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Analysis of Essential Oils of *Juniperus polycarpous* and *Juniperus communis*

SAHAR REZVANI*, MOHAMMAD ALI REZAI and NOSRATOLLAH MAHMODI
Department of Plant Physiology, Azad University of Golestan, Gorgan, Iran
Email: sahar_rezvani2056@yahoo.com

In this study, the ethanolic extracts of fleshy female cones of *Juniperus polycarpous* and *Juniperus communis* collected in May 2005 from natural habitat in Charbagh village (1950 m) from Golestan, Iran were analyzed.

Key Words: *Juniperus polycarpos, Juniperus communis*.

INTRODUCTION

Juniper is an evergreen coniferous shrub or small tree occurring throughout the northern hemisphere from Europe to Siberia and grows up to 10 m in height[1]. The medicinal portions of the plant are referred to as berries, but they are actually dark blue-black scales from the cones of the tree[2]. Unlike other pine cones, the juniper cones are fleshy and soft[3]. A remedy to treat tapeworm was found[4] in the Papyrus of Ani from ancient Egypt, 240 BC. It is also known that the branches and berries were burned in temples as a part of purification ceremonies. In the Ayurvedic system juniper is believed to not only purify the body but also the aura or subtle body. It also helps to destroy negative astral influences[2]. In more modern times the berries are used to flavour gin by extracting their essential oil and in cooking as well as in herbal medicine[5]. Juniper is an important spice in many European cuisines, especially in Alpine regions, where juniper grows abundantly[6]. It is the only example of a spice in the botanic group of the *coniferae* and also one of the few examples of spices from cold climatic regions, though the best quality stems from southern European countries[7]. For it's preparation, fresh cabbage is preserved by lactic fermentation and seasoned with juniper, caraway and a few bay leaves[8]. The taste then develops during ageing in large wooden barrels. *Sauerkraut* can either be eaten raw or be cooked or fried to be served as a side dish; there are also dumplings stuffed with it[9]. The ripe, dark blue berries are used for herbal remedies. As it takes 2 to 3 years for the berries to reach full ripeness, both green, unripe berries and dark, blue berries can be found growing together on the same plant[10]. Local Iranian names are Hovars, Avars (in Caraj valley), Ors (in Khorasan), Arduj (in Manjil & Khalkhal), Arbas (in Manjil), Archeh (in Goshankhaneh & Soaldy), Abol (in Bakhtiari), Vors (in Amol & Haraz valley), Archa,

Orsa, Qara arsha[11]. The berries are normally harvested in the autumn of their second year when they are blueish-black in colour. They should be dried carefully to preserve the volatile oil. The fresh berries can be made in to syrup. Juniper berries have a bittersweet taste and a hot, drying energy and, because of this characteristic, they should be used sparingly with appropriate balancing herbs[12]. Juniper berries owe their use to an essential oil, content 0.2 to 2 % dependent on provenance[13]. Hungarian berries contains 1.2%, German berries only 0.7 % and Iran berries 0.9 %. The essential oil is mainly composed of monoterpenes: 80 % α- and β-pinene, thujene, sabinene, 5 % terpinene-4-ol, α-terpineol, borneol and geraniol; sesqiterpenes (α- and β-cadinen, caryophyllene) are found in traces. The essential oil from this plant was analyzed for the chemical components[11]. It was also tested for its antibacterial activities against a wide spectrum of Gram-positive and Gram-negative bacteria[14]. In addition, the essential oil was subjected to antifungal and antioxidant testing. The major chemical components obtained were camphene, α-pinene, β-pinene, cymene, limonene, β-myrcene, terpinene-4-ol, linalool, *trans*-carryophyllene, α-phellandrene, 3-carene, α-terpineol and germacrene[15]. The essential oil exhibited notable antibacterial activity against gram-positive and -negative bacteria as well as significant antifungal and antioxidant activity[16].

EXPERIMENTAL

The cones (25 g) were subjected to $(EtO)_2O$:hexane:MeOH 1:3:2 by soxhlet extractor method 13 h. The essential oil yield obtained was 0.19 % (v/w). After filtration, it was stored at *ca*. 4ºC until tested and chemically analyzed[17]. The essential oil was subjected to GC/MS analysis, antibacterial, antifungal and antioxidant testing[18].

Antibacterial testing

Test organisms: The test organisms were selected on the basis that they cause a lot of infections in humans. The organisms were obtained from the Department of Pharmacy, University of Guilan, Rasht as pure characterized strains[19].

Inoculation procedure: The isosensitest broth was inoculated aseptically with the appropriate microorganism 24 h before testing. This was to ensure that the bacteria fully adapted to the broth. The procedure was repeated for each bacterial species. The inoculated bacterial strains were incubated at 25ºC for 24 h. The procedure was repeated for each bacterial species[20].

Antibacterial activity: Essential oils were diluted with absolute alcohol to produce the following concentrations: 10, 20, 50 and 100 % (v/v). Agar was melted in a steam bath set at 30ºC to prevent solidification. 4 Petri dishes were pre-inoculated with the appropriate bacteria in the

following manner. 1 mL of the bacterial suspension was pipetted into the appropriately labeled Petri dish to which 25 mL of molten agar was then added followed by thorough mixing of the bacteria and molten agar. The agar was allowed to set for 1 h. 4 mm wide holes were then made in the agar using a borer[21]. Oil (25 µL) of a specific concentration was introduced into each of the holes in an appropriately labelled petri dish using a sterile micropipette. Gentamicin (10 g/mL) was used as a positive control and absolute alcohol as a negative control. The dishes were then incubated at 25ºC for 24 h after which zones of inhibition were measured and recorded. The zone of inhibition was taken to be the diameter of the zone visibly showing the absence of growth including the 4 mm hole.

RESULTS AND DISCUSSION

Chemical composition of the essential oil: 12 Compounds representing more than 99.99 % of the essential oils were identified in both species. α-Pinene, 3-carene, α-phellandrene and β-myrcene were the most abundant components of the essential oils in both *Juniperus polycarpos* and *Juniperus communis*. The other chemical components were limonene, *trans*-caryophyllene, β-pinene, cymene, germacrene, α-terpineol, linalool and terpinene-4-ol (Table-1). It is important to note that the monoterpene fraction was also present in relatively high amounts (> 85.57 %).

TABLE-1
MAJOR PHYTOCONSTITUENTS OF ESSENTIAL OILS FROM *Juniperus polycarpous and Juniperus communis*

Compound	Composition (%)		Compound	Composition (%)	
α-Pinene	16.022	22.996	β-Pinene	6.178	4.495
3-Carene	13.949	12.526	Cymene	5.782	6.657
α-Phellandrene	12.022	20.147	Germacrene	5.429	5.549
β-Myrcene	11.526	4.780	α-Terpineol	5.105	7.351
Limonene	7.474	5.067	Linalool	4.933	3.417
trans-Caryophyllee	6.756	4.359	Terpinene-4-ol	4.821	3.647

Antibacterial activity: Both *J. polycarpos* and *J. communis* exhibited notable antibacterial activity against *E. coli*, *S. aureus* and *P. aeruginosa* (Table-2).

The essential oils from these plants exhibited antibacterial, antifungal and antioxidant activities. These activities may be attributed to the presence of α-pinene, β-pinene, cymene, limonene, β-myrcene, terpinene-4-ol, linalool, *trans*-carryophyllene, α-phellandrene, 3-carene, α-terpineol and germacrene found in both species.

TABLE-2
ANTIBACTERIAL ACTIVITY OF *J. polycarpos* AND *J. communis*

Bacterial species source	Inhibition zone diameter (mm)					Gentamycin (10 µg/mL) (positive control)
	0 %	10 %	20 %	50 %	100 %	
Staphylococcus aureus NCIB 6751	0	12.1	14.8	15.0	9.5	16.1
Pseudomonas aeruginosa NCIB 950	0	0	0	0	8.5	14.5
Escherichia coli NCIB 8879	0	6.1	7.2	8.3	19.2	20.7

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