Antimicrobial Activity and Main Chemical Composition of Two Smoke Condensates from *Peganum harmala* Seeds

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The smoke of *Peganum harmala* seeds is traditionally used in Iran as a disinfectant agent. The aim of this study was to determine the antimicrobial activity of two smoke condensates from *Peganum harmala* seeds. Furthermore the composition of smoke preparations was studied using gas chromatography and mass spectroscopy analysis. The most prevalent compound detected in a dichloromethane extract was harmine. Standard harmine as well as the dichloromethane extract showed antimicrobial activity against all test strains. Harmine was not detected in an *n*-hexane extract and we did not observe antimicrobial activity from this smoke preparation at the tested concentrations.

Key words: Antimicrobial Activity, Harmine, *Peganum harmala*, Smoke

Introduction

Peganum harmala L. is a wild-growing flowering plant belonging to the Zygophyllaceae family and it is found abundantly in the Middle East and North Africa (Zargari, 1989). *P. harmala* seeds are commonly named "Esphand" in Iran. From ancient times, it has been considered an important medicinal plant. The *P. harmala* seeds are known to possess hypothermic and hallucinogenic properties (Lamchouri *et al.*, 1999; Kuhn and Winston, 2000). It has been used traditionally as an abortifacient agent in the Middle East and North Africa (Shapira *et al.*, 1989). There are several reports in the literature indicating a great variety of pharmacological activities for *P. harmala*, such as antimicrobial, antitumor, antinociceptive and MAO-inhibitor activities (Abdel-Fattah *et al.*, 1995; Prashanth and John, 1999; Monsef *et al.*, 2004). The smoke of Esphand is traditionally used as a disinfectant agent in Iran (Amin, 1991). To our knowledge, based on a literature search, no studies have been conducted on the antimicrobial properties of smoke condensates from *P. harmala* seeds. The objective of this investigation was to determine the inhibitory effect of two organic smoke preparations from Esphand against different strains of bacteria and fungi. The chemical compositions of these preparations were also studied using GC/MS. Harmine was identified as the individual alkaloid in Esphand smoke.

Materials and Methods

Microorganisms

Clinical isolates of *Bacillus subtilis*, *Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella typhi*, *Serratia marcescens*, *Shigella dysenteriae*, *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Pseudomonas aeruginosa* were obtained from Shariati Hospital, University of Tehran, Iran. The identification of these strains was carried out using conventional methods. *Aspergillus niger* (PIM), *Candida albicans* (ATCC 14053) and *Cryptococcus neoformans* (kf 33) were supplied by the Laboratory of General Microbiology, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran.

Plant material and preparation of smoke extracts

The seeds of *Peganum harmala* L. (Zygophyllaceae) were collected from Abyaneh (Isfahan Province), Iran, in June 2003 and authenticated by

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Dr. H. R. Monsef-Esfahani. Voucher specimens were deposited in the Herbarium of Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran. For the manufacture of smoke extracts, smoke from smoldering seeds (100 g) was passed to a condensing tower where it was captured in organic solvents. Dichloromethane and *n*-hexane were used as the solvents. The extracts were evaporated to yield a dark-brown viscous residue, and reserved for antimicrobial experiments.

Gas chromatography-mass spectroscopy (GC/MS)

The composition of the smoke extracts was identified and quantified by GC/MS on a Thermo-Quest # 2000 instrument using a DB-1 capillary column (30 m × 250 μm × 0.1 μm). The gas chromatograph column was programmed to increase the temperature from 50 °C (isothermal, 1 min) to 260 °C at a rate of 2.5 °C/min with the mass spectrometer operating at 70 eV and an ion source temperature of 250 °C. The constituents of the smoke extracts were identified by comparing their GC retention times with known compounds. Confirmation was obtained by comparing the MS fragmentation pattern of each component with those of the authentic compounds (Eight Peak Index of Mass Spectra, 1983; Adams, 1995).

Antimicrobial activity of smoke extracts

The antimicrobial activities of the smoke extracts and harmine (Sigma Chemical Co., USA) were studied by a conventional disc-diffusion method. An antimicrobial assay was performed based on the dry mass of the smoke extracts. Sterilized blank discs containing different amounts of each smoke extract (0.156, 0.312, 0.612, 1.25, 2.5, 5 mg) were applied to the inoculated Muller-Hinton agar plates. The petri dishes were incubated at 37 °C for 24 h (bacteria), at 25 °C for 48 h (yeasts) and at 25 °C for 72 h (mycelial fungus). After incubation, the zones of inhibition around the discs were measured and compared. The assays were performed in triplicate. Gentamycin standard discs were supplied from Padtan Teb Co., Tehran, Iran and used as positive control for antibacterial assays. Discs containing freshly prepared nystatin (100 μg) were used as a positive control for antifungal assays. Blank discs were impregnated with *n*-hexane and dichloromethane and used as negative control in all experiments.

Results and Discussion

The GC/MS analysis showed that the smoke extracts were composed of many compounds. Thirty-five compounds representing 94.7% and 30 con-

Table I. Main compounds of smoke preparations from *Peganum harmala* seeds.

No.	Compound	KI	*n*-Hexane extract (%)	Dichloromethane extract (%)	Identification method[a]
1	Dodecane	1198	3.8	–	KI, Mass
2	Tetradecane	1397	5.6	1.6	KI, Mass
3	Methyl dodecanoate	1506	12.4	–	KI, Mass
4	Hexadecane	1593	5.6	2.9	KI, Mass
5	2-Octanol benzoate	1675	1.1	3.1	Mass
6	Heptadecane	1698	5.4	6.2	KI, Mass
7	Methyl tetradecanoate	1705	5.8	–	KI, Mass
8	2,6,10,14-Tetramethyl pentadecane	1708	–	2.6	Mass
9	Octadecane	1795	7.9	7.6	KI, Mass
10	2,6,10,14-Tetramethyl hexadecane	1810	1.4	3.7	Mass
11	Nonadecane	1895	6.0	8.1	KI, Mass
12	Methyl hexadecanoate	1904	4.4	–	KI, Mass
13	Dibutyl phthalate	1906	–	3.6	Mass
14	Eicosane	1995	6.8	9.3	KI, Mass
15	Methyl oleate	2071	2.6	1.5	Mass
16	Henicosane	2087	5.4	12.9	KI, Mass
17	Docosane	2195	5.3	–	KI, Mass
18	Harmine	2210	–	14.1	Mass
19	Tricosane	2295	3.4	5.1	KI, Mass
Sum	Major components	–	82.8	80.13	–

[a] KI, Kovat's retention index; Mass, mass spectroscopy data.

Table II. Antimicrobial activity of smoke preparations from *Peganum harmala* seeds[a].

Microorganism	Zone of inhibition [mm]								
	Dichloromethane extract						Harmine	Gentamycin	Nystatin
	5 mg	2.5 mg	1.25 mg	0.625 mg	0.312 mg	0.156 mg	(5 mg)	(30 μg)	(100 μg)
Staphylococcus epidermidis	22.0 ± 1.0[c]	19.3 ± 0.6	13.7 ± 0.6	8.3 ± 0.6	–	–	25.3 ± 1.5	25.3 ± 1.2	ND[b]
Bacillus subtilis	15.0 ± 1.0	13.3 ± 1.2	10.3 ± 0.6	11.3 ± 0.6	8.3 ± 0.6	–	29.7 ± 1.5	30.0 ± 1.0	ND
Staphylococcus aureus	15.7 ± 1.5	11.7 ± 1.5	10.3 ± 0.6	11.3 ± 0.6	–	–	20.0 ± 1.0	17.0 ± 1.0	ND
Salmonella typhi	10.3 ± 0.6	–	–	–	–	–	15.0 ± 1.0	16 ± 1.0	ND
Shigella dysenteriae	13.0 ± 1.0	10.0 ± 1.0	–	–	–	–	–	17.0 ± 1.0	ND
Klebsiella pneumoniae	9.3 ± 0.6	–	–	–	–	–	13.7 ± 0.6	15.6 ± 1.2	ND
Pseudomonas aeruginosa	9.0 ± 1.0	–	–	–	–	–	9.3 ± 0.6	22.3 ± 1.2	ND
Escherichia coli	9.7 ± 0.6	9.3 ± 0.6	–	–	–	–	16.0 ± 1.0	–	ND
Serratia marcescens	8.3 ± 0.6	–	–	–	–	–	17.7 ± 0.6	10 ± 1.0	ND
Candida albicans	16.0 ± 1.0	11.7 ± 1.2	7.3 ± 0.6	–	–	–	25.0 ± 1.0	ND	11.7 ± 0.6
Aspergillus niger	12.7 ± 1.2	8.7 ± 1.5	–	–	–	–	28.7 ± 1.5	ND	8.7 ± 0.6
Cryptococcus neoformans	20.3 ± 1.5	15.3 ± 0.6	12.0 ± 1.0	–	–	–	33.0 ± 1.0	ND	10.0 ± 1.0

[a] No antimicrobial activity observed from the *n*-hexane smoke extract.
[b] Not determined.
[c] Data are mean ± SD of three distinct experiments.

stituents amounting 93% were identified in *n*-hexane and dichloromethane extracts, respectively (Khoshakhlagh, 2002). The major compounds and their proportion of each smoke extract are shown in Table I. The antimicrobial effects of smoke extracts at different concentrations are shown in Table II. The effective preparation against the test organisms was the dichloromethane extract. The most prevalent compound detected in the dichloromethane smoke preparation was harmine (14.1%). Standard harmine represented the most potent antibacterial activity, with inhibition zones of: 33 mm for *C. neoformans*, 30 mm for *B. subtilis*, between 9 and 29 mm for the other test strains. Other major components detected in the dichloromethane fraction were henicosane (12.9%), eicosane (9.3%), nonadecane (8.1%), octadecane (7.6%) and heptadecane (6.2%) in addition to tricosane and other compounds, which were present in amounts of less than 6%. Harmine was not detected in the *n*-hexane extract and we did not observe antimicrobial activity from this extract at the tested concentrations. Furthermore, the dichloromethane smoke condensate did not show antibacterial activity against any of the strains at the lowest content (0.156 mg), whilst the higher contents of this extract showed good antimicrobial activity, especially against *S. epidermidis* and *C. neoformans*.

Extracts from *P. harmala* seeds have been reported to exhibit antibacterial activity (Prashanth and John, 1999). This study demonstrates antimicrobial activity of a smoke preparation of Esphand which parallels the traditional use of *P. harmala* smoke as a disinfectant agent. To the best of our knowledge, this is the first study on the antimicrobial activity of the smoke condensates from *P. harmala* seeds.

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Abdel-Fattah A. F. M., Matsumoto K., Gammaz H. A. K., and Watanabe H. (1995), Hypothermic effect of harmala alkaloid in rats: involvement of serotonergic mechanism. Pharmacol. Biochem. Behav. **52**, 421–426.

Adams R. P. (1995), Identification of Essential Oil Components by GC-MS. Allured Publishing Corporation, Carol Stream, Illinois.

Amin Gh. (1991), Popular Medicinal Plants of Iran, Vol. 1, 1st ed. Research Deputy, Iranian Health and Medical Education Ministry, Tehran, Iran, pp. 85–90.

Eight Peak Index of Mass Spectra (1983), 3rd ed. Mass Spectrometry Data Centre, Royal Society of Chemistry, The University of Nottingham, Nottingham, UK.

Khoshakhlagh N. (2002), The compositions of volatile fractions of *Peganum harmala* seeds and its smoke. PhD Thesis, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran.

Kuhn M. A. and Winston D. (2000), Herbal Therapy and Supplements, a Scientific and Traditional Approach. Lippincott, New York, pp. 347–350.

Lamchouri F., Settaf A., and Cherrah Y. (1999), Antitumour principles from *Peganum harmala* seeds. Therapie **54**, 753–758.

Monsef H. R., Ghobadi A., Iranshahi M., and Abdollahi M. (2004), Antinociceptive effects of *Peganum harmala* L. alkaloid extract on mouse formalin test. J. Pharm. Pharm. Sci. **7**, 65–69.

Prashanth D. and John S. (1999), Antibacterial activity of *Peganum harmala*. Fitoterapia **70**, 438–439.

Shapira Z., Terkel J., Egozi Y., Nyska A., and Friedman J. (1989), Abortifacient potential for the epigeal parts of *Peganum harmala*. J. Ethnopharmacol. **27**, 319–325.

Zargari A. (1989), Medicinal Plants, Vol. 1. Tehran University Press, Tehran, pp. 637–639.