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Evaluation of Antibacterial Properties of Iranian Medicinal-Plants against *Micrococcus luteus*, *Serratia marcescens*, *Klebsiella pneumoniae* and *Bordetella bronchoseptica*

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Abstract: From 195 plant samples in 76 families used by Iranian Native People for curing infectious maladies, methanolic extracts of 64 samples in 37 families showed antibacterial activity at least against one bacterial species of *Bordetella bronchoseptica*, *Micrococcus luteus, Klebsiella pneumoniae* and *Serratia marcescens*. High activity belonged to *Terminalia chebula, Myrtus communis*, *Dianthus coryophyllus*, *Rhus coriaria*, *Lawsonia inermis* and *Alpinia officinarum* against *B. bronchiseptica*; *T. chebula, M. communis*, *D. coryophyllus, Glycyrrhiza glabra*, *Ranunculus asitaticus*, *R. coriaria*, *Rheum ribes*, *Chrozophora verbasafalia*, *Ephedra intermedia*, *Cinnamomum zeylanicum* and *Citrullus colcocynthis* against *M. luteus*; *R. ribes*, *R. coriaria* and *G. glabra* against *K. pneumoniae*. No plant showed high or medium activity against *Serratia marcescens*. Umbelliferae with 7, Labiatae with 6 and Compositae with 4, had most number of active samples per plant family. Lowest MIC belonged to *T. chebula* (ripen seeds) and *M. communis* (leaves) as 0.93 mg ml^{-1}, against *B. bronchiseptica* and to *T. chebula* (unripe seeds) as 0.46 mg ml^{-1} against *M. luteus* and to *R. ribes* and *R. coriaria* as 1.87 mg ml^{-1} against *K. pneumoniae*. Most susceptible bacterium was *B. bronchiseptica* and most resistant bacterium was *S. marcescens*. All of the active extracts were well stable at room temperature up to 18 months and did not show any reduction of activity against the sensitive bacterial isolates.

Key words: Antibacterial, iranian medicinal-plants, *Micrococcus luteus*, *Serratia marcescens*, *Klebsiella pneumoniae*, *Bordetella bronchoseptica*

INTRODUCTION

During the last two decades, due to the increasing development of drug resistance in pathogenic microorganisms as well as the appearance of undesirable side effects of certain antibiotics and the emergence of previously uncommon infections[1-8] antimicrobial properties have been reported in a wide range of plant extracts with the goal to discover new chemical classes of antibiotics that could resolve these problems[9-22]. Traditionally, usage of plants in curing illnesses has deep roots in man's history[23]. Ethnopharmacological uses of plants prevail among Iranian Native People (INP). They use plants in treating burns, dermatophytes and infectious diseases. The curative plants vary between these natives in different localities. In order to screen and determine antibacterial effects of these plants, four different bacterial species including a gram positive, *Micrococcus luteus* and three gram negative bacteria *Serratia marcescens*, *Klebsiella pneumoniae* and *Bordetella bronchoseptica* were used. *M. luteus* causes minor infections especially in patients with suppressed immune system. *S. marcescens* is a common opportunistic pathogen causing pneumonia, bacteremia and endocarditis-especially in narcotics addicts and hospitalized patients. *K. pneumoniae* is present in the respiratory tract of about 5% of normal individuals; it can produce extensive hemorrhagic necrotizing consolidation of the lung. It occasionally produces urinary tract infection and bacteremia with focal lesions in debilitated patients. It also causes hospital-acquired infections. *B. bronchoseptica* occasionally inhibits the respiratory tracts, in which causes infections as a common opportunistic pathogen. It also infects respiratory tracts of canines, in which it may cause "Kennel cough" and pneumonitis[24]. In a two-year study, 195 species of INP-medicinal plants were tested in an *in vitro* bioassay method against these bacteria.

MATERIALS AND METHODS

Plant material: One hundred ninety five plant samples belonging to 76 families, being used by INP, were collected from South-East regions of Iran and identified

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by Mrs. P. Rashid Farrokhi in the Herbarium of Plant Systematic Laboratory of the College of Agricultural Sciences, Bahonar University of Kerman, Iran, where voucher specimens of plants were deposited. Twenty species, used in INP but not grown in Iran, were prepared through the mentioned Herbarium. According to the information gathered about the ethnopharmacological usages, the plant organs used in this study were the same as used by INP.

Preparation of the extracts: The plant parts were dried in shade or an oven at 40°C and powdered with mortar and pestle or by coffee blender. The methanol extracts were prepared by maceration of the plant material with methanol for 3 days at room temperature and this procedure was repeated twice. The respective extracts were filtered and dried under reduced pressure at a temperature below 45°C to yield a dense residue. Sample transferred to glass vials and lyophilized overnight before use.

Preparation of test samples: In the study of the antimicrobial activity, the methanol extracts were diluted in dimethylsulfoxide (DMSO): methanol (1: 1, v/v) solvent. As a precaution not to miss trace amounts of antimicrobials, for preliminary screening, a relatively high concentration of 20 mg ml^{-1} of each extract was prepared for bioassays.

Test microorganisms: The plant extracts were assayed for antimicrobial activity against five registered bacterial-isolates which were obtained from the Persian Type Culture Collection, Tehran, Iran (PTCC). The bacteria included two Gram-positive bacterial isolates: *Micrococcus luteus* (PTCC No. 1110) and *M. luteus* (PTCC No. 1170) and three Gram-negative bacteria: *Bordetella bronchiseptica* (PTCC No. 1025), *Klebsiella pneumoniae* (PTCC No. 1053) and *Serratia marcescens* (ATCC No. 27117). The bacteria were rejuvenated on Mueller-Hinton-Agar medium (MHM, Merk, Germany) and subcultured as needed.

Antimicrobial bioassay: For bioassays, suspension of approximately $1.5x10^8$ bacterial cells ml^{-1} in sterile normal saline were prepared as described by Forbes *et al.*[25] and about 1.5 ml of it was uniformly seeded on MHM in 12x1.2 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. Respective concentrations were administered to fullness in each well. Culture plates, were incubated at 37°C for 48 h. Bioactivity was determined by measuring Diameter of inhibition zones (DIZ) in mm. Each experiment was repeated three times and the mean of the diameter of the inhibition zones was calculated. Controls included use of solvent without test compounds, although no antibacterial activity noted in the solvent employed for the test.

Determination of Minimum Inhibitory Concentrations (MIC): To measure the MIC values, concentrations of 15, 7.5, 3.75, 1.87, 0.93 and 0.46 mg ml^{-1} of the methanolic extracts were prepared in DMSO: methanol (1/1: v/v) solvent and assayed against the bacteria as mentioned earlier. The MIC was defined as the lowest concentration able to inhibit any visible bacterial growth. All data represent average of three replicated experiments.

Determination of Shelf life or Stability of the active extracts: To measure the stability of the active extracts in both soluble and dry states, 20 mg ml^{-1} of each sample was prepared in DMSO: methanol (1:1, v/v) solvent and 20 mg dry samples placed in small vials. These samples were kept at room temperature and tested for antibacterial activity against the most sensitive bacterium at 14 days intervals up to 18 months.

RESULTS

From 195 plant samples in 76 families used by INP 64 samples in 37 families showed antibacterial activity at least against one of the bacterial species (Table 1). The activities were rated into three classes as: poor (DIZ<12mm), medium (DIZ 12 to <15 mm) and high (DIZ>15 mm or higher). Plants with high activity were as follows: *Terminalia chebula, Myrtus communis*, *Dianthus coryophyllus*, *Rhus coriaria*, *Lawsonia inermis* and *Alpinia officinarum* effective against *Bordetella bronchiseptica* (PTCC No. 1025); *T. chebula, M. communis*, *D. coryophyllus, Glycyrrhiza glabra*, *Ranunculus asitaticus*, *R. coriaria*, *Rheum ribes*, *Chrozophora verbasafalia*, *Ephedra intermedia* and *Cinnamomum zeylanicum* against *Micrococcus luteus* (PTCC No. 1110); *Citrullus colcocynthis* effective against *M. luteus* (PTCC No. 1170); and *R. ribes*, *R. coriaria* and *G. glabra* effective against *Klebsiella pneumoniae* (PTCC No. 1053). No plant showed high or medium activity against *Serratia marcescens* (ATCC No. 27117), only *Petroselinum sativum* and *Ziziphus spini-christi* had poor activity against it. Umbelliferae with seven, Labiatae with six and Compositae with four, had most number of active samples per plant family. Lowest MIC belonged to *T. chebula* (ripen seeds) and

Table 1: Evaluation results against four bacterial species of *Micrococcus luteus*, *Serratia marcescens*, *Klebsiella pneumoniae* and *Bordetella bronchoseptica* of 195 plant samples in 76 families used by Iranian Native People, from which 64 plant samples in 37 families showed inhibitory effects at least against one bacterial species. Antibacterial activity indicated by Diameter of inhibition zones (DIZ, mm), Minimum Inhibitory Concentration (MIC), plant species, families, used organs and bacterial species are listed. Plants whose DIZ values indicated in bold are rated as high actives, DIZ values underlined are rated as medium actives and DIZ values shown in regular font-style are rated as poor actives against the respective bacteria

			a		b		c		d		e	
Plant species	Plant families	OT	DIZ	MIC	DIZ	MIC	DIZ	MIC	DIZ	MIC	DIZ	MIC
Alhaji camelorum	Papilionaceae	SG	14	15	-	-	-	-	12	7.5	-	-
Alpinia officinarum	Zingiberaceae	FR	16	3.75	12	15	-	-	12	7.5	-	-
Althaea officinalis	Malvaceae	RO	10	15	-	-	9	-	-	-	-	-
Althaea officinalis	Malvaceae	FL	9	15	-	-	-	-	-	-	-	-
Amaranthus paniculatus	Amaranthaceae	FL	-	-	10	15	-	-	-	-	-	-
Amomum subulatum	Zingiberaceae	SE	14	15	14	7.5	-	-	10	15	-	-
Anthemis nobilis	Compositae	FL	-	-	-	-	9	-	-	-	-	-
Apium celleri	Umbelliferae	LE	10	15	-	-	-	-	-	-	-	-
Berberis integerrima	Berberidaceae	FR	12	7.5	14	7.5	-	-	-	-	-	-
Callendula officinalis	Compositae	FL	-	-	-	-	9	-	-	-	-	-
Camellia sinensis	Theaceae	LE	10	15	-	-	9	-	-	-	-	-
Cassia fistula	Cesalpinaceae	FR	10	15	-	-	-	-	-	-	-	-
Chrozophora verbasafalia	Euphorbiaceae	LE	-	-	16	7.5	-	-	-	-	-	-
Cinnamomum zeylanicum	Lauraceae	SB	14	7.5	15	7.5	-	-	14	3.75	-	-
Citrullus colcocynthis	Cucurbitaceae	FR	10	15	9	15	16	3.75	9	15	-	-
Citrus medica	Rutaceae	SE	9	15	-	-	-	-	-	-	-	-
Colchicum luteum	Colchicaceae	WP	-	-	-	-	10	-	-	-	-	-
Cuminum cyminum	Umbelliferae	SE	10	7.5	9	15	-	-	10	15	-	-
Cuscuta epithymum	Convolvulaceae	SE	11	15	-	-	-	-	-	-	-	-
Cydonia oblonga	Rosaceae	SE	10	15	-	-	-	-	-	-	-	-
Dianthus coryophyllus	Caryophyllaceae	WP	18	3.75	18	7.5	-	-	12	7.5	-	-
Echinops cephalotes	Compositae	LE	9	15	-	-	-	-	-	-	-	-
Echium amoenum	Boraginaceae	FL	9	15	-	-	-	-	-	-	-	-
Ephedra intermedia	Ephedraceae	LE	-	-	16	7.5	-	-	13	7.5	-	-
Eucalyptus globolus	Myrtaceae	LE	10	15	-	-	-	-	-	-	-	-
Glycyrrhiza glabra	Papilionaceae	RO	9	15	18	3.75	-	-	16	3.75	-	-
Heracleum persicum	Umbelliferae	FR	12	7.5	-	-	-	-	-	-	-	-
Juglans rejia	Juglandaceae	FL	10	15	-	-	-	-	-	-	-	-
Lawsonia inermis	Lythraceae	LE	18	3.75	-	-	10	15	-	-	-	-
Malva silvestris	Malvaceae	FL	10	15	-	-	-	-	-	-	-	-
Mentha longifolia	Labiatae	FL	9	15	-	-	-	-	-	-	-	-
Myristica fragrans	Myristicaceae	SE	12	15	-	-	-	-	10	7.5	-	-
Myrtus communis	Myrtaceae	SE	16	7.5	20	3.75	-	-	-	-	-	-
Myrtus communis	Myrtaceae	LE	26	0.93	20	3.75	12	15	-	-	-	-
Nepeta menthoides	Labiatae	RO	9	15	-	-	-	-	-	-	-	-
Nigella sativa	Ranunculaceae	SE	-	-	-	-	9	-	-	-	-	-
Nymphaea alba	Nymphaceae	FL	10	15	10	15	-	-	9	15	-	-
Ocimum album	Labiatae	LE	10	15	-	-	-	-	-	-	-	-
Peganum harmala	Zygophyllaceae	SE	-	-	9	15	-	-	-	-	-	-
Petroselinum sativum	Umbelliferae	SE	-	-	-	-	-	-	-	-	10	15
Portulaca oleracea	Portulaceae	SE	9	15	-	-	-	-	-	-	-	-
Punica granatum	Punicaceae	FL	9	15	-	-	-	-	-	-	-	-
Ranunculus asitaticus	Ranunculaceae	WP	13	7.5	18	7.5	-	-	-	-	-	-
Rheum ribes	Polygonaceae	RO	10	15	17	3.75	-	-	18	1.87	-	-
Rubus idaeus	Rosaceae	LE	13	7.5	-	-	-	-	-	-	-	-
Rhus coriaria	Anacardiaceae	FR	18	3.75	18	7.5	-	-	18	1.87	-	-
Ruta graveolens	Rutaceae	LE	-	-	-	-	9	-	-	-	-	-
Salix aegyptica	Salicaceae	FL	10	15	-	-	-	-	-	-	-	-
Salvia officinalis	Labiatae	WP	11	7.5	-	-	-	-	11	15	-	-
Semicarpus anacardium	Anacardiaceae	LE	11	15	12	15	-	-	10	15	-	-
Smilax china	Liliaceae	ST	10	15	-	-	-	-	-	-	-	-
Stachys lavandula folia	Labiatae	LE	10	7.5	-	-	-	-	-	-	-	-
Tamarix gallica	Tamaricaceae	LE	9	15	-	-	-	-	-	-	-	-
Terminalia chebula	Combretaceae	RS	25	0.93	24	0.93	-	-	-	-	-	-
Terminalia chebula	Combretaceae	US	13	7.5	30	0.46	-	-	10	7.5	-	-
Thymus kotschyanus	Labiatae	LE	9	15	14	15	10	-	-	-	-	-
Trachyspermum ammi	Umbelliferae	SE	9	15	-	-	-	-	9	15	-	-
Trachysermum copticum	Umbelliferae	SE	12	15	12	15	-	-	12	3.75	-	-
Trigonella foenum graecum	Umbelliferae	SE	9	15	-	-	-	-	9	15	-	-
Traxacum vulgare	Compositae	RO	8	15	-	-	-	-	9	15	-	-

Table 1: Continued

			a		b		c		d		e	
Plant species	Plant families	OT	DIZ	MIC	DIZ	MIC	DIZ	MIC	DIZ	MIC	DIZ	MIC
Viola odorata	Violaceae	FL	10	15	-	-	-	-	-	-	-	-
Zingiber officinale	Zingiberaceae	RH	14	7.5	-	-	14	7.5	-	-	-	-
Ziziphus spini-christi	Rhamnaceae	LE	-	-	-	-	10	-	-	-	10	15
Ziziphus spini-christi	Rhamnaceae	FR	-	-	-	-	10	-	-	-	10	15

DIZ= Diameter of Inhibition Zone (mm) at 20 mg ml^{-1}. MIC= Minimal Inhibitory Concentration (mg ml^{-1}). OT= Organs tested, as FL: Flower, FR: Fruit, LE: Leaves, RH: Rhizome, RO: Roots, SB: Stem Bark, SE: Seeds, SG: Stem Gum, ST: Stem and WP: Whole Plant. a= *Bordetella bronchiseptica* (PTCC No. 1025), b= *Micrococcus luteus* (PTCC No. 1110), c= *M. luteus* (PTCC No. 1170), d= *Klebsiella pneumoniae* (PTCC No. 1053), e= *Serratia marcescens* (ATCC No. 27117)

M. communis (leaves) as 0.93 mg ml^{-1} against *B. bronchiseptica* and to *T. chebula* (unripe seeds) as 0.46 mg ml^{-1} against *M. luteus* and to *R. ribes* and *R. coriaria* as 1.87 mg ml^{-1} against *K. pneumoniae*. Most susceptible bacterium was *B. bronchiseptica* being sensitive to 52 plant samples and most resistant bacterium was *S. marcescens* being susceptible only to three samples. All other 131 plant samples which did not show any inhibitory effects are not listed in the table. At 20 mg ml^{-1} concentration the largest DIZ belonged to *M. communis* leaves (26 mm) and *Terminalia chebula* ripen seeds (25 mm). All of the active extracts were well stable at room temperature in both DMSO: methanol (1:1, v/v) solvent and dry state up to 18 months and did not show any reduction of activity against the sensitive bacteria as compared to the starting day.

DISCUSSION

Many published reports show the effectiveness of traditional herbs against microorganisms, as a result, plants are one of the bedrocks for modern medicine to attain new principles[26]. In this regard, plants have given western pharmacopoeia about 7000 different pharmaceutically important compounds and a number of top-selling drugs of modern time, e.g. quinine, artemisinin, taxol, camptothecin, etc.[27].

Acquired resistance to antibiotics in bacteria over 25 years now constitutes a serious threat to public health[8,7]. The phenomenon is further compounded by demographic factors (e.g. population growth and urbanization) which generate fertile conditions for the transmission of infections and new opportunities for inter-species traffic of pathogens to man. Problem of antibiotic resistance in both hospital-acquired (nosocomial) and community-acquired bacterial infections have made many antibiotics virtually obsolete. As the reports show, no antibiotic can last effective too long. To combat the problem of microbial antibiotic resistance, one logical way is providing new agents. It is vital that research strategies be oriented toward discovery and development of antimicrobial agents urgently required in the future.

I suggest the most active extracts presented (Table 1) for evaluation against methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant enterococci (VRE) isolates[28, 29] two of the most problematic bacteria.

Smith *et al.*[30] express that "The emergence of bacterial resistance threatens to return us to the era before the development of antibiotics" and I like to express that "For not loosing the battle in the war against antimicrobial-resistant bacteria, one major way is to extend man's knowledge about the new antimicrobial sources". The results of such studies form the avenue for further investigations to find new drugs for therapeutic usages.

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