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GC-MS Analysis and Antimicrobial activity of the essential oil of the trunk exudates from *Pistacia atlantica kurdica*

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Abstract:
The volatile oil from the crude gum of Kurdica, from *Pistacia atlantica Kurdica*, was isolated by hydrodistillation and analyzed through a combination of gas chromatography (GC-FID) and gas chromatography/mass spectrometry (GC-MS). The essential oil formed 20% of the weight of the crude gum. The major constituent was α-Pinene. The extracted essential oil was then screened for antimicrobial activity against 9 strain of *Helicobacter pylori* (*H. pylori*) and some other Gram-negative and Gram-positive bacteria, the MIC values ranged 500-1000 mg/mL.

Keywords: *Pistacia atlantica kurdica, Pistacia atlantica, Pistacia lentiscus*, mastic gum, essential oil, GC/MS. Antimicrobial activity.

1. INTRODUCTION

Anacardiaceae is a small family with about 400 species of mainly tropical trees and shrubs. Leaves are alternate, pinnate in all the European species without stipules. Flowers are small with 3-7 sepals and petals, or without petals. All the members of this family have a resinous sap. The most known species of this family are *Anacardium occidentale* and *Pistacia vera*, the trees that are cultivated for their nuts (Cashew and Pistachio) [1].

The genus *Pistacia* from the Anacardiaceae family consists of eleven species of trees and shrubs found in some Mediterranean countries and in Southern and Central America [2].

Substantial work has been done on *Pistacia vera* and *Pistacia lentiscus*, [3-9] the evergreen shrub that is known for centuries for its resin, so-called "mastic gum", however, nothing has been done on the composition of the essential oil of *Pistacia atlantica* (*P. atlantica*) and its subspecies *P. atlantica. kurdica* (*P. a. kurdica*), *P. a. kurdica* is widely spread around the Zagros Mountains and particularly in Western and Northern Iran and Eastern and Northern Iraq, Southern Turkey and Northern Syria so called Kurdistan. *P. a. kurdica* shows a discontinuous pattern of distribution over the region and is an important constituent of the natural vegetation in this area. It is the major source of a gum that has not been known well to the world and here we refer to that as Kurdica. *P. a. kurdica* is a native plant of this region. Zohary [10] classified *P. a. kurdica* as a sub-species of *P. atlantica* because of the presence of leaf rachis wing that narrower than the type of *P. atlantica*. However, Yaltirik [11] has classified this plant as a different species *Pistacia khinjuk*, because the leaves are light green on both sides (instead of being dark green above and pale below as in *P. atlantica*), and the nuts are much smaller than *P. atlantica*'s fruit.

The essential oils of some of the species of the genus *Pistacia* have been characterized [3-9], however, the species of *atlantica* and its subspecies have not been characterized. We have analyzed the essential oil of *P. a. kurdica*.. by GC-MS and screened it against 9 strains of *H. pylori* and some other Gram-negative and Gram positive bacteria.

2. MATERIAL AND METHODS

2.1. Extraction of Essential Oils and analysis

The gum of *P. a. kurdica*. was collected on the first month of summer in Kurdistan province in Iran. The gum was hydrodistilled for 5 hours to obtain a volatile oil in 20% yield based on the fresh weight of gum. The oils were stored at low temperature (-20° C) prior to analysis. GC-FID analysis was performed on a Varian 3700 gas chromatograph coupled to a Shimadzu C-R3A integrator and fitted with a fused silica capillary column (25QC/BP5) obtained from SGE, Australia (25m x 0.25mm i.d., 0.25 µm film thickness). The analytical conditions were: carrier gas hydrogen (ca. 1mL/min) injector temperature 260° C, detector (FID) temperature 280° C, oven temperature 40° C (2 min. hold) to 280° C (5 min. hold) at 4° C/min. Programmed-temperature Kovats retention indices (RI) were obtained by GC-FID analysis of an aliquot of the essential oil spiked with n-alkane mixture containing each homologue from n-C8 to n-C30. GC-MS analysis was performed in electron impact mode (EI, 70 eV) using a VG AutoSpec system interfaced with a Hewlett Packard 5890 Series II gas chromatograph, fitted with a capillary column (BPX5) supplied by SGE, Ausalia (25m x 0.25mm i.d., 0.25 µm film thickness). Oven temperature settings were the same as those described for GC-FID analysis, with He (ca. 1.4 mL/min) used as carrier gas.

2.2 Bacterial Strains and Culture Conditions

2.2.1 H. pylori

H. pylori strains 26695, J99, RSB6, P10, SS1, SS2000, N6, NCTC11637 and RU1 were obtained from the culture collection of the University of New South Wales and were used for the MIC determinations. The strains were grown on Blood Agar Base No. 2 (Oxoid, Basenstoke, UK) supplemented with 7% defibrinated horse blood, amphotericin B (2μg/mL) and Skirrow's selective supplement, consisting of the antibiotics Trimethoprim (5 μg/mL), Polymixin B (2.5 μg/mL), and Vancomycin (10 μg/mL). Bacterial cultures were incubated in an atmosphere of 10% CO_2 in air, 95% relative humidity at 37° C.

Isosensitest (Oxoid Basenstoke UK) broth was prepared containing 0.5% Tween 80 and 1.00% Essential Oil of Kurdica (EOK). Serial dilutions were made until the EOK concentration of 0.0625% was achieved. Dilution tubes containing 1ml of mixture were stored at 4° C. Similar procedure was carried out with α-pinene 99% (Sigma-Aldrich) instead of EOK for comparison.

The inoculum was prepared with Isosensitest broth from 36 h cultures grown on CSA, using above mentioned *H. pylori* strains diluted to an optical density (OD) of 0.1 (0.5 McFaellan standard) at A_{600} with Isosensitest broth containing 0.5% Tween-80. Each culture was incubated for 2 h to allow recovery of the bacteria. The serial dilutions of EOK and α-pinene were then inoculated with 1ml of diluted culture and incubated using tissue culture flask at 37°C in a CO_2 incubator for 48h. CSA plates were then inoculated with samples from the dilutions using a spiral platter and incubated at 37°C, in a Stericult incubator (Forma Scientific, USA) at 37°C with 95% relative humidity and 10% CO_2 to reduce oxygen and provide microaerophillic environment with each test being performed in duplicate. Control cultures were prepared in the same manner without adding EOK/ α-pinene.

The Minimum Bacterial Concentration (MBC) was defined as the concentration of EOK/ α-pinene that killed the entire inoculum and was equivalent to the Minimum Inhibitory Concentration (MIC).

2.2.2 All other Gram-negative and Gram-positive bacteria

Agar Plates

All other Gram-negative and Gram-positive bacteria were grown on Luria Bertani medium. Plates were incubated at appropriate temperature for 12-18 h in an aerobic/anaerobic environment. Agar plates were used for maintaining cultures and also as a source of inoculum for liquid cultures.

Liquid Culture

LB broth was used to grow all Gram-positive and Gram-negative that is listed below (Table 1). The cultures were grown for 12-18 h in aerobic/anaerobic environment. Liquid cultures were used to determine MIC.

Table 1. Gram-positive and Gram-negative bacteria obtained from UNSW

Gram-negative bacteria	***Incubation***
Escherichia coli type 1	UNSW 048200 37° C
Salmonella typhimurium	UNSW 086300 37° C
Serratia marscens	UNSW 052001 37° C
Pseudomonas aeruginosa	UNSW 029101 37° C
Alcaligenes faecalis	UNSW 034000 37° C
Enterobacter aerogenes	UNSW 045800 37° C
Pseudomonas fluorescens	UNSW 036800 30° C
Proteus vulgaris	UNSW 027800 37° C
Porphyromonas gingivalis (W83)	UNSW 37° C

Gram-positive bacteria	***Incubation***
Bacillus cereus	UNSW 052300 37° C
Staphylococcus aureus	UNSW 056201 37° C
Streptococcus faecalis	UNSW 055440 37° C
Staphylococcus epidermidis	UNSW 001402 37° C
Bacillus subtilis	UNSW 030702 30° C
Corynebacterium sp.	UNSW 000400 37° C

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Staphylococcus epidermidis	UNSW 001402 37° C
Bacillus subtilis	UNSW 030702 30° C
Corynebacterium sp.	UNSW 000400 37° C

3. RESULTS AND DISCUSSION

3.1. Chemical Analysis

Table 2 shows the composition of the essential oils of *P. a. kurdica* in the order of elution time. The major constituent of both *P. lentiscus* [3-7] and *P. a. kurdica* is α-pinene, ranging from 21.70 to 78.90 for *P .lentiscus* and 97.20 for *P. a. kurdica* that gives a unique characteristic to this species. ß-Pinene also has been reported in all analytical data from the literature [3-8] and from our results, ranging 1.26-38.70. ß-Myrcene is present in all reported data but ours and the Turkish one, ranging from 0.10-12.27 [8]. Limonene is reported in literature for *P. lentiscus* as ranging from 0.95-11.52, and 0.06 in *P. a. kurdica*, according to our results. Some of the reported data are also not reliable as in some reports [3-7] 1.7-10% of the total composition of the essential oils are not known.

Typical chromatograms of the essential oils of *P.a. kurdica*, is shown in Figure 1. The prominent component 97.2% is α-pinene that shows a unique characteristic for sub-species of *P. atlantica*

Figure 1. Typical chromatogram of the essential oil of kurdica gum

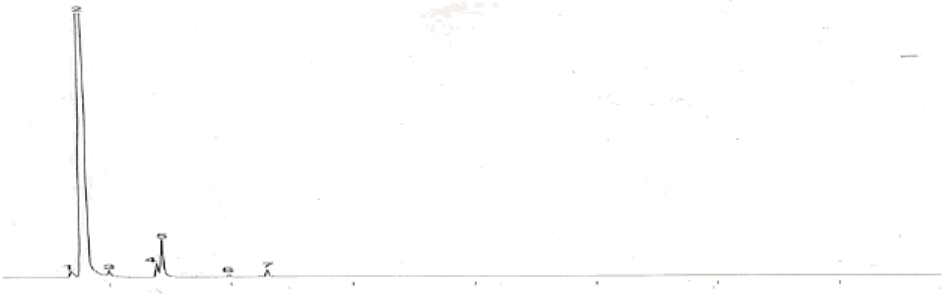

Table 2. The composition of essential oil of kurdica gum in the order of elution time

Peak No.	*Compounds*	*RA[a] (%)*	*RI[b] (Exp)*	*RI[c] (lit)*	*MWt*	*Identification*
1	α-Thujene	0.07	920	931	136	1
2	α-Pinene	97.18	935	939	136	1
3	Camphene	0.41	946	953	136	1
4	Sabinene	0.16	972	976	136	1,2
5	β-Pinene	1.26	975	980	136	1
6	Δ3-Carene	0.11	1010	1011	136	1,2
7	Limonene	0.06	1089	1088	136	1,2

[a] RA; relative area (raw peak area relative to total peak area) . [b] RI (Exp); programmed temperature retention indices as determined on BP-5 column using a homologous series of *n*-alkanes (C_8-C_{30}) as internal standard and H_2 as carrier gas; [c] RI (lit); values from literature data using He as carries gas, with T denoting programmed temperature values; MWt: molecular weight; values with subscript "d" confirmed from GC-MS (CI) data; [d] 1; based on retention index, 2; based on comparison of mass spectra with literature data (NIST, NISTREP) or authentic sample, 3; retention time identical to authentic compound.

Table 3. The MIC values of EOK and pure α-pinene for Gram-negative bacteria

Gram-negative bacteria	*MIC EOK mg/mL*	*MIC for alpha pinene mg/mL*
Escherichia coli type 1	500	500
Salmonella typhimurium	1000	1000
Serratia marscens	1000	1000
Pseudomonas aeruginosa	500	500
Alcaligenes faecalis	1000	1000
Enterobacter aerogenes	500	500
Pseudomonas fluorescens	500	1000
Proteus vulgaris	500	500
Porphyromonas gingivalis (W83)	500	1000

Table 4. The MIC values of EOK and pure α-pinene for Gram-positive bacteria

Gram-positive bacteria	*MIC EOK mg/mL*	*MIC for alpha pinene mg/mL*
Bacillus cereus	1000	1000
Staphylococcus aureus	1000	1000
Streptococcus faecalis	1000	1000
Staphylococcus epidermidis	500	1000
Bacillus subtilis	500	1000
Corynebacterium sp.	1000	1000

3.2. Antibacterial Activity

The EOK is active almost equally against *H. pylori* and all other Gram-positive and Gram-negative bacteria ranging from 500-1000 mg/mL (Table 3 and 4). No significant differences were found between almost pure α-pinene and EOK so as to indicate any synergistic effect of the other constituents of EOK such as camphene, sabinene, limonene etc. As the major constituent of EOK is α-pinene, which has a very low toxicity [12-13], this essential oil would be a good candidate for usage in health products.

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