JNFS
Journal of Nutrition and Food Security
Shahid Sadoughi University of Medical Sciences
School of Public Health
Department of Nutrition
Nutrition & Food Security Research Center

eISSN: 2476-7425 pISSN: 2476-7417 JNFS 2019; 4(3): 186-190 Website: jnfs.ssu.ac.ir

Antimicrobial Activity and Chemical Composition of Pistachia Atlantica Gum Sub Sp. Kurdica. Essential Oil

Hasan Ellahi; MSc[1], Elham Khalili Sadrabad; PhD[1], Seyed Hossein Hekmatimoghaddam; MD[2], Ali Jebali; PhD[3], Jalal Sadeghizadeh-yazdi[4]; PhD, Fatemeh Rastiani; MSc[1] & Fateme Akrami Mohajeri; PhD[*1]

[1] *Zoonotic Diseases Research Center, Department of Food Hygiene and Safety, School of Public Health, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
[2] *Department of Advanced Medical Sciences and Technologies, School of Paramedicine, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
[3] *Department of Laboratory Sciences, School of Paramedicine, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
[4] *Departmeny of Food Sciences and Technology, School of Public Health, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*

ARTICLE INFO

ORIGINAL ARTICLE

Article history:
Received: 30 Dec 2017
Revised: 3 Mar 2018
Accepted: 26 May 2018

***Corresponding author:**
Fateme.akrami@gmail.com
Zoonotic Diseases Research Center, Department of Food Hygiene and Safety, School of Public Health, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.

***Postal* code:** 1981619573
***Tel*:** +989136517764

ABSTRACT

Background: *Staphylococcus aureus*, *Salmonella enterica*, *Escherichia Coli* (*E. Coli*) and *Listeria monocytogenes* are considered as important foodborne pathogens. *Pistachia atlantica* sub sp. *Kurdica*, called wild pistachio, has been known as an antimicrobial compound. The aim of this study was to determine the antimicrobial activity and chemical composition of this essential oil (EO) on some of foodborne pathogens. ***Methods:*** The EO of *Pistachia atlantica* was obtained by hydro-distillation and analyzed by GC-MASS. The antibacterial effects of *Pistachia atlantica* were evaluated at two concentrations of 10 and 15 µL against *Staphylococcus aureus*, *E. Coli*, *Salmonella enterica*, and *Listeria monocytogenes* using disk diffusion method. The analysis was done by SPSS. ***Results:*** In the current study, α-pinene (92.5%) and ß-pinene (1.62%) were the main components of *Pistachia atlantica* EO. The EO was most effective on *Salmonella enterica*, whereas, its effect on *Listeria monocytogenes* was the weakest. The results showed a significant difference in reducing *Salmonella enterica* in comparison to others ($P < 0.05$). ***Conclusion:*** The EO has inhibitory effects on the studied bacteria. Therefore, this EO can be used as a natural preservative to extend the shelf life of foods.

Keywords: *Pistachia atlantica; antimicrobial; Listeria monocytogenes; Staphylococcus aureus; Escherichia Coli; Listeria monocytogenes*

Introduction

Food can be contaminated by pathogenic bacteria including *Salmonella enterica*, *Staphylococcus aureus*, *Escherichia Coli (E. Coli)*, and *Listeria monocytogenes*, which can cause several problems in gastro intestinal tract. During the last decades, extensive use of antimicrobial

This paper should be cited as: Ellahi H, Khalili Sadrabad E, Hekmatimoghaddam SH, Jebali A, Sadeghizadeh-yazdi J;, Rastiani F, et al**. *Antimicrobial Activity and Chemical Composition of Pistachia Atlantica Gum Sub Sp. Kurdica. Essential Oil.*** *Journal of Nutrition and Food Security (JNFS)*, 2019; 4 (3): 186-190.

drugs has led to bacterial resistance in human beings (Moshafi *et al.*, 2004). Essential oils (EO) are aromatic oils extracted from plants by different methods (Burt, 2004). Essential oils have been recognized as natural and harmless antimicrobial agents for the environment and Generally Recognized As Safe (GRAS) in food application (Nawel *et al.*, 2013). The antibacterial effects of EOs depend on the type of plant, meteorological conditions, place of growth, as well as drying and processing methods. The use of oil plants in Iran as an antioxidant or antibiotic was traced back to ancient times (Akrami *et al.*, 2015). *Pistachio atlantica* subsp. *Kurdica*, called Baneh, is one of the wild pistachio species (grows in Zagros forests), which belongs to the *Anacardiacea* family (Karimi *et al.*, 2009). Three subspecies of *cabulica*, *kurdica*, and *mutica* grow in Iran. Over 1,200,000 hectares of western, central, and eastern parts of Iran are covered by *kurdica* and *mutica* subspecies (Farhoosh *et al.*, 2008). *Pistacia atlantica* tree grows in the western (mainly), central, and eastern part of Iran. The local people use this tree to produce ingredients in some food, jams, and gum (Hatamnia *et al.*, 2014). The gum obtained from this tree consists of oleoresin. This essential oil contains α-Thujene, α-Pinene, Camphorene, Sabinene, β-Pinene, Δ3-Carene Limonene, as well as alpha-tropineol, armanderen, and caprylic acid (Sharifi and Hazell, 2011). The results of previous studies show that the presence of resin in Pistachio has an inhibitory effect on the growth of *clostridium botulinum*, *Staphylococcus aureus*, *E. Coli*, and *Streptococcus* (Aksoy *et al.*, 2006, Daifas *et al.*, 2004). In another study, the antimicrobial activity of *Pistachio atlantica* Subsp., i.e., *kurdica* species were found to have positive results on helicobacter pylori (Sharifi and Hazell, 2011).The aim of the current study was to investigate the inhibition effect of *Pistachio atlantica* Subsp. *Kurdica* gum essential oil on *staphylococcus aureus*, *Salmonella*, *E. Coli*, and *Listeria monocytogenes*.

Materials and Methods

Preparation of the essential oil of Pistachia atlantica gum Subsp. Kurdica: Pistachia atlantica sub sp. *Kurdica* gum was collected from Kordestan province in Iran. The herbarium approval of the plant was confirmed by the Institute of Medicinal Plants, Medical University of Tehran, Iran. The gum was placed inside the Clevenger along with distilled water and the extraction was carried out by steam distillation method. The ratio of the achieved essence to gum weight was also calculated. The oil was kept at low temperature for further analysis (Mohajeri *et al.*, 2018, Rahimi *et al.*, 2019).

GC-MASS analysis of essential oil: The components of gum EO were isolated by Gas Chromatography/Mass Spectrometry (GC model 9-A, Shimadzu, Japan, and GC/MS model Varian 3400, USA) using a DB-5 column with a length of 30 m and an inner diameter of 0.25 mm. Later, these components were identified by comparing their mass spectrum with the range of combinations found in the computer database and valid compositions. The oven temperature was increased from 60 to 250 °C with an increment rate of 5 °C per minute. At the end, it was kept for 10 minutes at 250 °C. Helium carrier gas with a flow rate of 1.1 mL/min and ionization energy of 70 electron volts was used. The separation ratio was 1 to 50 and the injection volume was 100 µ (Rahimi *et al.*, 2019, Sharifi and Hazell, 2011).

Determination of antimicrobial properties of Pistacia atlantica gum EO by disk diffusion method: In this study, four standard bacterial strains were used including *Listeria monocytogenes* (PTCC 1298), *Escherichia coli* (ATCC 2592), *Staphylococcus aureus* (ATCC 25923), and *Salmonella enterica* (PTCC 14028). At first, a microbial suspension equivalent to 0.5 McFarland turbidity standards (approximate density of 1.5×10^8 CFU/mL) was prepared from each strain. Then, 100 µL of each bacterial suspension with a density of 10^6 CFU/mL was cultured by spreading on the surface of the Mueller-Hinton agar medium. A 5-mm diameter sterile paper disk was placed on the medium and 15 µL of pure EO was injected into the disk. Plates were incubated for 24 hours at 37° C. Then, the diameter of the inhibition zone was measured. All treatments were carried out in triplicate (Akrami *et al.*, 2015).

Data analysis: The experimental data were collected and analyzed using one-way ANOVA by SPSS. In all calculations, P-value of less than 0.05 was considered statistically significant.

Table1. Chemical composition of *P. atlantica* subsp. *Kurdica* gum essential oil

Type of chemical composition	Percentage	Retention Time (%)
Tricyclene	0.15	11.15
α-pinene	92.08	11.78
Camphene	0.67	12.61
Sabinene	0.36	13.79
ß-pinene	1.61	14.06
Myrcene	0.27	14.62
δ-2-carene	0.35	15.63
ρ-cymene	0.26	16.61
Limonene	0.51	16.77
1,8-cineole	0.17	16.95
Terpinolene	0.11	16.69
Verbenone	0.20	26.19
Isiborny1 acetate	0.30	29.53
Total Identified	97.02	

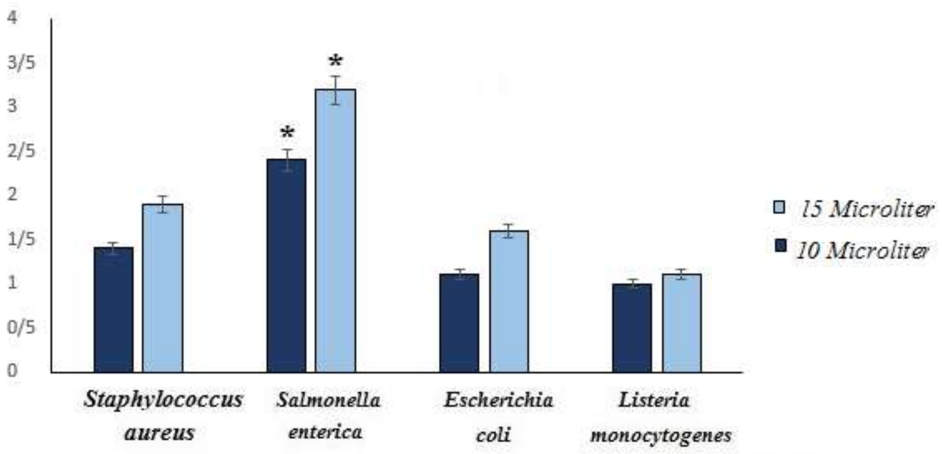

Figure 1. Growth inhibition of *Staphylococcus aureus*, *Salmonella enterica*, *Escherichia coli*, and *Listeria monocytogenes* at a concentration of 10^6 CFU/mL by the *Pistacia atlantica* gum EO after 24 hours of exposure

*Significant at the level of $P < 0.05$

Results

Chemical composition of *Pistacia atlantica* gum EO

The oil yield of the *Pistacia atlantica* gum EO was evaluated as 10% (w/w). About 97.02% of the components of gum EO were identified (approximately 13 compounds); of which α-pinene (92.08%) and β-pinene (1.61 %) were predominant (**Table 1**). Given that α-pinene was the main ingredient of *Pistacia atlantica* gum EO, the antimicrobial effects may be due to the presence of this component.

Antibacterial activity in disk diffusion method

The growth rates of *Staphylococcus aureus*, *Salmonella enterica*, *Escherichia coli*, and *Listeria* *monocytogenes* at concentration of 10^6 CFU/mL after 24 hours of exposure with *Pistacia atlantica* gum EO are shown in **Figure 1**. The maximum inhibition zone diameter was related to *S. enterica*, while the minimum value was attributed to *L. monocytogenes*. The results showed a significant difference in reduction of *Salmonella enterica* in comparison to others ($P < 0.05$). This result indicated that by increasing the concentration, the diameter of inhibition zone increased significantly ($P < 0.05$).

Discussion

Nevertheless, making definite conclusion in this regard is difficult, since other compounds such as ß-pinene, sabinene, and camphene exist in *Pistacia atlantica* gum EO, which may have contributed to the inhibitory effect (Delazar *et al.*, 2003, Mecherara-Idjeri *et al.*, 2008). Minaiyan et al. reported 38 compounds in the *Pistacia atlantica* tree's EO, among which α-pinene (41.23%), β-pinene (6.85), and trans-verbenol (5.39%) had the highest concentrations (Minaiyan *et al.*, 2015). In a similar study on the *Pistacia atlantica* composition in Marven region, Iran, α-pinene comprised about 70% of the EO (Delazar *et al.*, 2003). This difference in amount may be due to the different species or geographic regions (Benhammou *et al.*, 2008, Ghalem and Mohamed, 2009).

The agar diffusion test showed that *Salmonella enterica* had the highest sensitivity to the EO, while *Listeria monocytogenes* had the least sensitivity. So, it can be concluded that the *Pistacia atlantica* gum EO had an inhibitory effect on both gram positive and gram negative bacteria. In a similar study, the antimicrobial effect of *Pistacia lentiscus* leaf EO against gram-negative bacteria (such as *pseudomonas* and *salmonella*) and gram-positive bacteria (such as *Staphylococcus aureus*, *Bacillus* and *Enterococcus*) was investigated by disk diffusion method. *Salmonella* and *Enterobacter* had a higher sensitivity to the EO (Derwich *et al.*, 2010). The results of our study are in agreement with those reported by Gholem and Mohamed research. Their findings indicated that α-pinene had an antibacterial agent in the *Pistacia atlantica's* gum (Ghalem and Mohamed, 2009). The effect of EO on different bacteria may vary according to the laboratory conditions, the gum harvesting season, the gum's freshness or being processed, as well as the equipment and method of the extraction. Benhammou et al. studied the effect of *Pistacia atlantica*'s gum on eight bacteria (*E coli*, *Klebsiella pneumoneae*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Enterobacter cloacea*, *Proteus mirabilis*, *Listeria monocytogenes*, and *Staphylococcus aureus*) using disk diffusion method. They showed that *S. typhi* and *S. aureus* had the most sensitivity, while *K. pneumoneae* and *L. monocytogenes* were the most resistant bacteria (Benhammou *et al.*, 2008). A study on the effect of *Pistacia atlantica*'s gum EO on *Streptococcus mutans* by disc diffusion method showed that by increasing the concentration from 20 to 50 mg/mL, the inhibition zone diameter increased (Delazar *et al.*, 2003).

Conclusion

The results of this study indicated that *Pistachia atlantica* subsp. *kurdica* had inhibitory effects on *Staphylococcus aureus*, *Salmonella enterica*, *Escherichia coli*, and *Listeria monocytogenes*. Thus, by studying the organoleptic effects of *Pistachia atlantica*, this compound can be used as a natural preservative for increasing the shelf life of food products.

Authors' contribution

Akrami, Hekmati moghaddam, Jebali, Sadeghizadeh Yazdi, and Khalili conceived and designed the experiments. Ellahi and Rastiani carried out the experiment. All authors contributed to the final version of the manuscript.

Acknowledgement

This manuscript is part of a MSc. thesis in food hygiene and safety department at Public Health School at Shahid Sadoughi University of Medical Sciences, Yazd, Iran.

Conflict of interests

The authors declare no conflict of interests regarding the publication of this paper.

References

Akrami F, et al. 2015. Antioxidant and antimicrobial active paper based on Zataria (Zataria multiflora) and two cumin cultivars (Cuminum cyminum). *LWT-Food Science and Technology*. **60 (2)**: 929-933.

Aksoy A, Duran N & Koksal F 2006. In vitro and in vivo antimicrobial effects of mastic chewing gum against Streptococcus mutans and mutans streptococci. *Archives of Oral Biology*. **51 (6)**: 476-481.

Benhammou N, Bekkara FA & Panovska TK 2008. Antioxidant and antimicrobial activities of the Pistacia lentiscus and Pistacia atlantica extracts. *African Journal of Pharmacy and Pharmacology*. **2 (2)**: 022-028.

Burt S 2004. Essential oils: their antibacterial properties and potential applications in foods—a review. *International Journal of Food Microbiology*. **94 (3)**: 223-253.

Daifas DP, et al. 2004. Effects of mastic resin and its essential oil on the growth of proteolytic Clostridium botulinum. *International Journal of Food Microbiology*. **94 (3)**: 313-322.

Delazar A, Nazemieh H, Modaresi M & Afshar J 2003. Study on essential oil obtained from oleoresin of Pistacia atlantica var. mutica.

Derwich E, Manar A, Benziane Z & Boukir A 2010. GC/MS analysis and in vitro antibacterial activity of the essential oil isolated from leaf of Pistacia lentiscus growing in Morocoo. *World Applied Sciences Journal*. **8 (10)**: 1267-1276.

Farhoosh R, Tavakoli J & Khodaparast MHH 2008. Chemical composition and oxidative stability of kernel oils from two current subspecies of Pistacia atlantica in Iran. *Journal of the American Oil Chemists' Society*. **85 (8)**: 723.

Ghalem B & Mohamed B 2009. Antimicrobial activity evaluation of the oleoresin oil of Pistacia vera L. *African Journal of Pharmacy and Pharmacology*. **3 (3)**: 092-096.

Hatamnia AA, Abbaspour N & Darvishzadeh R 2014. Antioxidant activity and phenolic profile of different parts of Bene (Pistacia atlantica subsp. kurdica) fruits. *Food Chemistry*. **145**: 306-311.

Karimi H, Zamani Z, Ebadi A & Fatahi M 2009. Morphological diversity of Pistacia species in Iran. *Genetic Resources and Crop Evolution*. **56 (4)**: 561-571.

Mecherara-Idjeri S, Hassani A, Castola V & Casanova J 2008. Composition of leaf, fruit and gall essential oils of Algerian Pistacia atlantica Desf. *Journal of Essential Oil Research*. **20 (3)**: 215-219.

Minaiyan M, Karimi F & Ghannadi A 2015. Anti-inflammatory effect of Pistacia atlantica subsp. kurdica volatile oil and gum on acetic acid-induced acute colitis in rat. *Research Journal of Pharmacognosy*. **2 (2)**: 1-12.

Mohajeri FA, et al. 2018. The effect of Zataria multiflora Boiss Essential oil on the growth and citrinin production of Penicillium citrinum in culture media and cheese. *Food and Chemical Toxicology*.

Moshafi MH, Mehrabani M & Zolhasab H 2004. Antibacterial activity studies of Salvia Mirzayanii and Salvia Atropatana against six standard gram-positive and gram-negative bacteria. *Journal of Kerman University of Medical Sciences*. **11 (2)**.

Nawel M, ElAmine D, Hocine A & Boufeldja T 2013. Comparative analysis of essential oil components of two Daucus species from algeria and their antimicrobial activity. *International Research Journal of Biological Sciences*. **2 (1)**: 22-29.

Rahimi V, et al. 2019. Chemical Composition and Antifungal Activity of Essential Oil of Zataria Multiflora. *Journal of Nutrition and Food Security*. **4 (1)**: 1-6.

Sharifi MS & Hazell SL 2011. GC-MS Analysis and Antimicrobial activity of the essential oil of the trunk exudates from Pistacia atlantica kurdica. *Journal of Pharmaceutical Sciences and Research*. **3 (8)**: 1364.