The Ethnopharmacological Study on Antibacterial Activity of some Selected Plants Used in Iranian Traditional Medicine

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Abstract

Background: Iran is a land of great heritage of ancient medical scholars. Herbal medicines, as a basement of treatment of diseases have been clearly described in the medical texts of these scholars including Rhazes, Avicenna, and others. Numerous plants are introduced in these texts to treat those diseases likely to be infective ones. Few attempts have been made to evaluate ethnopharmacological aspects of these plants.

Objective: In the present study selected specimens of plants described in ancient Iranian medical texts to treat infective conditions were evaluated for antibacterial activity. The hypothesis of this study was that the plants described in the ancient medical texts possess antibacterial properties.

Methods: In this study, ten specimens of these plants were selected from various texts. Their scientific terminologies were prepared based on various respected sources. The antibacterial activity of hydroalcoholic extracts of these herbs, as well as their MICs and MBCs were evaluated according to standard techniques.

Results: Results of this study showed that almost all of 10 specimens showed considerable antibacterial activity. The MIC and MBC of some herbs showed strong activity against gram positive and gram negative microorganisms. MICs and MBCs of *Heracleum persicum* and antibacterial activity of *Zea mays* are reported for the first time in this study.

Conclusion: The antibacterial activity of the selected plants supports their old uses as anti-infective agents. The results of this survey also showed antibacterial activity of *Arnebia euchroma* (Royle) Johst. and *Teucrium polium* L. traditionally used as poultice for infective wounds. *H. persicum* traditionally used in Iran as spice can play a valuable role in preventing food poisoning. The data of this study supported the importance of ethnopharmacological approach and opened also the new doors to future researches in this field.

Keywords: Herbal Medicine, Ethnopharmacology, Anti- Infective Agents

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Introduction

Iran with its five major climates has been a unique land for growing more than 7500 plant species, many of them are categorized as herbal medicine [1]. Herbal medicines, as a basement of treatment of various diseases and ailments, have been described by ancient well known Persian medical scholars including Rhazes, Heravi, Avicenna, and many others [2]. This medico- pharmaceutical information penetrated into European universities during medieval and had its own influence on the European renaissance [3]. Plants described by these renowned scholars certainly had a long range of substances for treatment of numerous diseases. These plants have been used for millennia in the traditional practice of Iranian cultures. Ethnobotanical and ethno-pharmacological information have been considered as an effective way in evaluation and confirmation of traditional pharmacological claims as well as a scientific approach in the development of drugs from natural origin [4].

Antibacterial drugs are among the first line of medicaments in many countries including Iran. Many antibacterial agents currently used to treat infectious diseases have been originally derived from medicinal plants [5]. Numerous antimicrobial and antibacterial studies have been carried out on medicinal plants based on the ethnobotanical and ethnopharmacological information [6, 7, 8]. Plenty of anti-infective plants were described by ancient Iranian scholars in their books and treatises. Few attempts have been made to consider ethno-pharmacological aspects of these herbs in a modern sense.

In the present study selected specimens of plants described in ancient Iranian medical texts to treat infective conditions were evaluated for antibacterial activity. The hypothesis of this study was that the plants described in the ancient medical texts possess antibacterial properties.

Materials and Methods

Study design

An ethno-pharmacological survey was carried out in the renowned ancient Iranian medical texts [9-13] to select medicinal plants documented in these texts to treat those diseases likely to be infective ones through a comparative study with what currently defined as bacterial infections in modern texts [14].

Selection and collection of specimens

Ten specimens with fidelity values were selected. The selected samples have been used for treatment of skin wounds, kidney disorders, and other infective diseases and ailments. Plant samples were obtained from local market as usually used in folk medicine. Identification was achieved by matching their old names against their scientific terminology in a group under the supervision of a botanist using various respected sources [15-19]. A voucher sample of the obtained specimens was deposited in the herbarium of the Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran. According to the policy of the herbarium, no specific number is given for such a sampling but the samples are kept for occasional checking during the study. The characteristic properties of the selected plants are presented in Table 1.

Plant extracts

Plant samples were powdered and kept in the air tight containers. Crude extracts were prepared from plant parts via maceration method with 80% ethanol. A quantity of 20 g of each powdered sample was macerated in

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Table 1- Botanical and common names, part used, and traditional medical uses of the herbs selected through literature survey

Botanical name	Family	Part used	Persian name	Traditional uses
Amaranthus lividus L.	Amarnthaceae	Seed	Tājkhorus	Infectious and putrefactive wounds (Rhazes)
Arnebia euchroma (Royle) Johst.	Boraginaceae	Root	Havāchubeh	Poultice (Avicenna); Diarrhea, Infectious ulcers (Aghili, Mo'men)
Cannabis sativa L.	Cannabaceae	Seed	Shāhdāneh	Purulent ear (Rhazes); Otalgia (Avicenna)
Coriandrum sativum L.	Umbelliferae	Fruit	Tokhm-e Geshniz	Diarrhea (Heravi, Mo'men); Erysipelas (Avicenna)
Glycyrrhiza glabra L.	Papilionaceae	Rhizome	Shirinbayan	Kidney infections (Avicenna, Heravi); Septic sore throat (Rhazes)
Heracleum persicum Desf.	Umbelliferae	Fruit	Golpar	Diarrhea, Wound (Avicenna, Rhazes)
Rhus coriaria L.	Anacardiacea	Epicarp	Somagh	Purulent ear (Avicenna, Mo'men, Aghili); Diarrhea (Avicenna, Aghili, Heravi, Mo'men, Rhazes);
Teucrium polium L.	Labiatae	Flowering part	Kalpureh	Fresh wounds, malignant ulcers, (Avicenna)
Zataria multiflora Boiss.	Labiatae	Aerial part	Āvishan-e Shirāzi	Halitosis, Acne (Heravi); Food poisoning (Mo'men)
Zea mays L.	Graminae	Corn crest	Zorrat or Jāwars (Gāwars) Hindi	Diuretic, Astringent (Avicenna, Heravi), anti-diarrhea (Heravi)

80% ethanol in room temperature for 4 days. The extrac of each plant was then evaporated under vacuum and stored at room temperature. The percent yield of each sample was evaluated as dry extract weight/ dry starting material weight × 100. Extracts were diluted at desirable concentrations in 80% ethanol and sterilized by filtration prior to antimicrobial assays.

Bacterial strains

The following bacterial strains (*Bacillus cereus* PTCC 1274, *Staphylococcus aureus* 6538-P and *Escherichia coli* ATCC 8739 and *Pseudomonas aeruginosa* ATCC 9027) were donated by the Department of Pharmaceutical and Food Control, Faculty of Pharmacy, Tehran University of Medical Sciences. Stock cultures of bacteria were kept in 20% glycerol in PBS (phosphate buffered saline) at -70 °C. Active cultures were generated by inoculating 100 µl of the thawed microbial stock suspensions into 5ml nutrient broth (Merck, Germany) followed by overnight incubation at 37°C. Freshly synchronized cultures of bacterial strains were prepared by successively transferring 100 µl of the vegetative cells into Muller Hinton broth and incubating for 24 h at 37°C. The cells were harvested by centrifugation at 1600*g* for 10min, washed with PBS, spun at 1600*g* again and diluted in sterile water to obtain 10^8 cfu/ml as estimated by the surface plate counting method [20].

Preliminary antibacterial test

The bacterial strains were transferred onto the surface of Mueller Hinton agar plates using sterile cotton swabs. Wells with 8mm diameters were cut in Muller Hinton agar plates using a sterile cork borer. Aliquots of 100 µl of 200 mg/ml of hydroalcoholic extracts of herbs were delivered into the wells, left 1h at 4 °C for better diffusion of the extracts into the media prior to overnight incubation at 37 °C. A well containing 80% ethanol was used as negative control. After incubation the inhibition zones around wells were measured in mm using a caliper.

Minimum inhibitory and bactericidal concentration tests

The concentrations of the most potent extracts were prepared in the range of 0.15 to 37.5 mg/ ml in Muller Hinton broth containing 10^6 cfu/ml of the standard strains of bacteria. Gentamicin was chosen as a positive control and diluted with Muller Hinton broth to make concentrations between 0.78 to 100 µg/ ml. After incubation at 37 °C over night, the test tubes were examined for possible growth and minimum inhibitory concentrations (MICs) of each extract was determined as the lowest concentration that ended with no growth. Tubes containing concentrations above the MICs were streaked onto Muller Hinton agar plates to achieve minimum bactericidal concentrations (MBCs) of individual extracts against the tested strains.

Results

In this study, 10 plant species were selected according to an ethno-pharmacological survey in some ancient Iranian medical texts. Table 1 provides the botanical names, common Persian names, plant parts and ethno-pharmacological uses of these species. Antibacterial activity of these species is displayed in Table 2. The minimum inhibitory concentrations (MIC) of the extracts and the minimum bactericidal concentrations (MBC) of them are brought in Table 3.

Almost all of 10 selected species showed considerable antibacterial activity. The MIC and MBC of some herbs (Table 3) showed strong activity against both gram positive and

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gram negative bacteria. Antibacterial potentials of *Heracleum persicum* Desf. and *Zea mays* L., as well as their MICs and MBCs, are being reported for the first time in this article. Antibacterial activity of some specimens including *Arnebia euchroma* (Royle) Johst., *Rhus coriaria* L., *Teucrium polium* L., and *Zataria multiflora* Boiss. is reported by other investigators [21-24]. Antibacterial data of this study is in good agreement with the reported data while the populations are not the same. The antibacterial activity of the selected plants supports their old uses as anti-infective agents.

Table 2- Preliminary antibacterial activity of some traditional herbs

Botanical name	Yield of extraction	Antibacterial activity (well inhibition zone) [a]			
		B. cereus	***S. aurous***	***E. coli***	***P. aeruginosa***
Amaranthus lividus L.	2 [b]	---	11 [c]	---	ND [d]
Arnebia euchroma Johst.	4	18	20	12	12
Cannabis sativa L.	3.7	11	10	---	ND
Coriandrum sativum L.	13	14	11	9	9
Glycyrrhiza glabra L.	17.8	ND	ND	ND	---
Heracleum persicum Desf.	15.5	25	18	11	11
Rhus coriaria L.	43.6	19	19	16	11
Teucrium polium L.	19.5	16	20	11	11
Zataria multiflora Boiss.	21	18	25	9	9
Zea mays L.	21.3	15	14	11	12
Ethanol 80		---	---	---	---

[a] Each well contains 20 mg extract.
[b] Percentage of extract yield (w/w) was estimated as (dry extract weight/ dry starting material weight) × 100
[c] The results given in millimeter are the conclusion of two replicates.
[d] Not detected.

Table 3- MICs and MBCs of the herbs selected through preliminary antibacterial test

Botanical name	Antibacterial activity [a]			
	B. cereus	***S. aurous***	***E. coli***	***P. aeruginosa***
Arnebia euchroma (Royle) Johst.	2.34 [b] (4.68) [c]	1.17 (2.34)	2.34 (4.68)	2.34 (4.68)
Heracleum persicum Desf.	ND [d]	0.15 (18.75)	0.15	37.5 (37.5)
Rhus coriaria L.	0.58	1.17 (4.68)	2.34	4.68 (9.37)
Teucrium polium L.	2.34 (9.37)	9.37	18.75 (18.75)	18.75 (18.75)
Zataria multiflora Boiss.	4.68	4.68 (9.37)	4.68 (9.37)	ND
Gentamicin	6.25×10^{-3}	3.12×10^{-3} (3.12×10^{-3})	6.25×10^{3} (12.5×10^{3})	ND

[a] The results are the conclusion of two replicates and presented in mg/ ml.
[b] Minimum inhibitory concentration (MIC)
[c] Minimum bactericidal concentration (MBC)
[d] Not detected.

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Discussion

Iran is a country with rich floral biodiversity and great heritage of medicinal plant uses [2]. Research on the traditional knowledge on medicinal herbs is a valuable mean to support their claims and a way for development of new drugs [4]. Infective diseases are usually characterized with clear symptoms, so that traditional physicians had been able to recognize such diseases and developed some effective therapeutics for these illnesses.

This study showed antibacterial activity of *A. euchroma* and *T. polium* which traditionally used in Iran as poultices for infective wounds. *Heracleum persicum* Desf. is traditionally used in Iran as anti-infective agent and as a well-known spice for foods [19]. Usage of this plant can play a valuable role in preserving probable food poisonings. There is a report on antibacterial activity of *H. persicum* Desf. [25]. In that report, the antibacterial activity of this plant is reported from Iran without any data on MIC and MBC of this herb. Furthermore, our study showed activity of *H. persicum* against both gram positive and gram negative microorganisms in contrast with that report which antibacterial activity was limited to gram positive bacteria [25].

This study also reveals the importance of ethno medical approach to support their claims and their data should be regarded fruitful.

Antibacterial activity in somehow is indicative of presence of some antibacterial compounds and many antibacterial agents have been originally derived from medicinal herbs [5, 6]. This study put forth the importance of preserving and encouraging these herbs according to the standards of scientific achievements and open evidence based views toward a more rational policy on medicinal plant research.

Many of these herbs should be regarded as a valuable heritage and as our intellectual properties.

Unfortunately, many of these species are in danger due to various factors. Further investigations should be carried out on these antibacterial agents.

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