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Antibacterial Activity of Some Medicinal Plants of Iran Against *Pseudomonas aeruginosa* and *P. fluorescens*

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Abstract: Increase of antibacterial resistance is a global growing-problem. Isolation of microbial agents less susceptible to regular antibiotics and recovery of increasing resistant isolates during antibacterial therapy is rising throughout the world which highlights the need for new principles. In treating burns, dermatophytes and infectious diseases, use of plants is common in traditional medicine of Iran. According to the collected information about herbal remedies of such plants, antibacterial activities of methanol extracts of the plants were determined by *in vitro* bioassays using agar diffusion-method against standard strains of *Pseudomonas aeruginosa* and *P. fluorescens* at 20 mg ml⁻¹. From 160 plant species in 65 families, 13 species (8.1%) in 12 families (18.4%) showed anti-Pseudomona activities. Activities included 6.2% against *P. aeruginosa* and 7.5% against *P. fluorescens*. Minimum inhibitory concentrations (MIC) of the actives were determined using two fold serial dilutions. Most active plants against both bacterial species were *Dianthus caryophyllus* L., *Terminalia chebula* (Gaertner) Retz. and *Myrtus communis* L. with the MIC of 3.75, 1.87 and 7.5 mg ml⁻¹ against *P. aeruginosa*; 0.46, 0.93 and 1.87 mg ml⁻¹ against *P. fluorescens*, respectively.

Key words: Antibacterial activity, plant extracts, Iranian medicinal-plants, *Pseudomonas*

INTRODUCTION

Over the past 20 years, there has been a lot of interest in the investigation of natural materials as sources of new antibacterial agents. Different extracts from traditional medicinal plants have been tested. Some natural products have been approved as new antibacterial drugs, but there is an urgent need to identify novel substances active towards pathogens with high resistance^[1,2].

Use of plants in treating burns, dermatophytes and infectious diseases or as antiseptic and anti-inflammatory is common in Iranian traditional medicine (ITM).

P. aeruginosa is the most prevalent burn-patients pathogen capable of causing life-threatening illnesses^[3]. Clinically significant infections include infection of wounds and burns, giving rise to blue-green pus also meningitis, when introduced by lumbar puncture and urinary tract infection when introduced by catheters and instruments or irrigating solutions^[4]. In infants or debilitated persons, the bacterium may invade the bloodstream and result in fatal sepsis. Some strains causing septicemia and pneumonia in cystic fibrosis and immunocompromised patients are becoming difficult to treat with currently available antimicrobial agents. This organism is one of the most common pathogens

associated with bacterial corneal ulcers. Keratitis due to this pathogen also has been observed in the wearers of extended-wear contact lenses^[5]. Due to multi-resistance of *P. fluorescens*, there is a lack of active antibiotics effective against this bacterium, resulting in an increase in nosocomial infections and high mortality^[27]. The bacterium is an opportunist associated with respiratory and urinary tract, wounds and bacteremia^[4].

In a two-year study, a survey was set to screen antibacterial activity of some plants used in ITM in curing various maladies. Based on the information gathered from ethno pharmacologists, herbal-drug sellers and rural native-healers, the plant organs used in this study were as used in ITM. Many other researchers have also reported detection of antibacterials from plants in recent years. McCutcheon *et al.*^[6] tested 100 methanolic extracts of the plants, used by British Colombian Native People, against 11 bacterial isolates. They found 85% of the plants with antibacterial activity. Pedersen *et al.*^[7] examined antibacterial activity of 27 medicinal plant extracts of Rubiaceae and found 11 actives. Mansouri *et al.*^[8] evaluated antibacterial activity of crude extract of *Myrtus communis* against 10 laboratory strains of bacteria. They noticed that the crude extract inhibited the growth of all tested bacteria except *Campylobacter jejuni*. According

to reports, increase of antibacterial resistance is a worldwide growing-problem. Isolation of microbial agents less susceptible to regular antibiotics and recovery of increasing resistant isolates during antibacterial therapy is rising throughout the world^[9-16]. One of the measures to minimize the increasing rate of resistance in the long-run is to have continuous in-depth investigation for new, safe and effective antimicrobials as alternative agents to substitute with no effective ones. Natural resources, especially plants and microorganisms are potent candidates for this aim.

At the present study methanol extracts of 160 plant species in 65 families were tested at 20 mg ml⁻¹ by *in vitro* bioassays using agar diffusion-method against standard strains of *Pseudomonas aeruginosa* and *P. fluorescens*.

MATERIALS AND METHODS

Plant material and extraction procedure: The medicinal plants used in this study were collected from different regions of Iran and identified in the Herbarium of Plant Systematic Laboratory of the College of Agricultural Sciences, Bahonar University of Kerman, Iran where a voucher specimen was deposited. Sixteen species used by Iranian people but not grown in Iran were obtained from the local stores in Kerman city. The fine powder of air dried specimens were extracted three times with boiled methanol and the extracts were then concentrated under reduced pressure to yield a dense residue^[17,18]. Each sample transferred to glass vials and kept at 4°C before use.

Test organisms and agar well diffusion method: The standard strains of *Pseudomonas aeruginosa* (PTCC No. 1074) and *P. fluorescens* (PTCC No. 1181) were used as test microorganisms and obtained from Persian Type Culture Collection (PTCC), Tehran, Iran. The bacteria were rejuvenated on Mueller-Hinton-Agar medium (MH, E. Merk, Germany) and subcultured as needed. For bioassays, suspension of approximately 1.5x10⁸ cells ml⁻¹ in sterile normal saline were prepared as described by Forbes *et al.*^[19] and about 1.5 ml of each was uniformly seeded on MH in 9x1.2 cm glass Petri dishes, left aside for 15 min and excess of suspension was then drained and discarded properly. Wells of 6 mm in diameter and about 2 cm apart were punctured in the culture media using sterile cork borers. As a precaution not to miss trace amounts of antimicrobials, a relatively high concentration of 20 mg ml⁻¹ of each extract was prepared in dimethyl sulfoxide (DMSO): methanol (1:1, v/v) solvent (DM solvent) and administered to fullness in each well. Culture plates, were incubated at 37°C in case

of *P. aeruginosa* and 29°C in case of *P. fluorescens*. After 48 hr bioactivity was determined by measuring Diameter of inhibition zones (DIZ) in mm. All samples were tested in triplicate. Controls included incorporated DM solvent without test compounds, although no antibacterial activity noted in the controls.

Determination of minimum inhibitory concentration: Minimum inhibitory concentrations (MIC) determined for the active methanol-extracts using two fold dilution series of 0.46-15 mg ml⁻¹ in DM solvent and tested on the appropriate bacterial species as described earlier.

RESULTS AND DISCUSSION

From 160 plant species in 65 families, 13 species (8.1%) in 12 families (18.4%) showed antibacterial activities as indicated in Table 1. The table also contains MIC values of the actives measured in two fold dilution-series of 0.46-15 mg ml⁻¹. Activities included 6.2% against *P. aeruginosa* and 7.5% against *P. fluorescens*. *Dianthus caryophyllus* (whole plant), *Terminalia chebula* and *Myrtus communis* were the most active plant against both species, with the MIC of 3.75, 1.87 and 7.5 mg ml⁻¹ against *P. aeruginosa* and 0.46, 0.93 and 1.87 mg ml⁻¹ against *P. fluorescens*, respectively. Highest level of activity against *P. fluorescens* was noted with *Lawsonia inermis* with MIC of 0.46 mg ml⁻¹. Since *T. chebula*, *D. caryophyllus* and *M. communis* extracts were most actives against *P. aeruginosa*, further work is required to determine their activity on more strains isolated from clinical samples.

According to many reports^[20-22], multiple resistances to *P. aeruginosa* are spreading hazards in the world. An alternative to combat the problem of microbial resistance is development of new antibacterials for substitution with ineffective ones. Accordingly, medicinal plants and microorganisms are the proper candidates and should receive continuous research attention. The use of higher plants to treat infections is an age-old practice in a large part of the world population. Furthermore, because of the side effects and the resistance that pathogenic microorganisms build against the common antibiotics, much recent attention has been paid to extracts and biologically active compounds isolated from plants used in herbal medicine^[23-26]. Giamarellos-Bourboulis *et al.*^[27] found plant derivatives of gamma-linolenic acid and arachidonic acid act bactericidally on a significant number of multi-resistant *P. aeruginosa* isolates, but did not mention the plant names. In many parts of Iran there is a rich tradition in the use of herbal medicine for treatment of various infectious diseases and since Iran possesses vast number

Table 1: Evaluation of antibacterial activity, indicated by Diameter of inhibition zones (DIZ, mm), of plants used in Iranian native medicine against *Pseudomonas aeruginosa* and *P. fluorescens*

Plant family	Plant species	English common name	OT	a		b	
				DIZ	MIC	DIZ	MIC
Anacardiaceae	<i>Semecarpus anacardium</i> L. f.	Markingnut tree	LE	10	15.00	10	15.00
Apiaceae	<i>Anethum graveolens</i> L.	Dill; dillweed	FR	-	-	12	7.50
Apiaceae	<i>Trachyspermum ammi</i> (L.) Link	-	SE	11	15.00	-	-
Boraginaceae	<i>Borago officinalis</i> L.	Borage	FL	-	-	12	15.00
Caryophyllaceae	<i>Dianthus caryophyllus</i> L.	Carnation	WP	12	3.75	18	0.46
Combretaceae	<i>Terminalia chebula</i> (Gaertner) Retz.	Myrobalan	RS	16	1.87	18	0.93
Combretaceae	<i>Terminalia chebula</i> (Gaertner) Retz.	Myrobalan	US	14	7.50	14	7.50
Compositae	<i>Marticaria chamomilla</i> L.	Marticaria	FL	-	-	11	15.00
Ephedraceae	<i>Ephedra intermedia</i> Schrenk. Ex C.A.Mey. <i>Lawsonia inermis</i> L.	Ephedra	LE	10	15.00	13	7.50
Lythraceae	Syn. <i>L. alba</i> L. Malvaceae	Henna	LE	-	-	17	0.46
	<i>Aithaea officinalis</i> L.	Marsh mallow	FL	-	-	11	7.50
Myrtaceae	<i>Myrtus communis</i> L.	Myrtle	LE	11	7.50	16	1.87
Myrtaceae	<i>Myrtus communis</i> L.	Myrtle	SE	11	7.50	16	1.87
Nymphaeaceae	<i>Nymphaea alba</i> L.	White water lily	FL	10	15.00	-	-
Urticaceae	<i>Urtica gracilentia</i> Greene	Mountain nettle	LE	10	15.00	-	-

OT: Organs tested, as FL: Flower, FR: Fruit, LE: Leaves, RS: Ripen Seed, SE: Seeds, US: Unripe Seed and WP: Whole Plant. a: *Pseudomonas aeruginosa* (PTCC No. 1074), b: *P. fluorescens* (PTCC No. 1181), DIZ: (Diameter of Inhibition Zones, mm), MIC: (Minimum Inhibitory Concentration, mg ml⁻¹). Blank DIZs=0, blank MICs= not tested since the corresponding DIZs were 0

of medicinal plants^[28], their antimicrobial and phytochemical studies would provide valuable information to the media of the world knowledge. The present survey forms the basis for investigation on fractionation, purification, structural determination of the most promising components for *in vivo* evaluation of toxicity of these plants in animal and human studies.

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