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ESSENTIAL OIL AND ANTIMICROBIAL EVALUATION OF THE *Pistacia eurycarpa**

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The compositions of microdistilled and hydrodistilled essential oils of the mastix of Pistacia eurycarpa *Yalt. (*Anacardiaceae*) were compared. The essential oils were analyzed by GC/MS:* α*- and* β*-pinenes were found as the major constituents. The antimicrobial activity of the hydrodistilled oil was determined due to the ethnomedical uses of the oleo-gum resin on skin diseases.*

Key words: *Pistacia eurycarpa*, essential oil, microdistillation, GC/MS, antimicrobial activity.

The genus *Pistacia* consists of small trees of the cashew nut family Anacardiaceae and is native to tropical and subtropical Asia where its members have long been cultivated for a variety of uses [1]. It is represented by six species in Turkey [2].

The trunk of *Pistacia* species produces a characteristic exudate called mastic gum (mastix) which is an oleo-gum resin, known and used since ancient times. *P. lentiscus* is the main source for this material which is widely cultivated and commercialized, whereas *P. terebinthus* and *P. atlantica* also produce similar exudates. The well known *P. vera* is mainly cultivated for its nuts known as "pistachio". The first uses of the mastic gum and oil for medicinal purposes date back from the time of *Dioscorides*, *Galenus*, and *Theophrastus* against rabies, snake bites, baldness, and scabies, as well as in prescriptions for stomach, intestine, bladder, and liver inflammations. Another important application was against oral and dental diseases. Recent interest on the chemistry and biological activity of the different parts of *Pistacia* species support evidence of their ancient uses [3–9].

As part of our investigation, the oleo-gum resin was obtained by incisions of the *P. eurycarpa* tree. It has been used against jaundice and skin infections as well as for the treatment of burns and scalds in extreme situations with success in Batman by the peasants.

The strong fragrance of the oelo-gum resin encouraged a preliminary evaluation of the volatiles using microdistillation [10–11], which were analyzed by GC/MS. Thereafter further hydrodistillation yielded an essential oil which was also analyzed by GC/MS using the same conditions and later compared with the microdistilled volatiles. This essential oil was subjected to an antimicrobial assay using various pathogenic microorganisms.

Literature survey showed that the nuts (seeds) of Jordanian *Pistacia eurycarpa* were recently investigated for its fatty acid composition [12].

The essential oils and chemical compositions of various parts (esp. gum-oleo resin) of *Pistacia* species have been subject to several investigations [5, 8, 13–16 and cited references].

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TABLE 1. Composition of the Volatiles of *Pistacia eurycarpa* Obtained by Different Methods

RRI	Compound	A, %	B, %	RRI	Compound	A, %	B, %
1014	Tricyclene	0.1	0.1	1586	Pinocarvone	0.1	0.2
1032	α-Pinene	**66.0**	**75.0**	1597	Bornyl acetate	1.1	0.7
1076	Camphene	0.7	0.9	1611	Terpinen-4-ol	0.1	0.1
1118	β-Pinene	**4.9**	**5.6**	1648	Myrtenal	0.6	0.3
1132	Sabinene	0.4	0.5	1663	*cis*-Verbenol	0.5	0.3
1174	Myrcene	0.1	0.2	1664	*trans*-Pinocarveol	2.9	1.6
1203	Limonene	1.3	1.6	1674	*p*-Mentha-1,5-dien-8-ol	1.2	0.6
1213	1,8-Cineole	0.3	0.2	1683	*trans*-Verbenol	-	2.6
1218	β-Phellandrene	-	0.1	1694	Neral	3.5	-
1224	*o*-Mentha-1(7),5,8-triene	0.2	0.1	1697	Carvotanacetone	0.1	Tr.
1246	(Z)-b-Ocimene	0.1	Tr.	1706	α-Terpineol	1.1	0.4
1280	*p*-Cymene	1.0	0.8	1709	α-Terpinyl acetate	0.1	-
1290	Terpinolene	0.1	Tr.	1719	Borneol	0.1	-
1439	γ-Campholene aldehyde	0.3	0.1	1725	Verbenone	1.0	0.7
1452	α,p-Dimethylstyrene	0.1	0.1	1751	Carvone	0.1	0.1
1499	α-Campholene aldehyde	2.3	0.9	1797	*p*-Methyl acetophenone	0.1	-
1522	Chrysanthenone	0.1	0.1	1804	Myrtenol	1.1	0.6
1536	Pinocamphone	0.2	0.2	1845	*trans*-Carveol	1.0	0.6
1553	Linalool	0.3	0.2	1864	*p*-Cymen-8-ol	0.3	0.1
1562	Isopinocamphone	0.1	Tr.	1882	*cis*-Carveol	0.1	Tr.
1571	Methyl citronellate	-	Tr.	Total		93.7	95.6

A: Microdistillation; B: Hydrodistillation; RRI: Relative retention indices calculated against n-alkanes; %: Calculated from TIC data; Tr: Trace (<0.1 %).

TABLE 2. Antimicrobial Activity of the Essential Oils of *Pistacia eurycarpa* (E. O.) Microdilution Broth Susceptibility Assay, MIC (μg/mL)

Pathogenic Microorganisms	E. O.	St.
Escherichia coli Gr (–) *anearobic*	62.5	62.5
Staphyloccocus aureus Gr (+)	125	15.62
Pseudomonas aeruginosa Gr (–)	250	250
Enterobacter aerogenes Gr (–)	62.5	125
Proteus vulgaris Gr (–) *anearobic*	15.62	31.25
Salmonella typhimurium Gr (–)	125	62.5
Candida albicans (yeast)	125	125*

St: Chloramphenicol, * Ketoconazole

This is the first report on the essential oil composition and biological activity of *P. eurycarpa*. The essential oils obtained by two different methods, namely microdistillation (A) and hydrodistillation (B), were compared. As seen in Table 1 both methods resulted in similar chemical compositions with comparable amounts. Microdistillation yielded thirty-eight volatile compounds representing 93.7% of the total with α- and β-pinene as the major components. Hydrodistillation yielded thirty-seven compounds but with quite higher amounts of the major components. High essential oil yield (14.2%) by hydrodistillation was also remarkable.

TABLE 3. Pathologies of Selected Microorgasims to Humans

Microorganism	Pathology
Escerichia coli	Wound infections, urinary tract infections, gastrointestinal system infections, dysentery, septicemia
Staphyloccocus aureus	Food poisoning, respiratory system infections, wound infections
Pseudomonas aeruginosa	Septicemia, eye, urinary tract and wound infections
Enterobacter aerogenes	Gastro infestinal system infections, urinary tract infections
Proteus vulgaris	Diarrhea, urinary tract infections, skin diseases, and wound infections
Salmonella typhimurium	Salmonellosis, Diarrhea, Food poisoning
Candida albicans	Candidiasis: infestinal system infections, urinary tract infections, wound and skin infections

The essential oil of the oleo-gum resin of *P. eurycarpa* strongly inhibited microorganisms associated with common wound infections and skin diseases (*Enterobacter aerogenes* and *Proteus vulgaris*). The Gr (–) pathogen *E. aerogenes* was inhibited at a concentration of 62.5 *m*g/mL and *P. vulgaris* at 15.62 mg/mL more effectively by than the control drug chloramphenicol. The essential oil also showed inhibitory effects towards the other pathogens used in this study which are listed in Table 2 with the relevant MICs.

More in-depth studies of the essential oils of *Pistacia* species against different pathologies are suggested. Bioassay guided fractionation and investigation of the bioactive components are subject to further studies.

EXPERIMENTAL

Plant Material. The mastix was collected by one of the authors (E. G.) from Batman, Sason, Yucebag koyu (Yucebag), on 27.07.2000. The voucher specimen of the identified plant material is deposited (00187) at the Herbarium of the Faculty of Science and Letters, Gazi University, Ankara.

Microdistillation. The mastix was first subjected to microdistillation by Eppendorf MicroDistiller® to obtain the volatile compounds. 500 mg mastix was added to a sample vial containing 10 mL distilled water. NaCl (2.5 g) and water (0.5 mL) was placed in the collecting vial. *n*-Hexane (350 μL) was added into the collecting vial to trap the volatile components. The sample vial was heated to 108ºC at a rate of 20ºC/min, kept at the same temperature for 90 min, and then heated to 112ºC at a rate of 20ºC/min and kept at this temperature for 30 min. Finally the sample was subjected to post-run for 2 min under the same conditions. The collecting vial was cooled to –1ºC during distillation. After the distillation was completed, the organic layer in the collection vial was separated and injected into the GC/MS system.

Hydrodistillation. The mastix (50 g) was gently warmed up and later subjected to hydrodistillation using a Clevenger-type apparatus for 3 h to yield a colorless essential oil (7.1 mL).

Analysis of the Essential Oils. Both essential oils were analyzed using a Hewlett-Packard G1800A GCD system. HP-Innowax FSC column (60 m × 0.25 mm ⌀, with 0.25 μm film thickness). Helium (0.8 ml/min) was used as carrier gas. GC oven temperature was kept at 60ºC for 10 min and programmed to 220ºC at a rate of 4ºC/min and then kept constant at 220ºC for 10 min to 240ºC at a rate of 1ºC/min. Mass range was recorded from *m/z* 35 to 425.

Injections were applied splitless. Injection port temperature was at 250ºC. MS were recorded at 70 eV. Relative percentage amounts of the separated compounds were calculated automatically from peak areas of the total ion chromatogram (TIC). Alkanes were used as reference points in the calculation of relative retention indices (RRI). Library search was carried out using both "Wiley GC/MS Library" and "TBAM Library of Essential Oil Constituents". The compounds identified are listed in Table 1.

Bioassay. Microdilution broth susceptibility assay was used for the evaluation of the antimicrobial activity [17]. The stock solution of essential oil was prepared in DMSO. A dilution series of the essential oil was prepared in a sterile distilled water in a 96-well microtiter plate up to 1.94 μg/mL in sterile distilled water. Freshly grown bacterial suspensions in double strength Mueller-Hinton broth and a yeast suspension of *Candida albicans* in yeast medium were standardized to 10^8 CFU/mL. Sterile distilled water served as growth control. 100 μL of each microbial suspension was then added to each well. The last row

containing only the serial dilutions of the antimicrobial agent (chloramphenicol for bacteria and ketoconazole for *C. albicans*) without microorganism was used as negative control. After incubation at 37°C for 24 h the first well without turbidity was determined as the minimal inhibition concentration (MIC). Human pathogens *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Enterobacter aerogenes*, *Proteus vulgaris*, and *Salmonella typhimurium* were obtained from the culture collection of the Microbiology Department in Anadolu University, and *Candida albicans* was obtained from the culture collection of Osmangazi University, Medical Faculty, Microbiology Department (Table 2).

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