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Full Length Research Paper

Essential oil from gum of *Pistacia atlantica* Desf.: Screening of antimicrobial activity

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The essential oil from the gum of *Pistacia atlantica* Desf. grown in Algeria was obtained by the hydrodistillation method, and its antimicrobial activities against the growth of clinical isolates of *Staphylococcus aureus, Escherichia coli* and *Streptococcus pyogenes* were evaluated using three different methods; agar disc diffusion and dilution broth methods and minimum inhibitory concentration (MIC) which was subsequently, determined. The results of the study revealed that essential oil resin of *P. atlantica* has antimicrobial activity against gram-positive and -negative bacteria which are resistant to commonly used antimicrobial agents, and they were considerably dependent on concentration.

Key words: Gum of *Pistacia atlantica* Desf., essential oil, antimicrobial activities, *Staphylococcus aureus*, *Escherichia coli*, *Streptococcus pyogenes.*

INTRODUCTION

Infectious diseases are the leading cause of death world-wide. Antibiotic resistance has become a global concern (Westh et al., 2004). The clinical efficacy of many existing antibiotics is being threatened by the emergence of multidrug-resistant pathogens (Bandow et al., 2003). *Staphylococcus aureus* is mainly responsible for post-operative wound infections, toxic shock syndrome, endo-carditis, osteomyelitis and food poisoning (Mylotte, 1987). *Streptococcus pyogenes*, like *S. aureus*, causes an array of suppurative diseases and toxinoses, in addi-tion to some autoimmune or allergic diseases (Todar, 2008). *Escherichia coli* is present in the human intestine and causes lower urinary tract infection, coleocystis or septi-caemia (Singh et al., 2000). These pathogens can acquire antimicrobial resistance to prevent effective dis-ease treatment (Reinert et al., 2001; Sundqvist and Kahl-meter, 2005) . For this reason research is ongoing for new antimicrobial agents, either by the design and synthesis of new agents or through the search of natural sources for as yet undiscovered antimicrobial agents (Cock, 2008). Essential oils have been traditionally used for the treat-ment of infections and diseases all over the world for centuries (Rios and Recio, 2005). "Gum" mastic, an oleo-resin exudate from the stem of *Pistacia lentiscus* has a long history of use as a therapeutic agent with many re-ported medicinal properties (Demirci, 2001). Amongst its therapeutic properties, it has been implicated in the relief of upper abdominal discomfort, stomachaches, dyspepsia and peptic ulcer (Al- Said et al., 1986; Huwez and Al-Hab-bal, 1986). *Pistacia* species have also been reported to possess stimulant and diuretic properties (Bentleyand and Trimon 1980). The antimicrobial activity of *P. lentis-cus* essential oils and its resin against different microorg-anisms has been reported by several researchers (Tassou and Nychas, 1995; Ben Douissa et al., 2005; Benhammou et al., 2008) but little is known of the bacte-ricidal effect of *P. atlantica* Desf. extracts, precisely its oleoresin oils.

In this study we evaluate the antibacterial activity of es-sential oils extracted from mastic gum, a resin obtained from the *P. atlantica* tree, against clinical isolates of *S. aureus, S. pyogenes* and *E. coli.*

MATERIALS AND METHODS

Plant material and essential oil extraction

The resin of *P. atlantica* (pistachio tree of the Atlas) was collected from the Ain Fkain region, 24 km from Mascara, west of Algeria be-tween May-June 2006, which corresponds to the period of oleoresin formation. The essential oil was extracted from the resin the resin by hydrodistillation with ethanol. The combined hydroalcoholic ex-

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tract was filtered through filter paper and evaporated to dryness under reduced pressure in a Rota-vapor and then stored in the dark at 4°C with no air contact. The extract was further used for screening purposes (Benhassaini et al., 2003).

Microbial strains

The gram positive bacteria (*S. aureus and S. pyogenes)* and the gram negative bacteria (*E. coli)* were obtained from urine and stool samples respectively from patients attending Dr Yessaâd Khaled Hospital at Mascara city, west of Algeria. The bacterial samples were identified by standard biochemical tests and morphological studies (Marcha et al., 1982; Euzéby, 1998) in the Microbiology Laboratory, Biology Institute, Mascara University, Algeria.

Antimicrobial screening

Three different methods were employed for the determination of antimicrobial activities; an agar disc diffusion and dilution broth methods, followed by minimal inhibitory concentration (MIC) determination.

Agar disc diffusion method

In vitro antibacterial activity of *P. atlantica* essential oil was determined by the agar disc diffusion method according to Benhassaini et al. (2003). Disc assays were found to be a simple, cheap and reproducible practical method (Maidment et al., 2006). 18 ml of sterilized Mueller Hinton agar medium was taken in each Petri dish and then spread with a suspension of the tested micro-organism (average concentration is 10^6 cells/ml). Sterilized Whatman's No. 3 filter papers (6 mm diameter) were thoroughly moistened with 15 µl of the oil and placed on the seeded agar plates and then incubated at 37°C for 24 h. The diameters of the inhibition zones were measured in millimeters.

Minimal inhibitory concentration (MIC) determination

The minimal inhibitory concentration (MIC) is the lowest concentra-tion of an antimicrobial agent that will inhibit the visible growth of a micro-organism after overnight incubation. Minimum inhibitory con-centrations are important in diagnostic laboratories to confirm resis-tance of micro-organisms to an antimicrobial agent and also to monitor the activity of new antimicrobial agents (Andrews, 2001). 3 dilutions each of *S. aureus, S. pyogenes* and *E. coli* strains 10^{-1}, 10^{-2} and 10^{-3} were prepared. The diluted bacterial strains were spread over the surface of the Petri dish containing Mueller-Hinton agar medium.4 discs of 6 mm diameter are placed on agar containing the following quantities of the resin oil dilution: 0.5, 1, 1.5 and 2 l. In the center of the Petri dish, a control disc is impregnated in para-llel with 2 l of ethanol. The Petri dishes are then incubated at 37°C for 24 h.

Dilution broth method

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. 1 ml of each dilution and 0.5 ml of bacterial strains were added to test tubes containing 8 ml of nutrient broth, maintained later in a Marie bath at 37°C under stirring for 24 h, then seeded by streaking the surface of the agar medium and incubated for 24 h at 37°C.

S. aureus *S. pyogenes* *E. Coli*

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

The MIC of penicillin G, paromomycin, gentamicin, cefazolin, amoxicillin, nitroxoline, vanomycine, oxacillin, chloramphenicol, colistin, neomycin, pristinamycin, trimethoprim-sulfamethoxazole and ampicillin were also determined in parallel experiments in order to control the sensitivity of the test micro-organisms.

RESULTS AND DISCUSSIONS

The antibiotic susceptibility pattern of the gram negative and positive bacteria was determined (Table 1, Plate 1). The most effective antibiotics for *S. aureus* were neomycin, chloramphenicol, trimethoprim - sulfamethoxazole and nitroxoline, but for the second gram positive bacteria, *S. pyogenes*, they were penicillin G, gentamicine and amoxicillin. While, the most effective antibiotics for the gram-negative (E. *coli*) bacteria were chloramphenicol, neo-mycin and cefazolin.

Disc diffusion is one of the most common assays used in the evaluation of antibacterial activity of essential oils. Figure 1 shows the *in vitro* antimicrobial property of the essential oil resin of *P. atlantica* of three bacterial strains, with their three dilutions exposed at different concentrations to oil resin of *P. atlantica.* Antimicrobial activity by disc diffusion method showed that the oil resin of *P. atlantica* was most active against *E. coli* followed by *S. aureus* and *S. pyogenes.* The oil resin at all volumes showed potent inhibitory activity against the tested micro-organisms, with the exception of 10^{-1} dilution of the strain *S. pyogenes* with 10^{-4} of essential resin where there were no reports of inhibition. The diameter of the inhibition zone of oil resin of *P. atlantica* varied from 0 to 9 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. atlantica* and the lowest for *S. pyogenes* (10^{-4} dilution) with 10^{-1} µg/ml concentration of oil resin of *P . atlantica.* A more significant inhibition was seen with a higher oil oleo-resin concentration. The oil resin at 10^{-2} and 10^{-3} µg/ml showed moderate activity. On one hand, the growths of tested bacteria in high concentrations of oil resin were highly inhibited, where it was considered that these orga-nisms were sensitive to the oil. On the other hand, at low concentrations, a very limited inhibitory effect was observed

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

Antibiotics	Micro-organisms: *E. coli*		*S. aureus*		*S. pyogenes*	
	Inhibition zone diameter (mm)	**Results**	**Inhibition zone diameter (mm)**	**Results**	**Inhibition zone diameter (mm)**	**Results**
Penicillin G	-	-	-	-	29	S
Paromomycin	-	-	6	R	-	-
Gentamicin	-	-	-	-	25	S
Cefazolin	17	S	10	I	-	-
Amoxicillin	-	-	-	-	20	S
Nitroxoline	09	R	20	S	-	-
Vanomycine	-	-	-	-	18	I
Oxacillin	13	I	10	I	-	-
Chloramphenicol	20	S	24	S	08	I
Colistin	-	-	-	-	04	R
Neomycin	29	S	19	S	-	-
Pristinamycin	-	-	-	-	02	R
Trimethoprim-sulfamethoxazole	2	R	24	S	-	-
Ampicillin	14	I	-	-	-	-

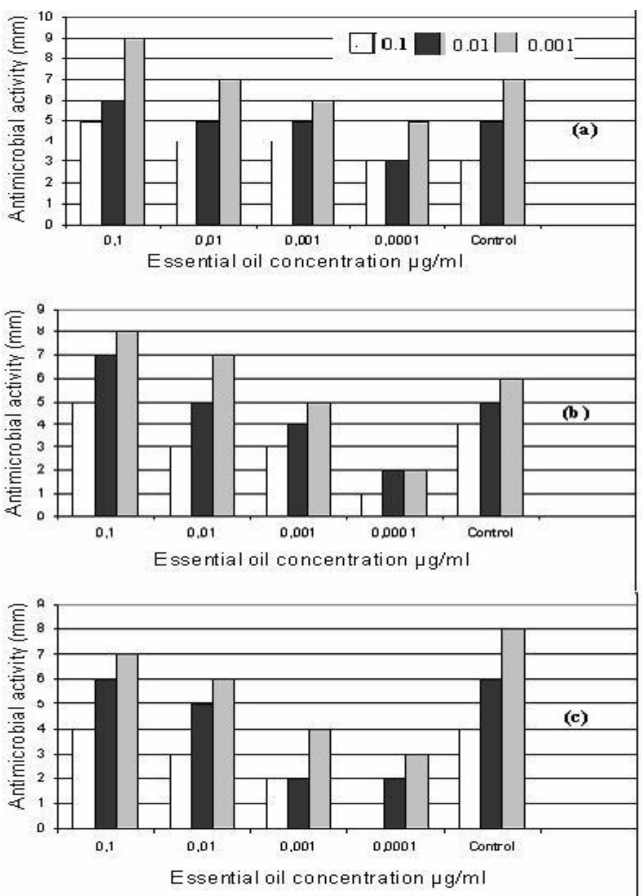

Figure 1. Antimicrobial activity evaluation of the essential oil resin of *P. atlantica* using agar disc diffusion method. Dilutions (0.1, 0.01, and 0.001); (a) *E. coli,* (b) *S. aureus,* and (c) *S. pyogenes*.

on the growth of micro-organisms in comparison with those witnessed.

The gram-positive bacterial strains (*S. aureus and S. pyogenes*) were more susceptible to the extracts as compared to the gram-negative bacteria (*E. coli*). This is in agreement with previous reports that plant extracts are more active against gram-positive than gram-negative bacteria (Iauk et al., 1996; Koutsoudaki et al., 2005; Özçelik et al., 2005; Kamrani et al., 2007 and Benhammou et al., 2008).

Similarly, when the minimal inhibitory concentration was evaluated using the resin oil of *P. atlantica* against gram-negative and positive bacteria, similar results to the disc diffusion method was produced.

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 oils on the micro-organisms (Figure 2). The MIC of the active essential oils of the resin oil of *P. atlantica* was tested at a volume ranging from 0.5 to 2.0 µl. The minimum inhibitory concentration (MIC) for the resin oil of *P. atlantica* showed an activity with values ranging from 3 - 11 g/ml against *E. coli,* 1 - 10 g/ml against *S. aureus* and 0 - 8 g/ml against *S. pyogenes* (Figure 2). *S. aureus* and *S. pyogenes* on the one hand were susceptible at 0.5 µg/ ml and *E.coli* on the other hand was tolerant to this concentration (0.5 µg/ml).

Gram-negative bacteria were more resistant to the essential oils and can be attributed in part to the great complexity of the double membrane-containing cell envelope in contrast to the single membrane structure of gram-positive bacteria (Bagamboula et al., 2004). The data

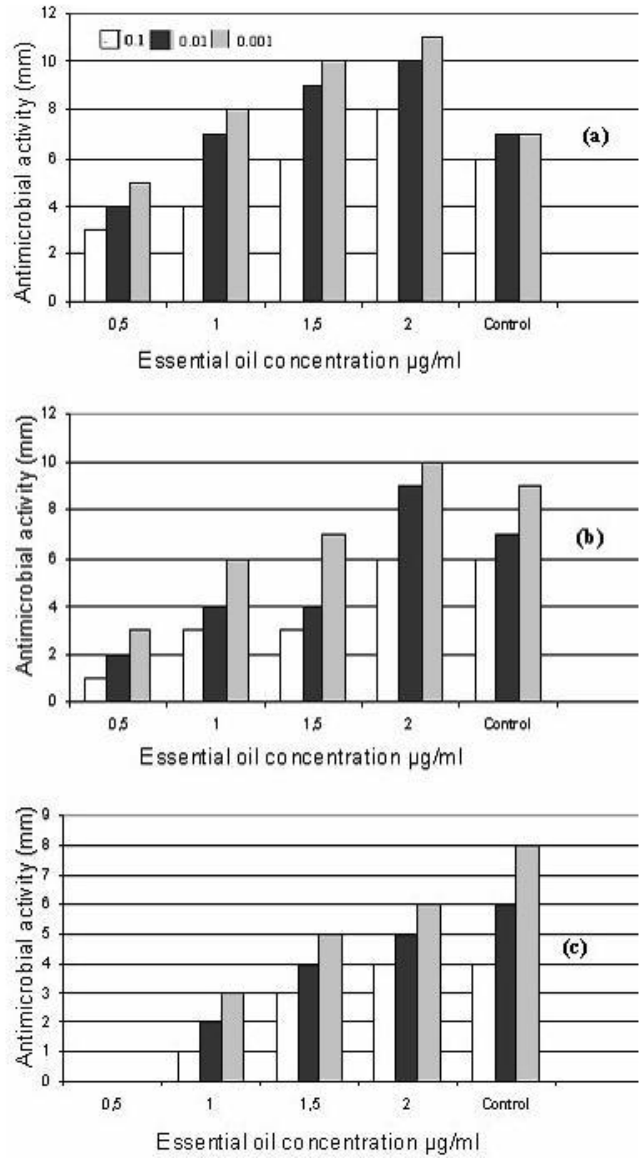

Figure 2. MIC evaluation of the essential oil resin of *P. atlantica* (0.1, 0.01 and 0.001), (a) *E. coli,* (b) *S. aureus,* and (c) *S. pyogenes.*

showed that, all bacterial strains were sensitive to 10^{-1} µg/ml (Table 2). With an increasing dose of essential oil resin of *P. atlantica* , the resulting diameter of the zone of inhibition increased for all the organisms.

The results of the study revealed that essential oil resin of *P. atlantica* have antibacterial activity against gram-positive as well as gram-negative bacteria. Some authors have reported that gram-positive micro-organisms are slightly more sensitive to essential oils when compared to gram-negative (Canillac and Mourey, 2001; Dermetzos and Perdetzoglou, 2001). This lower sensitivity of gram-negative organisms has been related to the presence of an outer membrane surrounding their cell wall, which restricts the diffusion of hydrophobic compounds through its lipopolyssacharide covering (Vaara, 1992).

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

Bacterial strain	**Essential oil (µg/ml)**				
	Control (0 µg/ml)		**10^{-1}**	**10^{-2}**	**10^{-3}**
E.coli	++	S/C	-	-	+
		D/C	-	-	+
S. aureus	++		-	+	++
S. pyogenes	++		-	+	++

S/C : sample concentration - D/C : double concentration ; ++ : comparable growth with control; + : slow growth; - : growth inhibition.

Some researchers reported that there is a relationship between the chemical structures of the most abundant in the tested essential oil and the antimicrobial activity. Essential oils rich in phenolic compounds such as *Pistacia* specie are widely reported to possess high levels of antimicrobial activity (Malekzadeh, 1974; Yalpani and Tyman, 1983; Conner and Beuchat, 1984; Marner et al., 19-91; Kubo et al., 1993; Ben Douissa, 2005). On the other-hand, it should be noted that the two major volatile constituents, -pinene and terpinolene contained in the *Pistacia* specie are compounds with interesting antibacterial activity (Tsokou et al, 2007).

Conclusion

In this study, the antimicrobial activity of the essential oils resin of *P. atlantica* was studied. The oil showed activity against *S. aureus, S. pyogenes* and *E. coli* which are used as gram-negative and -positive bacterial models, respectively. The 3 studied methods confirm that the resin and its essential oil have inhibiting effects according to the dilution of the strain on one hand and the concentration of resin and essential oil on the other.

The oil was found to have significant antibacterial activity and therefore can be used as a natural antimicrobial agent for the treatment of several infectious diseases caused by these germs, which have developed resistance to antibiotics.

REFERENCES

Al-Said MS, Ageel AM, Parmar NS, Tariq M (1986). Evaluation of mastic, a crude drug obtained from *Pistacia lentiscus* for gastric and duodenal anti-ulcer activity. J. Ethnopharmacol. 15: 271-278.

Andrews JM (2001). Determination of minimum inhibitory concentrations. J. Antimicrob. Chemother. 48 (1): 5-16.

Bagamboula CF, Uyttendaele M, Candan F, Daferera D, Unli GV, Polissiou M, Sokmen A (2004). Antimicrobial and antioxidative activities of the essential oils and methanol extracts of *S. cryptanha* (Montbret et Aucher ex Benth.) and *S. multicaulis* (Vahl.). Food Chem. 84: 519-525.

Bandow JE, Brotz H, Leichert LIO, Labischinski H, Hecker M (2003). Proteomic approach to understanding antibiotic action. Antimicrob Agents Chemother 47: 948-955.

Ben Douissa F, Hayder N, Chekir-Ghedira L, Hammami M, Ghedira K, Mariotte AM, Dijoux-Franca MG (2005). New study of the essential oil from leaves of *Pistacia lentiscus* L. (Anacardiaceae) from Tunisia.

Flavour Fragrance J. 20: 410-414.

Benhammou N, Bekkara AF, Panovska KT (2008). Antioxidant and antimicrobial activities of the *Pistacia lentiscus* and *Pistacia atlantica* extracts .Afri. J. Pharm. Pharmacol. 2:22–28.

Benhassaini H, Benabderrahmane M, Chikhi K (2003).Contribution à l'évaluation de l'activité antiseptique de l'oléorésine et des huiles essentielles du pistachier de l'Atlas sur certaines sources microbiennes: *Candida albicans* (ATC 20027), *Candida albicans* (ATCC 20032) et *Saccharomyces cerevisiae'*.: ethnopharmacologie. fév.N°. 30. pp. 38-46.

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.

Canillac N, Mourey A (2001). Antimicrobial activity of the essential oil of *Picea excelsa* on *Listeria*, *Staphylococcus aureus* and coliform bacteria. Food Microbiol. 18: 261-268.

Cock IE (2008). Antimicrobial Activity of *Aloe barbadensis* Miller Leaf Gel Components. Int. J. Microbiol. 4 (2). http://www.ispub.com/ostia/index.php?xmlFilePath=journals/ijmb/vol4n2/gel.xml

Conner DE, Beuchat LR (1984). Sensitivity of heat-stressed yeasts to essential oils of plants. Appl. Environ. Microbiol*.* 47:229-233.

De Billerbeck V-G, Roques C, Vanière P, Marquier P (2002). Activité antibactérienne et antifongique de produits à base d'huiles essentielles. Hygiènes. 10(3): 248-251.

Demirci F, Baser KHC, Calis IE, Gokhan (2001). Essential Oil and Antimicrobial Evaluation of the *Pistacia eurycarpa*. Chemistry of Natural Compounds. July. 37(4) : 332-335.

Dermetzos C, Perdetzoglou DK (2001). Composition and antimicrobial studies of the essential oils of *Origanum calcaratum* Juss. and *O. scabrum* Boiss. et Heldr. From Greece. J. Essent. Oil Res*.* 3: 460-462.

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

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.

Hammer, KA, Carson, CF, Riley TV (1999). Antimicrobial activity of essential oils and other plant extracts. J. Appl. Microbiol*.*, 86: 985-990.

Helander IM, Alakomi HL, Latva-Kala K, Mattila-Sandholm T, Pol I, Smid EJ, Gorris LGM, Wright AV (1998). Characterization of the action of selected essential oil components on gram-negative bacteria. J. Agric. Food Chem*.*, 46, 3590-3595.

Huwez FU, Al-Habbal MJ (1986). Mastic in treatment of benign gastric ulcers. Gastroenterol Jpn. 21:273–274.

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

Janssen AM, Scheffer JJ, Baerheim SA (1987). Antimicrobials activities of essential oils. Pharmaceutics Week lad (Scientific Edition) 9: 193-197.

Kamrani YY, Amanlou M, Esmaeelian B, Bidhendi MS, 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.

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.

Kubo I, Muroi H, Kubo A (1993). Antimicrobial activity of long-chain alchol's against *Streptococcus* mutants. J. Agric. Food. Chem. 48: 2143-2145.

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

Malekzadeh F (1974) .An antimicrobial compound in two *Pistacia* species. Mycopathologia .October. 54(1): 73-77.

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

Marner FJ, Freyer A, Lex J (1991).Triterpenoids from gum mastic, the resin of *Pistacia lentiscus*. Phytochemistry 30: 3709-3712.

Mylotte JM, McDermott C, Spooner J (1987). Prospective study of 114 consecutive episodes of *Staphylococcus aureus* bacteria. Rev. Infec. Dis. 9: 981–907.

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.

Reinert RR, Luetticken R, Briefs C, Neuberger N, Lemperle M, Al-lahham A (2001). Antibiotic Resistance of *Streptococcus pyogenes* and *Streptococcus pneumoniae* Strains Isolated from RTI of Outpatients in Germany, 2000-2001. Interscience Conference on Antimicrobial Agents and Chemotherapy 41st ICAAC Chicago. USA. December 16-19. Abstract No. C2-693.

Rios JL, Recio MC (2005) Medicinal plants and antimicrobial activity. J. Ethnopharmacol. 100: 80–84.

Shapiro S, Meier A, Guggenheim B (1994). The antimicrobial activity of essential oils and essential oil components towards oral bacteria. Oral Microbiol. Immunol. 9: 202-208.

Singh R, Chandra R, Bose M, Luthra PM (2000). Antibacterial activity of *Curcuma longa* rhizome extract on pathogenic bacteria. Curr. Scien. 83(6): 737-740.

Sundqvist M, Kahlmeter G (2005). *E. coli* and *S. aureus* antimicrobial resistance surveillance and the effect of excluding duplicate isolates in a consecutive database 1990–2003.15th European Congress of Clinical Microbiology and Infectious Diseases. Copenhagen / Denmark, April 2-5.

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. Biodeterior. Biodegrad. pp. 411- 420.

Tepe B, Daferera D, Sokmen M, Polissiou M, Sokmen A (2004).In vitro antimicrobial and antioxidant activities of the essential oils and various extracts of *Thymus eigii* M. Zohary et P.H. Davis. J. Agric. Food Chem. 52: 1132-1137.

Tepe B, Donney E, Unlu M, Candan F, Daferera, D, Unlu, GV, Polissiou, M, Sokmen A (2004). Antimicrobial and antioxidative activities of the essential oils and methanol extracts of *S. cryptanha* (Montbret et Aucher ex Benth.) *S. multicaulis* (Vahl.). Food Chem. 84, 519-525.

Todar K (2008). Todar's Online Textbook of Bacteriology. University of Wisconsin – chapter. Bacterial Pathogens of Humans. Page 3. www.textbookofbacteriology.net.

Tsokou A, Georgopoulou K, Melliou E, Magiatis P, Tsitsa E (2007).Composition and Enantiomeric Analysis of the Essential Oil of the Fruits and the Leaves of *Pistacia vera* from Greece. Molecules. 12: 1233-1239.

Vaara M (1992).Agents that increases the permeability of the outer membrane. Microbiol. Rev. 56: 395-411.

Velickovic DT, Randjelovic NV, Ristic MS, Smelcerovic, A.A and Velickovic, A.S. (2002). Chemical composition and antimicrobial action of the ethanol extracts of *S. pratensis* L. *S. glutinosa* L. *S. aethiopis* L. J. Serbia Chem. Soc. 67, 639-646.

Westh H, Zinn CS, Rosdahl VT, Sarisa Study Group (2004). An internatio- nal multicenter study of antimicrobial consumption and resistance in *Staphylococcus aureus* isolates from 15 hospitals in 14 countries. Microbiol. Drug Resist. June, 10(2): 169-176.

Yalpani M, Tyman JHP (1983).Long-chain phenols.24. The phenolic acids of *Pistacia vera*. Phytochemisrty 22: 2263-2266.