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The study of chemical composition of gum in Pistacia atlantica in Erbil region

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A B S T R A C T

Gum in *Pistacia atlantica* has elevated interest in medicine and has been reported to possess substantial antimicrobial activity. Thus, this study was carried out to analyze the active constituents present in gum of *Pistacia atlantica.* Twenty-six compounds in hexane extract were identified by Gas Chromatography - Mass Spectrometry (GC-MS) analysis. The GC-MS analysis has shown the presence of different phytochemical compounds in the gum of *Pistacia atlantica*. A total of 26 compounds were identified representing 84.49% of total hexanoic extract composition. The major compounds were (α-pinene 57.06%, β-pinene 9.83%, trans-Pinocarveol 2.95%, trans-Verbenol 3.97%, α-Phellandren-8-ol 3.81%). Our results show that gum obtained from *P. atlantica var. kurdica* contained various chemical constitutes which have been previously reported as health benefits.

1. INTRODUCTION

The genus *Pistacia* (Anacardiaceae) consists of dioecious trees and shrubs, all of which are anemophilous. Their distribution is mainly in the Mediterranean region; in west, central and east Asia, and in the south of North America, North Africa and the Middle East (Grundwag, 1976).

All *Pistacia* (Anacardiaceae) species are dioecious, pistillate and staminate flowers are formed on different trees. Both types of flowers are apetalous, and wind is the pollinating agent.

Pistacia species have been generally used as traditional medicine for various diseases such as blood clotting, toothache, gastralgia, periodontal disease, peptic ulcer, asthma, jaundice, antipyretic, diarrheic, throat infections, dyspesia, renal stones, and as astringent, antibacterial and antiviral antiinflammatory medicines (Grassmann, et al., 2000; Digˇrak, et al., 2001; Traboulsi, et al.,2002; Duru, et al., 2003).
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 (Satil et al., 2003). Some biological activities of essential oils have been known for long time (Digˇrak, et al., 2008).

The gum of the species is obtained as trunk exudate and has traditionally been used as chewing gum against lip-dryness, stomach disease (such as ulcer) and as an antiseptic for respiratory system. The gum is also used in the protection of user for glass-based products, metal as natural adhesive, porcelain, bone, wood, and 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 (Kordali, et al., 2003).

The main compounds of the essential oil from the gum of *P. lentiscus* growing in Greece were reported by Magatias et al. (1999) as R-pinene (66.5%), myrcene (8.4%), â-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 â-pinene (39.0%), R-pinene (22.0%), R-ylangene (4.0%), limonene (3.8%), nonanal (3.5%), borneol (3.0%), and verbenone (2%) (Kordali, et al., 2003).

P. atlantica 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. atlantica 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. atlantica kurdica* is a native plant of this region (Ahmed, 2017).

Gum" mastic, an oleoresin exudate from the stem of *Pistacia lentiscus* has a long history of use as a therapeutic agent with many reported medicinal properties (Ghalem and Mohamed 2009). Mastic gum from *Pistacia* has been used by traditional healers for the relief of upper abdominal discomfort, stomachaches, dyspepsia and peptic ulcer (Amhamdi et al., 2009).

The main objective of our research work was to analyze the phytochemicals compounds of the essential oils from the gum of genus *P. atlantica* from Barzan location.

2. MATERIALS AND METHODS

2.1. Sample collection:

Samples of P.atlantica L. were collected in Sherin Mountain (Kurdistan region / Iraq) in July 2015. Identification of the species was confirmed in Forestry department/ Agriculture College/ Salahaddin University. The gum was collected and stored at 4° C until used.

2.2. Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

The identification of the phytocompounds from the gum sample was performed using Perkin Elmer series Clarus 680 GC with a capillary column (Rxi-5ms, RESTEK: length of 30 m, internal diameter of 0.25 mm, film thickness of 0.25 μm) and Perkin Elmer series SQ 8T mass selective detector. Helium was used as carrier gas at a flow rate of 1 mL/min. Before the analysis, the samples were diluted with hexane and filtered through a 0.22 μm syringe-driven filter after dehydrated with anhydrous sodium sulfate. The temperature of the injector was kept at 250 °C and a sample volume of 1 μL was automatically injected by auto-sampler with a split ratio of 50:1. The GC oven temperature was programmed began with heating at 40 °C and held for 2 min, then raised to 100 °C at a rate of 3 °C/min and held for 1 min, and finally, raised to 270 °C at a rate of 4 °C/min and this final temperature was held for 1 min. The mass selective detector was operated in electron impact (EI) ionization mode. MS source and transfer line temperatures were kept at 280 °C and 270 °C, respectively. EI ionization energy was 70 eV and an m/z range from 40 to 550 was scanned. The solvent delay time of 4 min was used to avoid solvent influence.

3. RESULTS AND DISCUSSION

Due to the complexity of the results, a total of 26 components have been identified in the volatile profile of the gum of P. atlantica, representing 95.22% of the total oil components which were detected and are listed in Table 1 with their percentage composition and Kovat's indices. Our results (Table1) show that the P. atlantica gum contained about 57.06% α-pinene with retention time (11.15), 1.02% of Camphene (11.49), 1.18% of Dehydrosabinene (11.74), β-pinene (12.79) 9.83%, 1,3,8-p-Menthatriene (13.43) 0.75%, Delta-3-Carene (14.29) 0.54%, p-Cymene (14.98)1.31%. Also, Limonene (15.19) 0.91%, Eucalyptol (15.27) 0.31%, γ-Terpinene (16.62) 0.18%, Pulegone (18.12) 0.57%, Linalool (18.65) 0.2%, α-campholene aldehyde (19.85) 1.96%, trans-Pinocarveol (20.48) 2.95%. In addition, (S)-cis-Verbenol (20.6) 1.01%, trans-Verbenol (20.83) 3.79%, trans-p-Menth-2-en-1,8-diol (20.97) 1.25%, Pinocamphone (21.45) 0.26%, α-Phellandren-8-ol (21.88) 3.81%, Terpinen-4-ol (22.29) 0.35%, para-Cymen-8-ol (22.73) 0.99%, α-Terpineol (22.99) 0.44%, Myrtenal (23.21) 1.74%, Verbenone (23.89) 1.51%, Carveol (24.4) 0.68% and Bornyl Acetate (27.39) 0.62%.

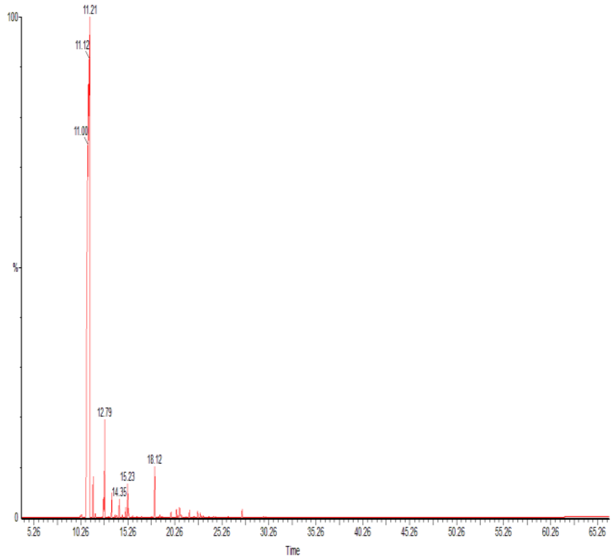

Figure 1. GC-MS chromatogram of *P. atlantica* gum showing the phytocompounds**.**

Table1. Chemical compounds isolated from selected Pistacia species.

No.	Compounds	Molecular Formula	Retention Time (min)	(%)
1	α-pinene	$C_{10}H_{16}$	11.15	57.06
2	Camphene	$C_{10}H_{16}$	11.49	1.02
3	Dehydrosabinene	$C_{10}H_{14}$	11.74	1.18
4	β-pinene	$C_{10}H_{16}$	12.79	9.83
5	1,3,8-p-Menthatriene	$C_{10}H_{14}$	13.43	0.75
6	Delta-3-Carene	$C_{10}H_{16}$	14.29	0.54
7	p-Cymene	$C_{10}H_{14}$	14.98	1.31
8	Limonene	$C_{10}H_{16}$	15.19	0.91
9	Eucalyptol	$C_{10}H_{18}O$	15.27	0.31
10	γ-Terpinene	$C_{10}H_{16}$	16.62	0.18
11	Pulegone	$C_{10}H_{16}O$	18.12	0.57
12	Linalool	$C_{10}H_{18}O$	18.65	0.2
13	α-campholene aldehyde	$C_{10}H_{16}O$	19.85	1.96
14	trans-Pinocarveol	$C_{10}H_{16}O$	20.48	2.95
15	(S)-cis-Verbenol	$C_{10}H_{16}O$	20.6	1.01
16	trans-Verbenol	$C_{10}H_{16}O$	20.83	3.79
17	trans-p-Menth-2-en-1,8-diol	$C_{10}H_{18}O_2$	20.97	1.25
18	Pinocamphone	$C_{10}H_{16}O$	21.45	0.26
19	α-Phellandren-8-ol	$C_{10}H_{16}O$	21.88	3.81
20	Terpinen-4-ol	$C_{10}H_{18}O$	22.29	0.35
21	para-Cymen-8-ol	$C_{10}H_{14}O$	22.73	0.99
22	α-Terpineol	$C_{10}H_{18}O$	22.99	0.44
23	Myrtenal	$C_{10}H_{14}O$	23.21	1.74
24	Verbenone	$C_{10}H_{14}O$	23.89	1.51
25	Carveol	$C_{10}H_{16}O$	24.4	0.68
26	Bornyl Acetate	$C_{12}H_{20}O_2$	27.39	0.62
Total				95.22

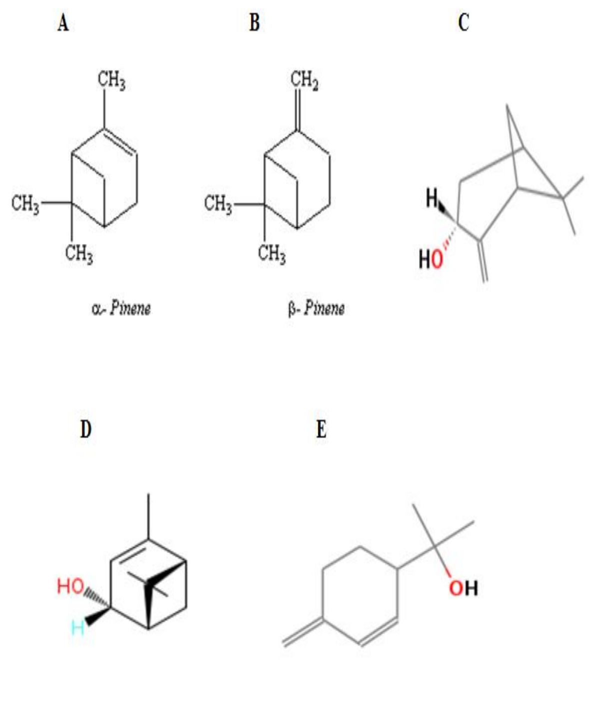

Figure 2. Chemical structure of α-pinene (A), β-pinene (B), trans-Pinocarveol (C), trans-Verbenol (D) and α-Phellandren-8-ol (E).

4. Discussion:

A total of 26 components have been identified in the volatile profile of the gum of Pistacia atlantica using GC-MS. The major constituents of the P. atlantica L. oil of were (α-pinene, β-pinene, trans-Pinocarveol, trans-Verbenol, α-Phellandren-8-ol). In other countries of the Mediterranean region, several studies have been studied the chemical composition of P. atlantica (Barrero et al., 2005; Mecherara-Idjeri et al., 2008). Koutsoudaki et al. (2005) studied the chemical components of P. lentiscus and found that α-Pinene, β-myrcene, β-pinene, limonene, and β-caryophyllene were the major components.

Many authors have studied the chemical composition of the essential oil of different species of Pistacia (Demirci et al., 2001; Duru et al., 2003; Delazar et al., 2004; Barrero et al., 2005). Our results are comparable with some of their findings, but differ from others. The differences in the essential oil composition of the various species may be due to environmental (geographical location) and growing conditions, while observed variations in the essential oil composition of the oleoresin could also be explained by the existence of chemotypes (Benhassaini et al., 2007).

Essential oil of P. atlantica has antimicrobial activity against gram-positive and -negative bacteria which are resistant to commonly used antimicrobial agents (Ghalem and Mohamed, 2009). Some researchers reported that there is a relationship between the chemical structures of the most abundant in the tested essential oil and the antimicrobial activity. Essential oils rich in phenolic compounds such as Pistacia specie are widely reported to possess high levels of antimicrobial activity (Marner et al., 1991; Douissa et al., 2005). On the other hand, it should be noted that the two major volatile constituents, α-pinene and terpinolene contained in the Pistacia specie are compounds with interesting antibacterial activity (Tsokou et al., 2007).

Most of the studies showed antimicrobial activity of these species especially P. lentiscus on a wide range of microorganisms including Gram-positive and -negative, aerobic and anaerobic bacteria, viruses and fungi. The findings indicated that α-pinene, verbenone, R-terpineol, linalool, carvacrol and flavones are major compounds related to antibacterial activity.

Hydrocarbon and oxygenated monoterpens are the major chemical constituents in essential oil and among hydrocarbon monoterpens, α-pinene has been reported as the main compound of some samples like P. vera (Ramezani et al., 2004), P. terebinthus (Özcan et al., 2009; Usai et al., 2006), P. lentiscus (Özcan et al., 2009) and P. atlantica (Barrero et al., 2005). In addition to α-pinene, other major components isolated from different parts of Pistacia species are as follows: (E)-β-Ocimene and limonene in fruits (Flamini et al., 2004; Roitman et al., 2011; Couladis et al., 2003). β-Ocimene and terpinen-4-ol in leaves and p-cymen, in young shoots of P. terebinthus (Roitman et al., 2011) bornyl acetate, terpinen-4-ol, sabinene, and myrcene in fruits, terpinen-4-ol, myrcene, p-mentha-1,8 diene, and ocimene from leaves (Barrero et al., 2005; Roitman et al., 2011; Tzakou et al., 2007), sabinene and p-mentha-1, diene in leaf buds, and Δ3-carene in unripe galls of P. atlantica (Tzakou et al., 2007).

Among triterpenes isolated from the resin of three sub-species of P. atlantica (kurdica, cabulica and mutica), 3-O-acetyl-3-epiisomasticadienolic acid has been identified as the most effective antimicrobial agent (Sharifi and Hazell, 2012). Antimicrobial activity of Pistacia lentiscus resin, the essential oil and gum from P. atlantica var. kurdica and its major constituent α-pinene and P. vera gum against Helicobacter pylori were recorded (Paraschos et al., 2011). Essential oil from leaf and gum of P. atlantica reported as acceptable antibacterial and antifungal activities (Yoram and Inbar, 2011). Sharifi and Hazell (2011) found that the major constituent was α-Pinene when they studied the compounds from P. atlantica. The extracted essential oil was then screened for antimicrobial activity against 9 strain of Helicobacter pylori and some other Gram-negative and Gram-positive bacteria, the MIC values ranged 500-1000 mg/mL.

5. Conclusion:

The presence of various bioactive compounds in the *P. atlantica* justifies the use of the gum for various diseases by traditional experts. However, isolation of individual phytochemical constituents and subjecting it to the biological activity will definitely give profitable results. From the results, it could be concluded that *P. atlantica* gum contains various bioactive compounds. Therefore, it is recommended as a plant of phytopharmaceutical importance.

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