Antimicrobial Activity of the Leaves of *Pistacia khinjuk*

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Abstract

Background: ***Pistacia khinjuk* is one of the three species *Pistacia* growing in Iran. The essential oils of some *Pistacia* plants contain variety of components with different therapeutical effects.**

Objective: The purpose of this study was to provide the examination of antibacterial and antifungal effects of chloroform, ethyl acetate, ethyl alcohol and diethyl ether extracts obtained from the leaves of *P. khinjuk*.

Method: for obtaining different extracts of *P. khinjuk*, 30 gr of leaves powder was macerated with 120 ml of each solvent (chloroform, ethyl acetate, ethyl alcohol, diethyl ether). The antibacterial and antifungal activities of different extracts was individually tested against a panel of microorganisms, including *Bacillus subtilis*, *Enterococcus faecalis*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli*, *Klebsiella pneumoniae*, *Candida albicans* and *Saccharomyces cerevisiae*

Results: Some major constituents of essential oil from the aerial parts of *P. khinjuk* are α-pinene, β-pinene, Myrcene, beta-caryophyllene, Germacrene B and Spathulenol. The extracts showed antimicrobial activity against bacteria (MIC = 0.02 - 0.5 mg/ml) and fungi (MIC = 0.06 - 0.4 mg/ml).

Conclusion: In conclusion the different leaves extracts of *P.khinjuk* (chloroform, ethyl acetate, ethyl alcohol, diethyl ether) like other species of *Pistacia* show antibacterial and antifungal activities.

Keywords: ***Pistacia khinjuk*, Antibacterial activity, Antifungal activity**

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Plant

The genus *Pistacia* belongs to the family Anacardiaceae. Among 15 known species of pistachios, only 3 species grow in Iran, including *Pistacia Vera, Pistacia khinjuk and Pistacia atlantica*. They are the most important species of pistachio and for this reason, Iran is known as the origin of pistachios. *P.khinjuk* is spread in places where the attitude is 700 - 2000 m [1].

Uses in traditional medicine

Some species of *Pistacia* have been used in folk medicine and these plants are used as antiflammatory, antipyretic, antibacterial, antiviral, in treatment diarrhea and throat infection [2 - 4].

Previously isolated compounds

Phenolic composition consisting of gallic acid and quinic acid [5], flavonoids (kaempferol, quercetin, myricetin, taxifolin, distylin, naringenin, cyanidin) [6-8], essential oils (pinene, ,camphene,limonene, myrecene, gurjenene, etc) [9-12] and terpenoids [13, 14].

Plant material

The leaves of *P. khinjuk* were collected from Kerend of Kermanshah (western part of Iran) in May 2008. The voucher specimen (No.2406) is deposited in the Herbarium of Agricultural Faculty of Razi University, Kermanshah, Iran. The leaves were air dried in shadow. The essential oil hydrodistilled in a Clevenger-type apparatus (Clevenger 1928) according to the British method and Gas chromatography (GC) and Gas Chromatography-mass spectrometry (GC-MS) have been reported in the literature [15].

Tested materials

For obtaining different extracts of *P. khinjuk*, 30 gr of leaves powder was macerated with 120 ml of each solvent (chloroform, ethyl acetate, ethyl alcohol, diethyl ether).Yields for different extracts were 16.9, 5.1, 12.9, 0.13 percent respectively.

Used microorganism

The antimicrobial activity of different extracts was individually tested against a panel of microorganisms, including *Bacillus subtilis* (ATCC 127111), *Enterococcus faecalis* (ATCC 29737), *Staphylococcus aureus* (ATCC 29737), *Staphylococcus epidermidis* (ATCC 12229), *Escherichia coli* (ATCC 8739), *Klebsiella pneumoniae* (ATCC 10031), *Candida albicans* (ATCC 10231) and *Saccharomyces cerevisiae* (ATCC 9763) .Bacterial strains were cultured overnight at 37 ºC in Mueller Hinton broth (Merck). Yeasts were cultured at 28 ºC for 22h in Sabouraud dextrose broth (Merck). For the determination of antimicrobial activities MIC (minimum inhibitory concentration) method was employed.

Results

The major constituents of essential oil from the aerial parts of *P. khinjuk*, their kovats indices and their percentage are given in table 1.

Antibacterial and antifungal activity of the different extracts is shown in the table 2. The MIC of the extracts against the test microorganisms were determined by the Micro dilution method [16]. Antibacterial and antifungal differences of the extracts was determined by One way variance analysis (ANOVA), using the SPSS 11.5 software package. Data were considered statistically significant at $p \leq 0.05$.

Discussion

Different extracts of the leaves of

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Table 1- Chemical composition (%) of identified compounds in the oil of *P. khinjuk*

	Component	Percent	[a]KI
1	Tricyclene	0.64	926
2	α-pinene	2.11	936
3	camphene	1.52	953
4	Sabinene	0.67	967
5	β-pinene	1.49	980
6	Myrcene	2.85	1000
7	α-3-carene	0.29	1010
8	βcymene	0.58	1024
9	Limonene	0.58	1029
10	γTerpinene	1.15	1057
11	Nonanol	1.13	1104
12	Comphenol	0.63	1125
13	Trans-pinocarveol	0.35	1138
14	Verbenol	0.41	1148
15	P Mentha-1,5-dien8-ol	0.75	1169
16	α -terpineol	0.58	1195
17	Bornyl Acetate	2.77	1286
18	δ-Elemene	1.52	1339
19	beta-caryophyllene	3.67	1421
20	α -Guaiene	2.24	1444
21	Aromadendrone<dehydro>	8.80	1464
22	Germacrene B	9.53	1500
23	Luparene	1.38	1520
24	Occidentalol cadinene <deltha>	3.77	1532
25	Thusopsanone	3.84	1653
26	Eudesmol	2.29	1659
27	Spathulenol	20.87	1584
28	α -Eudesmol	6.78	1666
29	Occidentalol Acetate	3.68	1687
30	Zizanol	6.51	1699
31	Khusimol	1.48	1722
32	Aristolone	0.55	1768
33	Muurolene<14-hydroxy- α >	0.28	1842
34	Dibutyl phthalate	0.33	1956
35	Pentacosune	0.54	2500
36	Heptacosane	0.35	2580

[a]KI: kovats indices on column

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Table 2- The minimum inhibitory concentration (MIC, mg/ml) of the leaves of *P.khinjuk* against bacteria and fungi

Microorganism	Extract			
	Chloroform	Ethyl acetate	Ethyl alcohol	Diethyl ether
B. subtilis	0.09	0.05	0.34	0.16
E. faecalis	0.21	0.38	0.19	0.36
S. aureus	0.04	0.13	0.09	0.42
S. epidermidis	0.25	0.06	0.25	0.13
E. coli	0.19	0.36	0.34	0.17
K. pneumoniae	0.02	0.13	0.04	0.16
C. albicans	0.10	0.34	0.43	0.38
S. cerevisiae	0.06	0.36	0.18	0.09

The results are the conclusion of three replicates.

P. khinjuk inhibited gram positive and gram negative bacteria probably due to its particular chemical pattern mainly the amounts of Germacrene B, α-pinene and β-pinene. Chloroform and ethyl alcohol extracts inhibited *K. pneumoniae* more than others bacteria. Ethylacetate and diethyl ether extracts inhibited *B. subtilis* and *S. epidermidis* respectively more than other bacteria.

Also the extracts of the leaves of *P. khinjuk* inhibited fungi. Among different extracts, chloroform extract inhibited growth of fungi more than others (MIC 0.10 for *C. albicans* and 0.06 for *S. cerevisiae*). In conclusion the leaves extracts of *P.khinjuk* (especially chloroform extract) like other species of *Pistacia* show antibacterial and antifungal activities.

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