G, Rojas L B, Velasco J, Díaz T, Usubillaga A (2007). Composition and Antibacterial Screening of the Essential Oils of Leaves and Roots of *Espeletiopsis angustifolia Cuatrec*, Nat. Prod. Commun. 2:1221-1224.

Mazutti M, Mossi AJ, Cansian RL, Corazza ML, Dariva CJ, OliveiraV (2008). Chemical profile and antimicrobial activity of Boldo (*Peumus boldus* Molina) extracts obtained by compressed carbon dioxide extraction. Braz. J. Chem. Eng. 25(02):427-434.

Perez-Jimenez J, Arranz S, Tabernero M, Dı´az- Rubio ME, Serrano J, Gon˜ i I,Saura-Calixto F (2008). Updated methodology to determine antioxidant capacity in plant foods, oils and beverages: Extraction, measurement and expression of results. Food Res. Int. 41:274-85.

Rejeb MN, Khouja ML, Ghrabi Z, Chemli R, Albouchi A, Khaldi A, Dahman M (2006). Guide des plantes médicinales et aromatiques. Maghreb Editions. Tunis. Tunisie. pp. 25-26.

Rojas J, Velasco J, Rojas LB, Díaz T, Carmona J, Morales A (2007). Chemical composition and antibacterial activity of the essential oil of *Baccharis latifolia* Pers. and *B. prunifolia* H. B. &K. (Asteraceae), Nat. Prod. Commun. 2:1245-1248.

Stellman JM, Dufresne C (2000). Encyclopédie de sécurité et de santé au travail. International labour organisation. Genève. pp. 417-420.

Sacchetti G, Maietti S, Muzzoli M, Scagliant M, Manfredini S, Radice M, Bruni R (2005). Chemical composition of the essential oil of *Stachys acerosa* and its antibacterial and antioxidant activities. Food Chem. 91:621.

Samaniego S´anchez C, Troncoso Gonz´alez AM, Garc´ıa-Parrilla MC, Quesada Granados JJ, L´opez Garc´ıa de la Serrana H, L´opez Mart´ınez MC (2007). Different radical scavenging tests in virgin olive oil and their relation to the total phenol content, Anal. Chim. Acta. 593:103-107.

Teyssou R (2007). Dictionnaire mémorable des remèdes d'autrefois. L'Harmattan, Paris, France. pp. 20-26.

Tej Yaakoubi M, Dhaou S (2007). Extraction et analyse de la composition acidique des huiles fixes de *Pistacia lentiscus* L. Revue des régions arides 1:30-39.

Trabelsi H, Aicha Cherif O, Sakouhi F, Villeneuve P, Renaud J, Barouh N, Boukhchina S and Mayer P (2011). Total lipid content, fatty acids and 4-desmethylsterols accumulation in developing fruit of *Pistacia lentiscus* L. growing wild in Tunisia. Food Chem. 131(2):434-440.

Zhao WH, Hu ZO, Okubo S, Hara Y, Shimamura T (2001). Mechanism of synergy between epigallocatechin gallate and lactams against methicillin-resistant *Staphylococcus aureus*. Antimicrob. Agents Chemother. 45:1737-1742.