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Full Length Research Paper

Antimicrobial activity evaluation of the oleoresin oil of *Pistacia vera* L.

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The hydrodistilled essential oils from *Pistacia vera*.L stem exudates have been tested against three bacteria (*Escherichia coli*, *Staphylococcus aureus* and *Proteus spp*) using three methods: agar disc diffusion, determination of MIC (minimum inhibitory concentration) and in the liquid phase by Maruzella method. The results obtained showed that essential oil resin of *P. vera* L. has antimicrobial activity against gram negative bacteria (*E. coli* and *Proteus spp.*) as well as gram positive bacteria (*S. aureus*), when *Proteus spp.* was the greatest inhibited of all the strains tested.

Key words: Essential oils, resin, *Pistacia vera* L., strains tested, antimicrobial activity.

INTRODUCTION

Among the aromatic plants belonging to the family of Anacardiaceae, the genus *Pistacia* is noteworthy for its numerous species and varieties of wild-growing plants. Many of these species are typical of the Mediterranean area. *Pistacia* has an economic value as it is the source of a traditional medicinal agent "gum" mastic, an oleoresin exudate from the stem of this plant (Dogan et al., 2003). It is a traditional natural remedy that has been used by very ancient Mediterranean civilizations like Greeks and Egyptians (Pellecuer et al., 1980; Peachey, 1995; Langenheim, 2003). In Algeria, it is found in four species, namely *Pistacia lentiscus*, *Pistacia terebinthus, Pistacia atlantica* and *Pistacia vera* (Belhadj, 1999). The latter, known as true pistachio (*Pistacia vera*) is characterized by a large tolerance to climatic variations; it can grow under slices, quite low rainfall and cope in all types of soils. In Algerian folk medicine, *P. lentiscus* has been used as an astringent, expectorant and cicatrisant agent (Benhammou et al., 2008).

About the other species of this plant, investigations have shown some pharmacological effects such as reducing blood pressure (Villar et al., 1987), anti-inflammatory (Giner et al., 2000; Giner et al., 2001) and antimicrobial action (Ali-Shtayeh and Abu Ghdeib, 1999; Magiatis et al., 1999).

The antiseptic activity of *P. lentiscus* essential oils and resin on different micro-organisms has been reported by several researchers (Tassou and Nychas,1995; Iauk et al., 1996; Ali-Shtayeh and Abu Ghdeib,1999; Marone et al., 2001; Ben Douissa et al., 2005; Benhammou et al., 2008) but the antimicrobial effect of *Pistacia vera* extracts precisely its oleoresin oils have not been studied so far (Duru et al., 2003; Kordali et al., 2003; Özçelik et al., 2005).

In this study, we aimed to detect a possible inhibitory effect of the oils extracted from oleoresin exudates by the *P. vera* stem on the growth of *Escherichia coli*, *Staphylococcus aureus* and *Proteus spp.* tested by using three methods: agar disc diffusion, determination of MIC (minimum inhibitory concentration) and in the liquid phase by Maruzella method.

MATERIALS AND METHODS

Plant material and extraction of the essential oil

Pistachio *(P. vera* L.) is a member of the Anacardiaceae family. The genus *Pistacia c*ontains about 11 species, of which *P. vera* is by far the most economically important (Alma et al., 2004). The *P. vera* tree is native to arid zones of Central and West Asia and distributed throughout the Mediterranean basin. The mastic gum is collected from the Institut Technique des Arbres Fruitiers – I.T.A.F - (Technical Institute for Fruit Trees) of Tighennif (Wilaya of Mascara) situated in the northwest of Algeria, during three different months; April, May and June, which corresponds to the period of oleoresin formation. The essential oil was extracted from resin by hydrodistillation with ethanol using a Clevenger apparatus. The combined hydroalcoholic extract was filtered through a filter paper and evapo-

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Table 1. Antibiogram results of *S. aureus, E. coli* and *Proteus spp.* with traditional antibiotics.

Antibiotic	Microorganisms					
	E.coli		*S. aureus*		*Proteus spp*	
	Inhibition zone diameter (mm)	Results	Inhibition zone diameter (mm)	Results	Inhibition zone diameter (mm)	Results
Ampicillin	11	I	40	S	23	S
Nitroxolin	23	S	22	S	11	I
Chloramphenicol	30	S	30	S	30	S
Erythromycin	12	I	11	I	-	-
Tetracycline	17	S	23	S	09	R
Trimetoprime-sulfamethoxazole	30	S	40	S	23	S
Docicyclin	17	S	-	-	-	-

R= resists; I = intermediary resistance; S= sensitive.

rated to dryness under reduced pressure in a Rotavapor (Heidolph Laborota 4000) and then stored in the dark at 4°C in an air tight container. The extract is further used for screening purposes.

Bacterial strains

All bacterial strains (*E. coli*, *Proteus spp.* and *S. aureus*) are provided by the Laboratory of medical analysis located in Dr. Yessaâd Khaled Hospital of Mascara city, situated in the west of Algeria from patients. *S. aureus* was isolated from the pus of a patient, *E. coli* from blood specimens while *Proteus spp.* is taen from a coproculture. The strains used in this work were identified and confirmed after morphological studies and by using biochemical tests in the Microbiological Laboratory of the Biological Institute of Mascara University (Euzéby, 1998; Marchal et al., 1982).

Determination of antimicrobial activity

Three methods were used to determine the antibacterial activity; agar disc diffusion method, determination of MIC (minimum inhibittory concentration) and dilution broth method.

The agar disc diffusion method was employed to determine the antimicrobial activities of the essential oil. Disc-assay was found to be a simple, cheap and reproducible practical method (Maidment et al., 2006). A suspension of each sample tested micro-organism diluted prior to 10^{-1}, 10^{-2} and 10^{-3} - (1 ml of 10^{8} cells/ml) was spread on a solid agar medium in Petri dishes (Mueller-Hinton agar). Filter paper discs (6 mm in diameter) were soaked in 13 µl of the resin oil and placed on the inoculated plates and allowed to dry for 15 min, then incubated at 37°C for 24 h. The diameters of the inhibition zones were measured in millimetres (Tepe et al., 2004).

Minimal inhibitory concentration (MIC) was taken from the concentration of the lowest dosed test tube showing visually no growth after 24 h. 10 µl from each visually ungrown test tube were subcultured on a Mueller-Hinton agar (Pauli and Kubeczka, 1996). *S.* aureus, *Proteus.spp.* and *E. coli* were diluted at 10^{-1}, 10^{-2} and 10^{-3}; a control is prepared with ethanol and oleoresin oil of *Pistacia vera.*

Each dilution of the slick strain is spread over the surface of the Petri dish containing the Mueller-Hinton agar medium liquid which must be dried at 37°C for 15 min.

Then four discs were placed on an agar containing the following quantities of the oleoresin oil dilution: 0.5, 1, 1.5, 2 and 2.5 µl. The control disc is impregnated with ethanol and placed in the Petri dish center, to be incubated toward the end at 37°C for 24 h.

Dilution broth susceptibility assay (Bouhadjera et al., 2005) was used for the antimicrobial evaluation. Stock solutions of the resin oils were prepared in ethanol by mixing 1 ml of the extracts with 9 ml of alcohol in test tubes to obtain the mother solution, followed by successive dilutions at 10^{-2} and 10^{-3}. The control was prepared by mixing 1 ml of distilled water with 9 ml of alcohol solution. 1 ml of each dilution and 0.5 ml of tested culture strains are added to 8 ml of a nutrient broth, maintained after in a Marie bath at 37°C under stirring for 24 h, then seeded by streaking the surface of the agar medium and incubated at 37 ° C for 24 h.

The antibiotic sensitivity of these isolates was examined. The antibiotics tested were: ampicillin, nitroxolin, chloramphenicol, erythromycin, tetracycline, docicyclin and trimetoprime-sulfamethoxazole.

RESULTS AND DISCUSSIONS

The results of the antibiogram (Table 1), showed that the *E. coli* strain has an intermediate resistance to ampicillin and erythromycin, sensitive to the remaining antibiotics tested. *Proteus spp.,* a second gram negative bacterium was sensitive to ampicillin, chloramphénicol and trimetoprime-sulfamethoxazole, resistant to tetracycline and showed intermediate resistance to nitroxolin.

On the other hand, *S. aureus* (gram positive bacterium) was sensitive to ampicillin, nitroxolin, chloramphénicol, tetracycline and trimetoprime-sulfamethoxazole and showed intermediate resistance to erythromycin. Plate 1 shows the antibiotic sensitivity testing against the three microorganisms.

In a preliminary experiment, we screened the effect of oil resin of *P. vera* against *S. aureus, E. coli* and *Proteus spp.* in disc diffusion assay.

Figure 1 summarizes the microbial growth inhibition by the oil resin of *Pistacia vera*, which showed good antibacterial activities against the three tested organisms. The results revealed that the oil resin showed antibacterial activity with varying magnitudes, depending on the size of inoculums and the concentration of resin oil. Diameter of inhibition zone of oil of resin of *P. vera* varied from 7 to 11.5

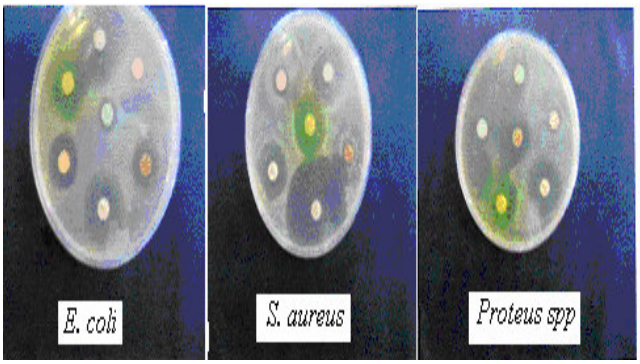

Plate 1. Antibiotic sensitivity testing against *S. aureus, E. coli* and *Proteus spp.*.

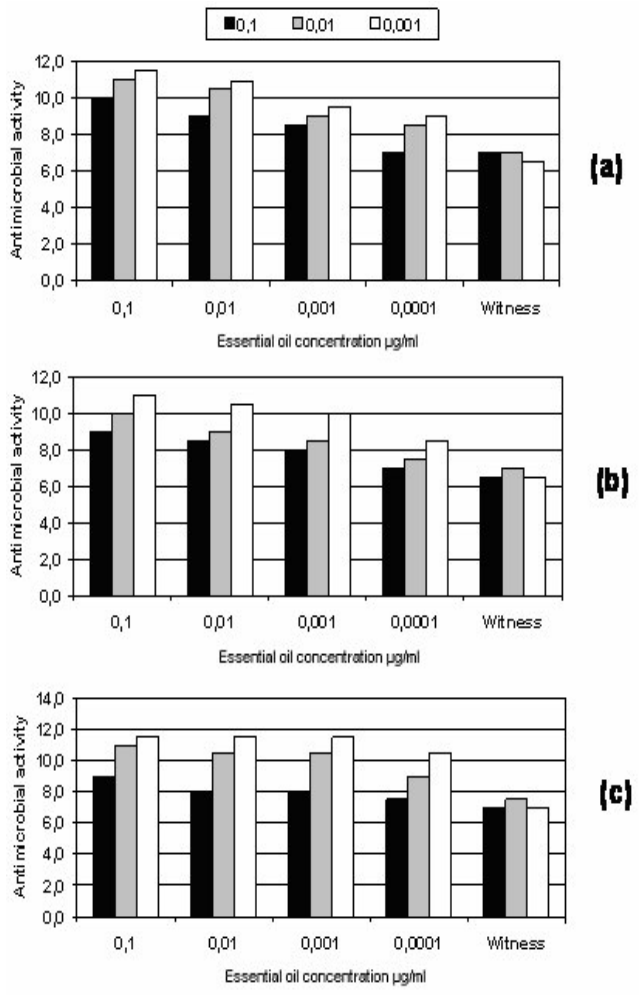

Figure 1. Antimicrobial activity evaluation of the essential oil resin of *P. vera* L. using the agar disc diffusion method; dilutions (0.1, 0.01 and 0.001); (a) *E. coli (b) S. aureus* and *(c) Proteus spp.*.

mm. The largest zone of inhibition was obtained for *E. coli* (10^{-3} dilution) with 10^{-1} µg/ml concentration of oil resin of *P .vera* and the lowest for *S. aureus* and *E. coli* (10^{-4} dilution) with 10^{-4} µg/ml concentration of oil resin of *P .vera.* A more significant inhibition was seen with a higher oil oleoresin concentration. At low concentrations, a very limited inhibitory effect was observed on the growth of microorganisms in comparison with those witnessed.

With increasing essential oil resin of *P. vera* concentration, an obvious inhibitory effect on the growth of *E. coli*, *Proteus spp.* and S. aureus, was significantly increased.

The addition of mastic gum oil in broth culture inoculated with *S. aureus*, *E. coli* and *Proteus spp.* inhibited the growth of these organisms. The rate of inhibition was greater, on gram negative bacteria (*E. coli* and *Proteus spp.*) than that observed on gram positive bacterium (*S. aureus*). In most cases the size of inoculum and the concentration of mastic gum oil affected the growth/survival of the organisms.

These results are almost similar to those shown by other works on the antimicrobial activity of oil mastic gum of *Pistacia vera* as well as those of similar species (Iauk et al., 1996; Koutsoudaki et al., 2005; Özçelik et al., 2005; Kamrani et al., 2007 and Benhammou et al., 2008).

The minimal inhibitory concentration (MIC) was defined as the lowest concentration of the test samples where the absence of growth was recorded (Glowniak et al., 2006). The results showed a variable effect of the essential oil on the microorganisms (Figure 2).

The MIC of the active oil resin of *P. vera* was tested at a volume ranging from 0.5 to 2.5 µl. The resin oil of *P. vera* showed an activity with MIC values ranging from 6.5 to 11 µg/ml against *E. coli,* 6.5 to 12 µg/ml against *Proteus spp.* and 6 - 12 µg/ml against *S. aureus*.

Like previous tests, the application of the dilution broth method confirms by its results shown in Table 2 the important antibacterial activity of the oil mastic gum of *P. vera* on these three microbial strains, as it seems that *Proteus spp.* is more sensitive than the other two.

The results indicated that the oil mastic gum of *P. vera* showed antibacterial activity, according to Alma et al. (2004), and Özçelik et al. (2005), mainly against the gram-negative bacteria (*E. coli* and *Proteus spp.*). The oil mastic gum also exhibited an effect against the gram-positive bacterium (*S. aureus*). However, this effect was less efficient than that presented against the gram-positive bacterium, since a higher MIC value was obtained with the gram-negative bacteria. Differences in MIC values of bacteria may be related to differential susceptibility of bacterial cell wall, which is the functional barrier to minor differences present in the outer membrane in the cell wall composition (Zhao et al., 2001). The gram-positive and gram-negative microorganisms differ in several aspects other than with respect to the structure of their cellular walls, mainly with regard to the presence of lipoproteins and lipopolysaccharides in gram-negative bacteria that form a barrier to hydrophobic compounds (Zhao et al., 2001; Mazutti et al., 2008).

According to Rang et al. (2001), these aspects have important implications in antibiotic action. On the other hand, these activities can be attributed, to a considerable

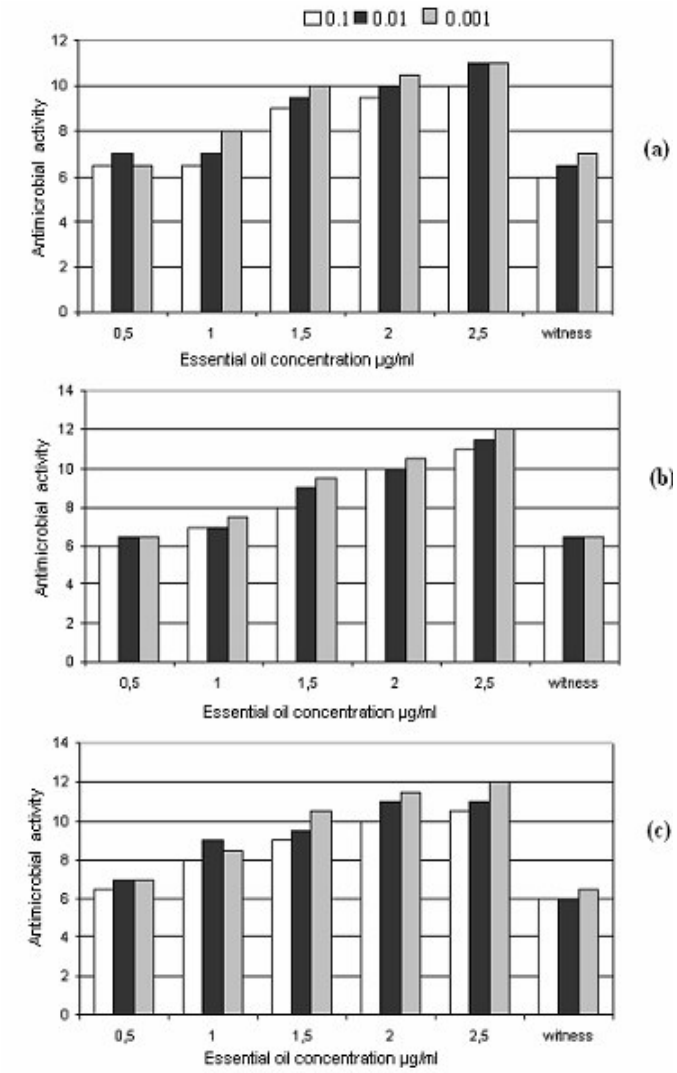

Figure 2. MIC evaluation of the essential oil resin of *P. vera* L.; (0.1, 0.01 and 0.001); (a) *E. coli, (b) S. aureus,* and *(c) Proteus spp.*

Table 2. MIC evaluation of the essential oil resin of *P. vera* using the dilution broth method against the three bacterial strains.

Bacterial strain	Essential oil (µg/ml)			
	Control	10^{-1} ,10^{-2}	10^{-3}, 10^{-4}	10^{-5}
E.coli	+++	+	++	+++
S. aureus	+++	+	++	+++
Proteus spp.	+++	+	+++	+++

++: Comparable growth with that Control +; slow growth.

degree, to the existence of its bioactive compounds such as carvacrol, camphene and limonene, which appeared to possess strong activities against all tested microbial strains (Alma et al., 2004).

Conclusion

The results of the antimicrobial activity tests indicate that the essential oil of *P. vera* mastic gum exhibited higher activity against the tested strains and confirms its traditional uses. However, oil mastic gum was found to inhibit both gram-positive and -negative bacteria. We believe that the present investigation together with previous studies provide a support to the antibacterial properties of this essential oil. These results suggest that the essential oil of *P. vera* mastic gum is beneficial to human health, having the potential to be used for medical purposes as a microbiostatic, antiseptic or as a disinfectant and to be utilized as anti-bacterial food additives.

REFERENCES

Ali-Shtayeh MS, Abu Ghdeib SI (1999). Antifungal activity of plant extracts against dermatophytes. Mycoses. 42: 665-672.

Alma MH, Nitz S, Kollmannsberger H, Digrak M, Efe FT, Yilmaz N. (2004). Chemical composition and antimicrobial activity of the essential oils from the gum of Turkish pistachio (*Pistacia vera* L.) J. Agric. Food Chem. Jun 6. 52(12):3911-4.

Belhadj S (1999). Pistachio situation in Algeria. FAO-IHEAM Nucis. Newsletter. 8: 30.

Ben Douissa, F. Hayder, Chekir-Ghedira, N. Mohamed, H. Ghedira ,K. Mariotte, A.M. Marie-Geneviève D.F (2005). New study of the essential oil from leaves of *Pistacia lentiscus* L. (Anacardiaceae) from Tunisia. Flavour Frag J. July/August. 20(4): 410-414.

Benhammou N, Atik Bekkara, F and Kadifkova Panovska, T (2008). Antioxidant and antimicrobial activities of the *Pistacia lentiscus* and *Pistacia atlantica* extracts. Afr. J. Pharm. Pharmacol. April. 2(2): 22–28.

Bouhadjera K, Bendahou ZM, Tabti B (2005). Anti-microbial Activity of Extracts from Algerian *Aristida pungens* L. Pak. J. Biol. Sci.. 8 (2): 206 -210.

Dogan O, Baslar S, Aydin H, Mert HH (2003). A study of the soil-plant interactions of *Pistacia lentiscus* L. distributed in the western Anatolian part of Turkey. Acta Bot. Croat. 62 (2):73–88.

Duru ME, Cakir A, Kordali S, Zengin H, Harmandar M, Izumi S, Hirata T (2003). Chemical composition and antifungal properties of essential oils of three Pistacia species. Fitoterapia 74: 170–176.

Euzéby JP (1998). *Enterobacteriaceae*. Dictionnaire de Bactériologie Vétérinaire. Centre Interuniversitaire de Calcul de Toulouse (CICT), France. http://www.bacdico.net.

Giner EM, Manez S, Giner RM (2000). The anti-inflammatory and anti-phospholipase A_2 activity of extract from lanostane-rich species. J. Ethnopharmacol. 73: 61-69.

Giner EM, Manez S, Recio C, Giner RM, Prieto JM (2001). Oleanonic acid, a 3-oxotritrepene from *Pistacia* inhibits leukotriene synthesis and has anti-inflammatory activity. Eur. J. Pharmacol. 428: 137-143.

Glowniak P, Łos R, Skalicka-Wozniak K, Widelski J, Burczyk J, Malm A (2006). Activity of *Crithmum maritimum* L. (Apiaceae) against Gram-positive bacteria. XIX(2)17. Annales universitatis Mariae Curie - Skłodowska . Polonia.

Iauk L, Ragusa S, Rapisarda A, Franco S, Nicolosi V M (1996). In vitro antimicrobial activity of *Pistacia lentiscus* L. extracts: Preliminary report. J. Chemotherapy 8:207–209.

Kamrani YY, Amanlou M, Esmaeelian B, Moradi BS, SahebJamei M (2007). Inhibitory Effects of a Flavonoid-Rich Extract of *Pistacia vera* Hull on Growth and Acid Production of Bacteria Involved in Dental Plaque. Int. J. Pharmacol. 3 (3): 219-226.

Kordali S, Cakir A, Zengin H, Duru ME (2003). Antifungal activities of the leaves of three Pistacia species grown in Turkey. Fitoterapia 74 : 164–167.

Koutsoudaki C, Krsek M, Rodger A (2005).Chemical Composition and Antibacterial Activity of the Essential Oil and the Gum of *Pistacia lentiscus* Var. *chia.* J. Agric. Food Chem. 53 (20): 7681 -7685.

Langenheim JH (2003). Plant resins: Chemistry, evolution, ecology, and ethnobotany. Timber Press, Portland.

Magiatis P, Melliou, E, Shaltsounis, AL, Chinou, IB, Mitaku S (1999). Chemical composition and antimicrobial activity of the essential oils of *Pistacia lentiscus* var. Chiao. Planta Med. 65: 749-752.

Maidment C, Dyson A, Haysom I (2006). A study into the antimicrobial effects of cloves (*Syzgium aromaticum*) and cinnamon (*Cinnamomum zeylanicum*) using disc-diffusion assay. Nutr. Food Sci. 36 (4): 225 – 230.

Marchal N, Bourdon JL, Richard C (1982). Les milieux de culture pour l'isolement et l'identification biochimique des bactéries. Doin éditeurs -Paris.

Marone P, Bono L., Leone E, Bona S, Carretto E , Perversi L (2001). Bactericidal activity of *Pistacia lentiscus* mastic gum against *Helicobacter pylori*. J. Chemother. 13(6): 611–614.

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. April – June.

Özçelik B, Aslan M, Orhan I, Karaoglu T (2005). Antibacterial, antifungal, and antiviral activities of the lipophylic extracts of *Pistacia vera*. Microbiol. Res. 160:159-164.

Pauli A, Kubeczka KH (1996). Evaluation of Inhibitory Data of Essential Oil Constituents Obtained with Different Microbiological Testing Methods; in "Essential Oils: Basic and Applied research", Ch. Franz, A. Mathe G, Buchbauer (eds): Proceedings of the 27th International Symposium on Essential Oils, Allured Publishing Corporation. pp. 33-36.

Peachey CP (1995). Terebinth Resin in Antiquity: Possible Uses in the Late Bronze Age Aegean Region. Masters Thesis Nautical Archaeology Program. Texas University, USA.

Pellecuer J, Jacob M, Simeon DM, Dusart G, Attisto M, Barthez M., Gourgas L, Pascal B, Tomei R (1980). Essais d'utilisation d'huiles essentielles de plantes aromatiques méditerranéennes en odontologie conservatrice. Plant Médicin Phytothér 14: 83-98.

Rang HP, Dale MM, Ritter JM (2001). Farmacologia, in Mazutti M, Mossi AJ, Cansian RL,Corazza ML, Dariva CJ, Oliveira V (2008).Chemical profile and antimicrobial activity of Boldo (*Peumus boldus Molina*) extracts obtained by compressed carbon dioxide extraction. Braz. J. Chem. Eng. April – June. 25(02): 427- 434.

Tassou CC, Nychas GJE (1995). Antimicrobial activity of the essential oil of mastic gum (*Pistacia lentiscus* var. *chia*) on Gram positive and Gram negative bacteria in broth and in model food system. Int. Biodeter. Biodegrad. 36 (3-4): 411-420.

Tepe B, Donmez E, Unlu M, Candan F, Daferera D, Vardar-Unlu G, Polissiou M, Sokmen A (2004). Antimicrobial and antioxidative activities of the essential oils and methanol extracts of *Salvia cryptantha* (Montbret et Aucher ex Benth.) and *Salvia multicaulis* (Vahl). Food Chem. March. 84(4): 519-525

Villar A, Sanz MJ Paya M (1987). Hypotensive effects of *Pistacia lentiscus* L. Int. J. Crud Drug Res. 25: 1-3.

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