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# Chemical Composition and Antimicrobial Activity of the Essential Oils from the Gum of Turkish Pistachio (*Pistacia vera* L.)

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The essential oil from the gum of Pistachio (*Pistacia vera L.* (*Anacardiaceae*)) grown in Turkey was obtained by the hydro-distillation method, and its chemical composition was analyzed by GC and GC-MS. Moreover, the antimicrobial activities of the oil against the growth of 13 bacteria and 3 pathogenic yeasts were evaluated using the agar-disk diffusion and minimum inhibitory concentration (MIC) methods. The results showed that the essential oil contained about 89.67% monoterpenes, 8.1% oxygenated monoterpenes and 1.2% diterpenes. α-Pinene (75.6%), β-pinene (9.5%), *trans*-verbenol (3.0%), camphene (1.4%), *trans*-pinocarveol (about 1.20%), and limonene (1.0%) were the major components. The antimicrobial results showed that the oil inhibited nine bacteria and all the yeasts studied, and the activities were considerably dependent upon concentration and its bioactive compounds such as carvacrol, camphene, and limonene. Moreover, the essential oil of the gum was found to be more effective yeastcide than Nystatin, synthetic yeastcide. Furthermore, the antibacterial activities of the oil were lower than those of standard antibiotics, ampicillin sodium, and streptomycine sulfate under the conditions studied.

**KEYWORDS: Antimicrobial activity; essential oil; *Pistacia vera*; turkey; gum; chemical composition**

## INTRODUCTION

Essential oils of plants and their other products from secondary metabolism have had a great usage in folk medicine, food flavoring, fragrance, and pharmaceutical industries (*1*, *2*). Some biological activities of essential oils have been known for long time (*3*−*7*).

Pistachio (*Pistacia vera* L.) is a member of the *Anacardiaceae* or cashew family. The genus *Pistacia* contains only 11 species, of which *P. vera* is by far the most economically important (*8*). It is a spreading and partially deciduous tree up to 10 m high and has leaves of 1−5 pairs of thick, oval leaflets, tiny brownish green flowers, and clusters of oblong fruit, so-called the pistachio kernel, which is of rich taste used for flavoring of cakes and candy (*8*).

The *P. vera* tree is native of arid zones of Central and West Asia and distributed throughout the Mediterranean basin. Only in the last century has the species been introduced away from its center of natural distribution and domestication, such as in Australia. It grows mainly in Iran, Iraq, Syria, Turkey, Greece, Tunisia, Italy, California, and Arizona (*8*).

*Pistacia* species have been generally used as traditional medicine for various diseases such as toothache, periodontal disease, blood clotting, gastralgia, dyspesia, peptic ulcer, asthma, jaundice, diarrheic, throat infections, renal stones, and as astringent, antiinflammatory, antipyretic, antibacterial, and antiviral medicines (*1*, *9*, *10*). Some recent studies showed that essential oils and crude extracts of leaves and gums of *Pistacia* species (specifically, *P. lentiscus*) had antimicrobial (*10*−*16*), antiinflammatory (*7*, *17*, *18*), and insecticidal (*19*) activities.

Magatias et al. (*9*) reported that the main compounds of the essential oil from the gum of *P. lentiscus* growing in Greece were α-pinene (66.5%), myrcene (8.4%), β-pinene (3.3%), linalool (2.8%), *trans*-caryophyllene (2.0%), limonene (1.3%), and methyl-*O*-cresol (1.1%). On the other hand, it was reported that essential oil of the gum from *P. lentiscus* originated from the west part of Turkey contained β-pinene (39.0%), α-pinene (22.0%), α-ylangene (4.0%), limonene (3.8%), nonanal (3.5%), borneol (3.0%), and verbenone (2%) (*16*).

The gum of the species is obtained as trunk exudate and has traditionally been used as chewing gum against some stomach disease (e.g., ulcer) and lip-dryness, and as an antiseptic for respiratory system. The gum is also used in the protection of

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luster for glass-based products, porcelain, bone, wood, and metal as natural adhesive, in alcoholic and nonalcoholic beverages as food additives, and in the production of toothpaste and dentistry as filler as well as in the cosmetic industry as fragrance (*1*, *16*).

There are some studies about essential oils from the resin (so-called mastic gum) of *P. lentiscus* (*9*, *16*). Also, some reports have been published on the chemical composition (*9*) and antimicrobial activities (*9*, *16*) of essential oil from the leaves of *P. vera* as well as some studies on the new monoterpenes (*20*) and triterpenes (*21*) of bled resin of *P. vera*. However, to our knowledge, no study is available on the chemical composition and the biological activities of the essential oils from the gum of *P. vera*. Therefore, studies on the chemical composition and antimicrobial activities of essential oil from the gum of *P. vera* originating from Turkey were undertaken.

## MATERIALS AND METHODS

**Plant and Chemical Materials.** In this study, the gum of Pistachio (*P. vera* L. (*Anacardiaceae*)) was collected on August 29, 2002 from a private pistachio garden in Gaziantep province, the south east part of Turkey, with an altitude of 700 m. Voucher specimens were deposited in the Herbarium of Faculty of Forestry, Kahramanmaras Sutcu Imam University. Additionally, two reference antibiotics, ampicillin sodium (ampicillin 10) and streptomycine sulfate (streptomycin 10) were used as positive control bactericides while nystatin 100U was used as a positive control yeastscide. They were purchased from Eczacibasi Chem. Co., Turkey. Furthermore, six compounds (4-terpinol, limonene, α-pinene, borneol, camphene, and carvacrol) of the essential oil were purchased from Merck (Germany) to use as reference compounds for the identification of some chemical composition essential oil and antimicrobial activities.

**Preparation of Essential Oil.** The essential oil of the gum (50 g) of pistachio was obtained by hydro-distillation method by using a Clevenger-type apparatus for 3 h. The yield, density (*d*), and refractive index ($n_D$) of the oil were determined as 11.10%, 0.89 g/cm$^3$, and 1.4693−1.4700, respectively, by conventional methods. The white-colored essential oil was dried over anhydrous sodium sulfate ($Na_2SO_4$) and stored at −18 °C.

**Chemical Analysis.** Qualification of the essential oil (11.5 mg) diluted in diethyl ether ($Et_2O$) (1 mL) was analyzed on a Finnigan-MAT 8200 Mass Spectrometer coupled with a Hewlett-Packard GC-5890II series GC by using An SE-54 fused silica capillary column (30-m × 0.25-mm i.d.; 0.25-μm film thickness). Helium (He), having a flow rate of 1.15 mL/min, was used as carrier gas. The GC oven temperature was kept at 60 °C for 5 min and programmed to 260 °C at a rate of 2 °C/min and then kept at 260 °C. The injector temperature was 250 °C. The amount of injection was 1 μL. The carrier gas was delivered at a constant pressure of 5 kg/cm$^2$. MS spectra were taken at El ion source of 70 eV. Split ratio was 1:5.

Retention indices for all the components were determined according to Van Den Dool method (*22*) using *n-alkanes* as standard. Identification of the components was based on comparison of their mass spectra with those of internal (computer) library, NIST libraries (*23*), some reference compounds, and those described by Adams (*24*).

Quantification of the essential oil was conducted by gas chromatography with flame ionization detector (GC-FID) on a Hewlett-Packard GC-589011 series GC. A 1-μL aliquot of oil was injected into the same column under the same GC conditions as described for the GC-MS study. However, the split ratio was 1:14.

**Determination of Refractive Index.** The $n_D$ of the essential oil was measured at 20 °C by means of Abbe Refraktometer A3 und A1 (A.Krus GmbH).

**Microorganisms.** The growth inhibitory activity of the essential oil was tested against 13 bacteria (*Corynebacterium xerosis* UC 9165, *Bacillus brevis NRS*, *Bacillus megaterium* DSM 32, *Bacillus cereus* EU, *Mycobacterium smegmatis* CCM 2067, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* Cowan 1, *Klebsiella pneumoniae* FMC 5, *Klebsiella oxytocica A*, *Enterococcus faecalis* ATTC 15753, *Micrococcus luteus* LA 2971, *Escherichia coil* DM, and *Yersinia enterocolitica* CMC 120) and 3 yeasts (*Kluvyeromyces fragilis* A 230, *Rhodotorula rubra* MC 12 and *Candida albicans* ATCC 1023). These microorganisms were provided from Microbiology Laboratory Culture Collection, Department of Biology, Kahramanmaras Sutcu Imam University, Turkey.

**Table 1.** Chemical Composition of the Essential Oil from Gum of *P. Vera*

| compounds | RI[a] | percent | identification |
|---|---|---|---|
| 1 tricyclene | 923 | 0.22 | a[b] |
| 2 α-thujene | 929 | 0.06 | a |
| 3 **α-pinene** | 937 | 75.62 | b[c] |
| 4 cyclofenchene | 941 | 0.09 | a |
| 5 **camphene** | 950 | 1.43 | b |
| 6 thuja-2,4(10)-diene | 955 | 0.33 | a |
| 7 verbenene | 966 | 0.01 | a |
| 8 sabinene | 974 | 0.28 | a |
| 9 **β-pinene** | 977 | 9.52 | a |
| 10 6-methyl-5-hepten-2-one | 987 | 0.05 | a |
| 11 myrcene | 994 | 0.02 | a |
| 12 α-phellandrene | 1001 | 0.02 | a |
| 13 $\Delta^3$-carene | 1008 | 0.02 | a |
| 14 α-terpinene | 1015 | tr[d] | a |
| 15 1-*p*-menthene | 1021 | 0.04 | a |
| 16 *p*-cymene | 1023 | 0.14 | a |
| 17 **limonene** | 1028 | 1.02 | b |
| 18 1,8-cineole | 1030 | tr | a |
| 19 *cis*-β-ocimene | 1040 | 0.08 | a |
| 20 *trans*-β-*ocimene* | 1050 | 0.06 | a |
| 21 γ-terpinene | 1058 | 0.02 | a |
| 22 *cis*-sabinene hydrate | 1067 | 0.05 | a |
| 23 3-pinen-2-ol | 1079 | 0.09 | a |
| 24 *p*-cymenene | 1087 | 0.07 | a |
| 25 α-pinene oxide | 1093 | 0.04 | a |
| 26 fenchol | 1108 | tr | a |
| 27 2-pinen-7-one | 1120 | 0.10 | a |
| 28 α-campholenal | 1122 | 0.39 | a |
| 29 nopinone | 1131 | 0.05 | a |
| 30 **trans-pinocarveol** | 1135 | 1.16 | a |
| 31 *cis*-verbenol | 1139 | 0.54 | a |
| 32 **trans-verbenol** | 1143 | 3.01 | a |
| 33 α-menthadien-8-ol-isomer | 1146 | 0.12 | a |
| 34 *trans*-pinocamphone | 1156 | 0.16 | a |
| 35 pinocarvone | 1158 | 0.08 | a |
| 36 borneol | 1162 | 0.10 | b |
| 37 *p*–l,5-menthadien-8-ol | 1165 | 0.04 | a |
| 38 *cis*-pinocamphone | 1169 | 0.06 | a |
| 39 terpinen-4-ol | 1174 | 0.09 | a |
| 40 *p*-cymen-8-ol | 1182 | 0.03 | a |
| 41 α-terpineol | 1186 | 0.05 | b |
| 42 myrthenal | 1188 | 0.22 | a |
| 43 myrthenol | 1191 | 0.42 | a |
| 44 verbenone | 1200 | 0.25 | a |
| 45 *p*-cymen-9-ol | 1203 | 0.03 | a |
| *46 trans*-carveol | 1215 | 0.19 | a |
| 47 carvone | 1239 | 0.03 | a |
| 48 carvotanacetone | 1243 | 0.02 | a |
| 49 isopiperitenone | 1266 | tr | a |
| 50 perilla aldehyde | 1268 | tr | a |
| 51 bornyl acetate | 1282 | 0.54 | a |
| 52 carvacrol | 1300 | 0.04 | b |
| 53 8,11,13-abietatriene | 2040 | 0.36 | a |
| 54 7,13-abietadiene | 2064 | 0.87 | a |
| monoterpene hydrocarbons | | 89.67 | |
| oxygenated monoterpenes | | 8.08 | |
| diterpenes | | 1.22 | |

[a] RI determined on SE-54 on basis of n-alkanes. [b] Identification was based on comparison of their GC-MS spectra and RI with those of internal (computer) NIST library and those described by Adams. [c] Identification was based on comparison of their GC-MS spectra and RI with those of internal (computer) NIST library and those described by Adams, along with those of model compounds. [d] tr, concentration is less than 0.01%.

**Biological Activity.** Antimicrobial activities of the essential oils of the gum from *P. vera* were determined using the agar-disk diffusion method, as will be described below. The bacteria were first incubated at 37 ± 0.1 °C for 24 h in nutrient broth (Difco), and the yeasts were incubated in sabouraud dextrose broth (Difco) at 25 ± 0.1 °C for 24 h. The cultures of the bacteria and yeast were injected into the Petri dishes (9 cm) in the amount of 0.1 mL ($10^6$−$10^7$/mL for the bacteria and $10^6$/mL for the yeasts). Then, Mueller hinton agar) and Sabouraud

**Table 2.** Antimicrobial Activities of the Essential Oil from the Gum of *P. vera* and Some Positive Control Antibiotics along with the Minimum Inhibitory Concentration (MIC) of The Oil

| | | inhibition zone (mm) | | | | | |
|---|---|---|---|---|---|---|---|
| | | essential oil | | | | | |
| microorganisms | CFU[a]/mL inoculum | MIC ($\mu$g/mL) | 2 $\mu$L/disc | 4 $\mu$L/disc | A10[b] | S10[c] | N100[d] |
| | | gram-positive bacteria | | | | | |
| *Corynebacterium xerosis* | $4.2 \times 10^7$ | 1.50 | 8 | 10 | 12 | 10 | NT[e] |
| *Bacillus brevis* | $4.0 \times 10^7$ | 1.50 | 8 | 11 | 14 | 16 | NT |
| *Bacillus megaterium* | $8.6 \times 10^6$ | 1.50 | 7 | 11 | 11 | 17 | NT |
| *Bacillus cereus* | $5.3 \times 10^7$ | >10 | *f* | | 15 | 18 | NT |
| *Miycobacterium smegmatis* | $2.5 \times 10^6$ | 1.00 | 7 | 9 | 19 | 15 | NT |
| *Staphylococcus aureus* | $4.0 \times 10^7$ | 2.00 | 9 | 13 | 25 | 22 | NT |
| *Micrococcus luteus* | $6.3 \times 10^7$ | 0.05 | 17 | 25 | 33 | | NT |
| *Enterococcusfaecalis* | $7.4 \times 10^6$ | 1.50 | 7 | 9 | 16 | 17 | NT |
| | | gram-negative bacteria | | | | | |
| *Pseudomonas aeruginosa* | $1.5 \times 10^7$ | >10 | | | 10 | 13 | NT |
| *Klebsiellapneumoniae* | $7.3 \times 10^6$ | >10 | | | 17 | 16 | NT |
| *Klebsiella oxytocica* | $6.9 \times 10^7$ | >10 | | | 15 | 14 | NT |
| *Escherichia coli* | $4.3 \times 10^7$ | 0.10 | 8 | 13 | 11 | | NT |
| *Yersinia enterocolitica* | $5.5 \times 10^7$ | 1.50 | 8 | 15 | 13 | 17 | NT |
| | | yeasts | | | | | |
| *Kluyveroinycesfragilis* | $1.7 \times 10^6$ | 0.05 | 12 | 23 | NT | NT | 15 |
| *Rhodotorula rubra* | $2.0 \times 10^6$ | 1.50 | 9 | 15 | NT | NT | 14 |
| *Candidaalbicans* | $1.5 \times 10^6$ | 0.05 | 16 | 26 | NT | NT | 19 |

[a] Number of Colony Forming Units. [b] Ampicillin (10 $\mu$g/disk). [c] Streptomycin (10 $\mu$g/disk). [d] Nystatin 100 Units (10 $\mu$g/disk). [e] Not tested. [f] No inhibition zone is determined. Blanks mean not investigated.

dextrose agar (sterilized in a flask and cooled to 45−50 °C) were homogeneously distributed onto the sterilized Petri dishes in the amount of 15 mL. Subsequently, the sterilized blank paper disks of 6-mm diameter were saturated with 2 and 4 $\mu$L of essential oil per disk. The disks thus treated were placed onto the agar plates, which had previously been inoculated with the above organisms. In addition, blank paper disks treated with ampicillin, streptomycin, and nystatin-saturated antibiotics were used as positive controls. Afterward, the plates combined with the disks were left at 4 °C for 2 h, the plates injected with yeast were incubated at 25 ± 0.1 °C for 24 h, and ones injected with bacteria were incubated at 37 ± 0.1 °C for 24 h. After 24 h, inhibition zones appearing around the disks were measured and recorded in mm. The initial number of microorganisms in the suspension was determined for the total yeasts and bacterial count during 24 h at 37 °C for bacteria and 48 h 25 °C for yeasts (*25*, *26*).

**Determination of Minimal Inhibitory Concentration (MIC).** A broth microdilution broth susceptibility assay was used, as recommended by NCCLS, for the determination of the MIC of essential oil and some reference components (*27*). All tests were performed in Mueller hinton broth (MHB) supplemented with Tween 80 detergent (final concentration of 0.5% (v/v)), with the exception of the yeasts (Sabouraud dextrose broth (SDB) + Tween 80). Bacterial strains were cultured overnight at 37 °C in MHB, and the yeasts were cultured overnight at 30 °C in SDB. Geometric dilutions ranging from 0.01 to 10.0 $\mu$g/mL of the essential oil were prepared including one growth control (MHB + Tween 80) and one sterility control (MHB + Tween 80 + test oil). Test tubes were incubated under normal atmospheric conditions at 37 °C for 24 h for bacteria and at 30 °C for 48 h for the yeasts. The bacterial growth was indicated by the presence of a white "pellet" on the well bottom.

## RESULTS AND DISCUSSION

**Chemical Composition. Table 1** represents the chemical composition of the essential oil from gum of *P. vera*. As is shown in this table, 54 compounds, representing almost 99% of the essential oil of the gum from *P. vera*, were characterized. The major components are as follows: α-pinene (75.6%), *β*-pinene (9.5%), *trans*-verbenol (3.0%), camphene (1.4%), *trans*-pinocarveol (1.2%), and limonene (1%). In other words, about 90% of the oil consists of monoterpene hydrocarbons, followed by oxygenated monoterpenes (8.1%) and somewhat diterpenes (1.2%) (**Table 1**). Also, it is interesting to notice that no any sesquiterpene can be detected in the essential oil on the contrary to the essential of the gum from *P. lentiscus* (*9*, *16*).

It is well-known that these sorts of variations are due to species as well as geographical origin, harvesting time and growing conditions. It can be concluded that α-pinene, *β*-pinene, limonene, and verbenol are common main components of the essential oil of the gums from both *P. vera* and *P. lentiscus* (*9*). It is interesting to say that the essential oil from the gums of *P. vera* has considerably lower amount of myrcene (only 0.01%) in comparison to *P. lentiscus* (5−8%), which decreases the quality of the oil (*28*). This is an advantage of the oil of *P. vera*.

**Antimicrobial Activity.** In this study, the antimicrobial activities of the essential oil of the gum from *P. vera*, having two different concentrations of 2 and 4 $\mu$L/disk, are compared with those of ampicillin, streptomycine, and nystatin used as positive controls as shown in **Table 2**. It is evident from the table that the antimicrobial (including antibacterial and antifungal) activities increase when increasing the oil concentration from 2 to 4 $\mu$L/disk. It is also determined that the oil inhibits the growths of all the bacteria and yeasts except for *B. cereus*, *P. aeruginosa*, and genus *K. pneumoniae*. Specifically, it is important that the growth of *E. coli*, one of the most common gram-negative food poisoning bacteria, is significantly inhibited by the essential oil at a concentration of 4 $\mu$L/disk used.

As can be seen from the same table, the oil with a concentration of 2 and 4 $\mu$L/disk has an inhibiton zone higher than 7 mm, which is considered as the limit inhibiton zone for being reasonable antibiotic (*9*), for all the microorganisms studied, with the exceptions *of B. cereus*, *P. aeruginosa*, and genus *K. pneumoniae*. The antimicrobial activities of the essential oil from *P. vera* against the same microorganisms are found to be somewhat less in comparison with those of ampicillin (10 $\mu$g/disk) and streptomycin (10 $\mu$g/disk) except for *B. megaterium* and *E. coli* under conditions studied. Also, the oil with the 2 $\mu$L/disk concentration and, specifically, the oil with a 4-$\mu$L/disc- concentration, shows much higher antifungal activity to Nystatin (NS 100U), positive control yeastscide, under the conditions studied.

**Table 3.** Minimum Inhibitory Concentration (MIC, $\mu$g/mL) of Several Main Components of the Essential Oils of the Gum from *Pistacia vera*

| microorganisms | 1[a] | 2[b] | 3[c] | 4[d] | 5[e] | 6[f] |
|---|---|---|---|---|---|---|
| gram-positive bacteria | | | | | | |
| *Corynebacterium xerosis* | >10 | 3.00 | >8.0 | >8.0 | 0.25 | 0.50 |
| *Bacillus brevis* | >10 | 2.50 | >8.0 | >8.0 | 0.05 | 0.05 |
| *Bacillus megaterium* | >10 | 2.50 | >8.0 | >8.0 | 0.05 | 0.20 |
| *Bacillus cereus* | >10 | 2.50 | >8.0 | >8.0 | 0.05 | 0.01 |
| *Mycobacteriumn smegmatis* | >10 | 4.00 | >8.0 | >8.0 | 0.10 | 0.01 |
| *Staphylococcus aureus* | >8 | 3.00 | >5.0 | >8.0 | 0.05 | 0.05 |
| *Micrococcus luteus* | >10 | 1.50 | >5.0 | >8.0 | 0.01 | 0.01 |
| *Enterococcusfaecalis* | >5 | >5 | >8.0 | >8.0 | 0.05 | 0.02 |
| gram-negative bacteria | | | | | | |
| *Pseudomonas aeruginosa* | >10.0 | 6.00 | >10.0 | >10.0 | 0.05 | 0.01 |
| *Klebsiellapneumoniae* | >10.0 | 2.50 | >8.0 | >8.0 | 0.10 | 0.10 |
| *Klebsiella oxytocica* | >5.0 | 2.50 | >8.0 | >8.0 | 0.10 | 0.10 |
| *Escherichia coli* | >5.0 | 2.50 | >8.0 | >8.0 | 0.20 | 0.20 |
| *Yersinia enterocolitica* | >5.0 | 3.00 | >5.0 | >5.0 | 0.30 | 0.20 |
| yeasts | | | | | | |
| *Kluyveromycesfragilis* | >5.0 | >5 | >5.0 | >5.0 | 0.01 | 0.01 |
| *Rhodotorula rubra* | >5.0 | >5 | >5.0 | >5.0 | 0.05 | 0.02 |
| *Candida albicans* | >10.0 | 4.00 | >8.0 | >8.0 | 0.05 | 0.02 |

[a] $\alpha$-Terpineol. [b] Limonene. [c] $\alpha$-Pinene. [d] Borneol. [e] Camphene. [f] Carvacrol.

Meanwhile, the MICs of the oil against several bacteria and yeasts are presented in **Table 2**. As shown in this table, the oil has variable levels of inhibition. *C. albicans*, *M. luteus*, *K. fragilis*, *E. coli*, and *M. smegmatis* are the most sensitive microorganisms to the essential oil of the gum from *P. vera* because of their low MIC values, ranging from 0.05 to 1.0 $\mu$g/mL. The oil has the same MICs (1.5 $\mu$g/mL) against the *Cottynehacterium xerosis*, *B. brevis*, *B. megaterium*, *B. cereus*, *E. faecalis*, *Y. enterocolitica* and *R. rubra*. Also, the highest MIC values (>10 $\mu$g/mL) are determined against *B. cereus*, *P. aeruginosa*, *K. pneumoniae* and *K. oxytocica*.

**Table 3** indicates the MICs of several components of the oil. It is evident from the table that carvacrol (phenolic compound) is found to be the most effective constituent, followed by camphene, limonene, $\alpha$-pinene, borneol and $\alpha$-terpineol. Therefore, this high efficacy of the oil from *P. vera* against the microorganisms stated above can be due, most probably, to carvacrol, camphene, and limonene, respectively (see **Table 3**).

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