International Scholars Journals

African Journal of Agricultural Marketing ISSN 2375-1061 Vol. 8 (9), pp. 001-004, September, 2020. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

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

Bachir Raho Ghalem* and Benali Mohamed

Faculty of Biotoxicology Laboratory Science, Institute of Biology, Djillali Liabès University of Sidi Bel Abbès, Algeria.

Accepted 05 May, 2020

The hydrodistilled essential oils from the exudates of *Pistacia vera* L. stems have been tested against three bacteria, *Escherichia coli*, *Staphylococcus aureus* and *Proteus* using three methods: agar disc diffusion method, determination of MIC (minimal inhibitory concentration) and in the liquid phase, by Maruzella method. The results obtained showed that *E. coli* was the greatest inhibitor of all the strains tested, and that Gram negative bacteria (*E. coli* and *Proteus* spp.) showed more inhibition than that observed on Gram positive bacteria (*S. aureus*) by the essential oil tested.

Key words: Essential oils, oleoresin of *Pistacia vera* L., strains tested, antimicrobial activity determination.

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 traditional medicinal agent "gum" mastic, an oleoresin exudates from the stem of this plant (Dogan et al., 2003). It is a traditional natural remedy used in very ancient civilizations in the Mediterranean like Greek and Egyptian (Pellecuer et al., 1980; Langenheim, 2003; Peachey, 1995). In Algeria, it is found in four species, namely *Pistacia lentiscus*, *Pistacia terebinthus, Pistacia atlantica* and *Pistacia vera*. According to ecology, the true pistachio (*P. vera*) is characterized by a large tolerance to climatic variations; it can grow under slices rainfall quite low and can cope in soils. In Algerian folk medicine, *Pistacia* has been used as an astringent, expectorant and cicatrisant agent (Benhammou et al., 2008).

About the other genus of this plant, investigations have shown some pharmacological effects such as reducing blood pressure (Villar and Paya, 1987), anti- inflammatory (Giner et al., 2001; Giner et al., 2000) and antimicrobial action (Ali-Shtayeh and Abu, 1999; Magiatis et al., 1999). The antiseptic activity of *P. lentiscus* essential oils and its resin on different microorganisms has been reported by several researchers (Tassou and Nychas, 1995; Iauk et al., 1996; Ali-Shtayeh and Abu, 1999; Marone et al., 2001; Benhammou et al., 2008; Douissa et al., 2005) 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; Özçelika et al., 2005) . In this study, we aimed to detect the inhibitory effect of the oils extracts from exudates of oleoresin from the *P. vera* stem on the growth of *Escherichia coli*, *Staphylococcus aureus* and *Proteus* tested by using three methods: agar disc diffusion method, determination of MIC (minimal inhibitory concentrations) and in the liquid phase, by Maruzella method.

MATERIALS AND METHODS

Plant material and extraction of the essential oil

The essential oil of *P. vera* was extracted from the stem oleoresin by water distillation. The mastic gum was collected from the Technological Institute of fruit trees –T.I.F.T- of Tighennif (Wilaya of Mascara) situated in the northwest of Algeria, in the months of April,

*Corresponding author. E-mail:bachir_raho@yahoo.fr. Tel: 00213771063841.

Table 1. Antimicrobial activity evaluation of the essential oil resin of *P. vera* with agar disc diffusion method.

Bacterial strain	Resin concentration (µg/ml)					
	Dilution	10^{-1}	10^{-2}	10^{-3}	10^{-4}	Standard
E.coli	10^{-1}	10	9	8.5	7	7
	10^{-2}	11	10.5	9	8.5	7
	10^{-3}	11.5	10.9	9.5	9	6.5
S.aureus	10^{-1}	9	8.5	8	7	6.5
	10^{-2}	10	9	8.5	7.5	7
	10^{-3}	11	10.5	10	8.5	6.5
Proteus spp.	10^{-1}	9	8	8	7.5	7
	10^{-2}	11	10.5	10.5	9	7.5
	10^{-3}	11.5	11.5	11.5	10.5	7

May and June, which corresponds to the period of the oleoresin formation.

Bacterial strains

All bacterial strains (*E. coli*, *Proteus* spp and *S. aureus*) were provided by the Laboratory of Medical Analysis-–located in Dr. Yessaâd Khaled Hospital (YKH) of Mascara City, situated in the west of Algeria for patients suffering from certain infectious diseases. *S. aureus* was isolated from the pus of a patient, *E. coli* from blood specimens while *Proteus* was taken from a coproculture, and then confirmed by biochemical tests and morphological studies in Microbiology Laboratory of Biology Institute in the Mascara University (Euzéby, 1998; Marchal et al., 1982).

Antimicrobial activity determination

We used three methods to determine the antibacterial activity; agar disc diffusion method, determination of MIC (Minimal Inhibition Concentrations) and in the liquid phase, by Maruzella method. The agar disc diffusion method was employed to determine the anti-microbial activities of the essential oils in question. 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 the solid media plates. Filter paper discs (6 mm in diameter) were soaked in 13 µl of the resin oil and placed on the inoculated plates and, after drying for 15 min, were 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. 10 µl from each visually no grown test tube was subcultured on Mueller-Hinton agar (Pauli and Kubeczka, 1996) . Each strain from the three *S. aureus*, *Proteus* spp and *E. coli* was diluted at 10^{-1}, 10^{-2} and 10^{-3} (1 ml of 10^{8} cells/ml); a standard was prepared with ethanol and oleoresin oil of *P. vera.* From each dilution a slick strain was spread over the surface of the Petri dish containing Mueller-Hinton agar medium liquid which was dried at 37°C for 15 min. Four discs were placed on agar containing the following quantities of the oleoresin oil dilution: 0.5, 1, 1.5, 2 and 2.5 l. Ethanol was added to the standard disc, placed in the Petri dish center, and was incubated toward the end at 37°C for 24 h. The principal technique of Maruzella method is to act in the liquid phase of increasing concentrations of oleoresin essential oil, after emulsifier addition (Singh et al., 2000; Larrondo et al., 1995). Serial dilutions (10^{-1}, 10^{-2} and 10^{-3} µml) were prepared from this essential oil solution. 1 ml of each dilution and 0.5 ml of tested culture strains were added to 8 ml of nutrient broth, maintained in a Bain Marie to 37°C under agitation for 24 h and then seeded by streaking the surface of agar medium and incubated at 37°C for 24 h.

RESULTS AND DISCUSSION

As indicated in Table 1, all strain bacteria were inhibited by essential oil resin of *P. vera*. A more significant inhibition was seen with a higher oleoresin oil concentration. At low concentrations, a very limited inhibitory effect was observed on the growth of microorganisms in comparison with those of the standard. With increasing essential oil resin of *P. vera* concentration, an obvious inhibitory effect on 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* , *Proteus* spp), than that observed on Gram positive bacteria (*S. aureus*). In most cases the size of inoculum and the concentration of mastic gum oil affect the growth/survival of the organisms. These results are almost similar to those shown in other work on the antimicrobial activity of oil mastic gum of *P. vera* as well as those of similar species (Iauk et al., 1996; Koutsoudaki et al., 2005; Kamrani et al., 2007; Benhammou et al., 2008 and Özçelik et al., 2005). Minimal inhibitory concentration (MIC) values were defined as the lowest concentration of oils that completely inhibited microbial growth. The results were expressed in micrograms per millilitre. The results for the MIC are shown in Table 2. The MIC values regarding the antimicrobial activity of oil mastic gum of *P. vera* against Gram negative bacteria *(E. coli* and *Proteus* spp.) and *S. aureus* (Gram positive bacteria) were determined to be

Table 2. MIC evaluation of the essential oil resin of *P. atlantica.*

Bacterial strain	Resin concentration (µg/ml)						
	Dilution	0.5	1	1.5	2	2.5	Standard
E.coli	10^{-1}	6.5	6.5	9	9.5	10	6
	10^{-2}	7	7	9.5	10	11	6.5
	10^{-3}	6.5	8	10	10.5	11	7
S. aureus	10^{-1}	6	7	8	10	11	6
	10^{-2}	6.5	7	9	10	11.5	6.5
	10^{-3}	6.5	7.5	9.5	10.5	12	6.5
Proteus spp.	10^{-1}	6.5	8	9	10	10.5	6
	10^{-2}	7	9	9.5	11	11	6
	10^{-3}	7	8.5	10.5	11.5	12	6.5

Table 3. MIC evaluation essential oil resin of *P. atlantica* with the three bacterial strains.

Bacterial strain	Essential oil (µg/ml)			
	Standard «0»	10^{-1} ,10^{-2}	10^{-3}, 10^{-4}	10^{-5}
E.coli	+ ++	+	++	+++
S. aureus	+++	+	++	+++
Proteus spp	+++	+	+++	+++

++: Comparable growth with that witness.
+: Slow growth.

1.5 and 2.0 µg/ml, respectively. 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 bacteria (*S. aureus*). However, this effect was less efficient than that presented against the Gram-negative bacteria, since a higher MIC value was obtained with the Gram-positive 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 outer membrane in the cell wall composition (Zhao et al., 2001). Like previous tests, the application of the liquid phase method confirmed(by its results showed in Table 3) 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 change of inhibitory effect depends on the natural substance concentration.

Conclusion

The results of the antimicrobial activity tests indicate that essential oil of mastic gum *P. Vera* exhibited higher activity against the tested strains and confirm its traditional uses. However, oil mastic gum was found to inhibit both gram-positive and gram-negative bacteria. We believe that the present investigation together with previous studies provide a support to the antibacterial properties of this essential oil. It can be used as an antibacterial supplement in developing countries towards the development of new therapeutic agents to treat several infectious diseases caused by these pathogens.

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): 3911-3914.

Douissa Ben F, Hayder, Chekir-Ghedira N, Mohamed H, Ghedira K, Mariotte AM, Marie-Geneviève DF (2005). New study of the essential oil from leaves of *Pistacia lentiscus* L. (Anacardiaceae) from Tunisia. Flavour and Fragrance J., 20(4): 410-414.

Benhammou N, Atik Bekkara F, Kadifkova Panovska T (2008).

Antioxidant and antimicrobial activities of the Pistacia lentiscus and *Pistacia atlantica* extracts. Afric. J. Pharm. Pharmacol., 2(2): 22–28.
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. Dictionary of Veterinary Bacteriology (Dictionnaire de Bactériologie Vétérinaire). Inter-University Computing Center of 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.
Iauk L, Ragusa S, Rapisarda A, Franco S, Nicolosi VM (1996). *In vitro* antimicrobial activity of *Pistacia lentiscus* L. extracts: Preliminary report. J. Chemotherapy, 8: 207–209.
Kamrani YY, Amanlou M, Esmaeelian B, Moradi Bidhendi S, 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 Jean H (2003). Plant resins: Chemistry, evolution, ecology, and ethnobotany. Timber Press, Portland.
Larrondo JV, Agut M, Calvo TM (1995). Antimicrobial activity of essences from labiates. Microbios, 82(332): 171-172.
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.
Marchal N, Bourdon JL, Richard C (1982). The culture media for the isolation and biochemical identification of bacteria (Les milieux de culture pour l'isolement et l'identification biochimique des bactéries) in french. Doin Editors - 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. Chemotherapy 13(6): 611–614.
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 K-H (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. Master Thesis. Nautical Archaeology Program, Texas University, USA.
Pellecuer J, Jacob M, Simeon DeBouchberg M, Dusart G, Attisto M, Barthez M, Gourgas L, Pascal B, Tomei R (1980). Attempts to use the essential oils of Mediterranean aromatic plants in conservative odontology. (Essais d'utilisation d'huiles essentielles de plantes aromatiques méditerranéennes en odontologie conservatrice) in french. Plant Médicin Phytothér., 14: 83-98.
Singh R, Chandra R, Bose M, Luthra PM (2000). Antibacterial activity of *Curcuma longa* rhizome extract on pathogenic bacteria. Curr. Scien., 83(6): 25.
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., 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 lactams against methicillin-resistant *Staphylococcus aureus*. Antimicrob Agents Chemother, 45: 1737- 1742.