ANTI-PSEUDOMONA AND ANTI-BACILLI ACTIVITY OF SOME MEDICINAL PLANTS OF IRAN

[1]GHOLAM HOSEIN SHAHIDI BONJAR, [2]ASHRAF KARIMI NIK, [3]MOHAMMAD REZA HEYDARI, [4]MOHAMMAD HASSAN GHASEMZADEH, [5]PARVIN RASHID FARROKHI, [6]MAHMOOD REZA MOEIN, [7]SHAHLA MANSOURI AND [8]ALIREZA FOROUMADI

[1]Department of Plant Pathology, College of Agricultural Sciences, Bahonar University of Kerman, [2]Department of Microbiology, College of Sciences, University of Azad, Kerman, [3]Food and Cosmetic Control Office of Fars Province, Shiraz, [4]Fars Curing Management, Shiraz, [5]Department of Agronomy and Plant Breeding, College of Agricultural Sciences, Bahonar University of Kerman, [6]Department of Pharmacognosy, School of Pharmacy, Shaheed Beheshti University of Medical Sciences, [7]Department of Microbiology, College of Medicine, Kerman University of Medical Sciences, [8]Department of Medicinal Chemistry, School of Pharmacy, Kerman University of Medical Sciences, Iran

ABSTRACT

The use of plants in treatment of burns, dermatophytes, and infectious diseases is common in traditional medicine of Iran. Based on ethno pharmacological and taxonomic information, antibacterial activities of methanol extracts of some medicinal plants of Iran were determined by *In Vitro* bioassays using agar diffusion-method against standard strains of *Pseudomonas aeruginosa, P. fluorescens, Bacillus subtilis, B. cereus* and *B. pumilis* at 20 mg/ml. From 180 plant species of 72 families, 78 species (43.3%) in 42 families (58.3%) showed antibacterial activities against *B. cereus* (88.4%), *B. subtilis* (39.7%), *B. pumilis* (37.1%), *P. fluorescens* (37.1%) and *P. aeruginos* (10.2%). The most active plant families were Apiaceae, Compositae and Labiatae with 9, 8 and 7 active plant species respectively. Minimum inhibitory concentrations (MIC) of the active plants were determined using two fold serial dilutions. Most active plant against Bacilli was *Myrtus communis* L. with MIC of 1.87 mg/ml. For Pseudomonas species, *Dianthus caryophyllus* L. and *Terminalia chebula* (Gaertner) Retz. were more active with the MIC of 0.46 mg/ml for *P. fluorescens* and of 1.87 mg/ml for *P. aeruginosa* respectively.

Keywords: Antibacterial activity, Plant extracts, Iranian Medicinal Plants, Bacillus, 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. Many 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 (1). 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. (2). Until natural products have been approved as new antibacterial drugs, there is an urgent need to identify novel substances active towards highly resistant pathogens (3, 4). In Iranian traditional medicine (ITM) the use of plants in treatment of burns, dermatophytes, and infectious diseases or as antiseptic and anti-inflammatory was common (5). *P. aeruginosa* is the most prevalent burn-patients pathogen capable of causing life–threatening illnesses (6). This bacterium can cause clinically significant infections such as wounds and burns infections, giving rise to blue-green pus; also meningitis, when it is introduced by lumbar puncture; and urinary tract infection when introduced by catheters and instruments or irrigating solutions (7). In infants or debilitated persons, the bacterium may invade the bloodstream and result in fatal sepsis (6). Some strains causing septicemia and pneumonia in cystic fibrosis and immunocompromised patients are becoming difficult to treat with currently available

Correspondence: Gholamhosein Shahidi Bonjar, Department of Plant Pathology, College of Agricultural Sciences, Bahonar University of Kerman, Kerman, Iran, E-mail: shahidi@mail.uk.ac.ir

antimicrobial agents (8). This organism is one of the most common pathogens associated with bacterial corneal ulcers. Keratitis due to this pathogen also has been observed in those who wear extended-wear contact lenses (6). Due to multi-resistancy of *P. fluorescens,* there is a lack of active antibiotics effective against this bacterium, resulting in an increase in nosocomial infections and high mortality (9). The bacterium is an opportunist associated with respiratory and urinary tract, wounds, and bacteremia (7). *B. subtilis* occasionally produces disease such as meningitis, endocarditis, endophthalmitis, conjunctivitis, or acute gastro-enteritis in immunocompromised patients. *B. cereus* is a spore forming pathogen associated with various opportunistic clinical infections (10). It has also been associated with food borne diseases caused by toxins. It is present in ready-to-eat vegetable-based foods (11). *B. pumilis* is an environmental contaminant (12), which was included in this study to get more comprehensive evaluation of spectral activity of the plants.

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 ethnopharmaco-logists, herbal-drug sellers and rural native-healers, the plant organs used in this study were as used in ITM.

Many investigations have also been reported detection of antibacterials from plants in recent years. From methanolic extracts of 100 plants, used by British Colombian Native People, against 11 bacterial isolates, 85% of the plants were found to have antibacterial activity (13). In another study from 27 medicinal plant extracts of Rubiaceae 11 plants were found to be active (14). In one study in Kerman (South east of Iran) antibacterial activity of crude extract of *Myrtus communis* against 10 laboratory strains of bacteria was evaluated. The crude extract of this plant inhibited the growth of all tested bacteria except *Campylobacter jejuni* (15). Antibacterial activity of *Ficus racemosa* Linn. leaf extracts against *E. coli*, *B. pumilis* and *B. subtilis* has also been reported (16).

In the present study methanol extracts of 180 plant species in 72 families at 20 mg/ml were tested against standard strains of *P. aeruginosa, P. fluorescens, B. subtilis, B. cereus* and *B. pumilis,* by agar diffusion method.

MATERIALS AND METHODS

Plant material and extraction procedure

The medicinal plants used in this study were collected from different regions of Iran from April to August of 1995- 2001 and identified by P. Rashid Farrokhi at 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 which are used but do not grow in Iran or were not found in the collection areas were obtained from the local stores in Kerman city and identified at the mentioned laboratory. 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 was transferred to glass vials and kept at 4° C before the use.

Antibacterial Screening

The following standard bacterial strains were used as test microorganisms and obtained from Persian Type Culture Collection (PTCC), Tehran, Iran. *Pseudomonas aeruginosa* (PTCC No. 1074), *P. fluorescens* (PTCC No. 1181), *Bacillus subtilis* (PTCC No. 1023), *B. cereus* (PTCC No. 1015) and *B. pumilis* (PTCC No. 1319). The bacteria were rejuvenated on Mueller-Hinton-Agar medium (MH, E. Merk, Germany) and subcultured as it was required. For bioassays, suspension of approximately $1.5x10^8$ cells/ml in sterile normal saline were prepared as described by the reported methods (19), and about 1.5 ml of each sample was uniformly seeded on MH in 9 x 1.2 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 for not missing any trace amounts of antimicrobials, a relatively high concentration of 20 mg/ml 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 the case of *P. aeruginosa* and *B. subtilis,* and at 29 °C in the case of *P. fluorescens, B. cereus* and *B. pumilis*. After 48 hr bioactivity was determined by the measurement of the diameter of inhibition zones (DIZ) in mm. All samples were tested in triplicate. Controls had solvent (DM) without test compounds.

Table 1. Evaluation of antibacterial activity, indicated by diameter of inhibition zones (DIZ, mm), of plants used in Iranian native medicine against *Bacillus subtilis*, *B. cereus*, *B. pumilis*, *Pseudomonas aeruginosa* and *P. fluorescens*.

				a		b		c		d		e	
Plant family	**Plant species**	**English common name**	**OT**	**DIZ**	**MIC**	**DIZ**	**MIC**	**DIZ**	**MIC**	**DIZ**	**MIC**	**DIZ**	**MIC**
Anacardiaceae	*Rhus coriaria* L.	Tanner's sumac	SE	19	1.87	17	3.75	18	3.75				
Anacardiaceae	*Semecarpus anacardium* L. f.	Markingnut tree	LE	12	15	12	20	12	15	10	15	10	15
Apiaceae	*Apium graveolens* L.	Wild celery	LE			10	20						
Apiaceae	*Anethum graveolens* L.	Dill; dillweed	FR	13	15							12	7.5
Apiaceae	*Coriandrum sativum* L.	Chinese parsley	SE	10	20	9	20						
Apiaceae	*Cuminum cyminum* L.	Cumin	FR	10	20	18	3.75	9	20				
Apiaceae	*Foeniculum vulgare* P. Mill. Syn. *F. foeniculum*	Sweet fennel	RO	10	20	12	15						
Apiaceae	*Heracleum tuberosum* Molina.	Yellow cow-parsnip	FR	10	20	18	7.5						
Apiaceae	*Pimpinella anisum* L. Syn. *Anisum vulgare*	Anise burnet	SE	12	15	24	0.93						
Apiaceae	*Trachyspermum ammi* (L.) Link	-	SE	10	20			10	20	11	15		
Apiaceae	*Trachyspermum copticum* Link	Trachyspermum	SE	15	3.75	17	1.87	12	15				
Berberidaceae	*Berberis georgii* Ahrendt.	Barberry	FR					9	20				
Berberidaceae	*Berberis vulgaris* L.	Barberry	FR	12	15	15	3.75	10	20				
Boraginaceae	*Borago officinalis* L.	Borage	FL					10	20			12	15
Boraginaceae	*Echium vulgare* L.	Viper's bugloss; blue devil; blueweed	FL			9	20						
Caryophyllaceae	*Dianthus caryophyllus* L.	Carnation	WP	17	1.87	15	7.5	15	3.75	12	3.75	18	0.46
Colchicaceae	*Colchicum luteum* Bak.	Yellow autumn crocus	WP			9	20						
Combretaceae	*Terminalia chebula* (Gaertner) Retz.	Myrobalan	RS	13	7.5	16	3.75	16	3.75	16	1.87	18	0.93
Combretaceae	*Terminalia chebula* (Gaertner) Retz.	Myrobalan	US			16	7.5	14	7.5	14	7.5	14	7.5
Compositae	*Anthemis nobilis* (L.) All.	Camomile	FL					10	20				
Compositae	*Anthemis arvensis* L.	Corn chamomile	FL			10	20						
Compositae	*Arctium lappa* L. Syn. *Lappa major*	Great burdock	RO			10	20						
Compositae	*Calendula officinalis* L.	Pot marigold	FL			9	20	9	20				
Compositae	*Carthamus tinctorius* L.	Safflower; bastard; false saffron	FL			17	7.5						
Compositae	*Echinops exaltatus* Schrad., Syn. *E. commutatus*	Tall globethistle	LE			10	20						
Compositae	*Marticaria chamomilla* L.	Marticaria	FL			9	20	10	20			11	15

Table 1. Continued

Cruciferae	*Lepidium campestre* (L.) Ait. f. Syn. *Neolepia campestris*; *Thlaspi campestre*	Pepperwort	LE			10	20						
Cruciferae	*Raphanus sativus* L.	Wild radish	SE					10	20				
Cucurbitaceae	*Citrullus coloccynthis* (L.) Schrad. Syn. *Colocynthis vulgaris*; *Cucumis colocynthis*	Colocynth	FR			9	20						
Cuscutaceae	*Cuscuta epithymum* L.	Clover dodder	SE			14	15						
Ephedraceae	*Ephedra intermedia* Schrenk. ex C.A.Mey.	Ephedra	LE	13	7.5	16	3.75	14	7.5	10	15	13	7.5
Euphorbiaceae	*Chrozophora tinctoria* (L.) A. Juss.	Giradol	LE			12	20	12	20				
Fabaceae	*Glycyrrhiza glabra* L.	liquorice	RO	18	3.75	18	3.75						
Graminae	*Bromus biebersteinii* Roemer & J.A. Schultes Syn. *Bromopsis biebersteinii*	Meadow brome	RO			10	20						
Iridaceae	*Iris* sp. L.	Iris	RO					10	20				
Juglandaceae	*Juglans regia* L.	English walnut	FL			10	20						
Labiatae	*Mentha longifolia* (L.)Huds.	Horsemint	LE			10	20						
Labiatae	*Nepeta mussinii* Henckel	Persian catmint	RO					10	20				
Labiatae	*Nepeta mussinii* Henckel	Persian catmint	LE			10	20	9	20				
Labiatae	*Origanum majorana* L. Syn. *Majorana hortensis*; *M. majorana*	Sweet marjoram	LE			10	20						
Labiatae	*Salvia officinalis* L.	Sage	WP	11	20	12	15						
Labiatae	*Stachys officinalis* (L.) Trev. Syn. *Betonica officinalis*	Wood betony	LE			9	20						
Labiatae	*Thymus vulgaris* L.	Thyme	WP	10	20	12	15						
Lauraceae	*Cinnamomum zeylanicum* BL.	Cinnamon	SB	17	3.75	22	0.93	18	3.75				
Leguminosae-Caesalpinioideae	*Cassia fistula* L. Syn. *C. fistulosa*	Golden shower	FR			16	7.5						
Leguminosae-Papilionoideae	*Alhagi maurorum* Medik. Syn. *A. camelorum*; *A. pseudalhagi*	Camel thorn	SG	10	20	15	7.5						
Leguminosae-Papilionoideae	*Astragalus glycyphyllos* L.	Milk vetch	SG			9	20						
Leguminosae-Papilionoideae	*Trigonella foenum-graecum* L.	Sicklefruit fenugreek	SE			19	1.87						
Lythraceae	*Lawsonia inermis* L. Syn. *L. alba* L.	Henna	LE			26	0.46					17	0.46
Malvaceae	*Althaea officinalis* L.	Marsh mallow	FL			10	20					11	7.5
Malvaceae	*Malva sylvestris* L. Syn. *M. Mauritiana*	High mallow	FL	10	20								
Myristicaceae	*Myristica fragrans* Houtt.	Nutmeg	SE	13	20	20	3.75						
Myrtaceae	*Eucalyptus globulus* Labill.	Tasmanian bluegum	LE	9	20	19	7.5						
Myrtaceae	*Myrtus communis* L.	Myrtle	LE	22	1.87	26	0.93	20	1.87	11	7.5	16	1.87

Table 1. Continued

Myrtaceae	*Myrtus communis* L.	Myrtle	LE	22	1.87	26	0.93	20	1.87	11	7.5	16	1.87
Myrtaceae	*Myrtus communis* L.	Myrtle	SE	12	15	14	7.5	14	7.5	11	7.5	16	1.87
Nymphaeaceae	*Nymphaea alba* L.	White water lily	FL			9	20			10	15		
Papaveraceae	*Papaver bracteatum* Lindley	Oriental poppy	FL			10	20						
Peganaceae	*Peganum harmala* L.	Harmal peganum	SE			18	3.75	10	20				
Polygonaceae	*Rheum ribes* L.	Currant-fruited rhubarb	RO	18	3.75	19	1.87	17	1.87				
Polygonaceae	*Rumex acetosa* L.	Sour dock	LE			10	20						
Portulacaceae	*Portulaca oleracea* L.	Pussley; pusley	SE			10	20						
Punicaceae	*Punica granatum* L.	Pomegranate	FL			17	3.75						
Ranunculaceae	*Nigella sativa* L.	Black cumin	SE			18	3.75						
Ranunculaceae	*Ranunculus aestivalis* (L. Benson) Van Buren & Harper	Summer buttercup	WP	11	20	13	15	10	20				
Rhamnaceae	*Ziziphus jujube* Mill. Syn. *Z. sativa*; *Z. vulgaris*; *Z. zizyphus*; *R. zizyphus*; *Z. jujba*	Jujube	FR	10	20	9	20	10	15				
Rosaceae	*Cydonia oblonga* P. Mill. Syn. *C. vulgaris*; *Pyrus cydonia*	Quince	SE			9	20						
Rosaceae	*Rosa gallica* L.	Gallica Roses	SE			10	20						
Rosaceae	*Rubus idaeus* L. Syn. *R. greeneanus*	Red raspberry	LE	13	15	12	20						
Rutaceae	*Ruta graveolens* L.	Common rue	LE			10	20	9	20				
Rutaceae	*Citrus medica* L.	Citron	SE			15	15						
Salicaceae	*Salix aegyptiaca* L. Syn. *S. medemii*	Mediterranean willow	FL			9	20						
Smilacaceae	*Smilax china* L.	China root	ST	14	15	16	7.5						
Solanaceae	*Capsicum annuum* (Dunal) Heiser & Pickersgill	Cayenne pepper	WP			12	20						
Tamaricaceae	*Tamarix gallica* L.	Manna plant	LE			10	20						
Theaceae	*Camellia sinensis* (L.) Kuntze Syn. *C. thea*; *Thea bohea*	Tea plant	LE			10	20	10	20				
Urticaceae	*Urtica gracilenta* Greene	Mountain nettle	LE			10	20			10	15		
Violaceae	*Viola odorata* L.	Garden violet	FL			10	20						
Zingiberaceae	*Alpinia officinarum* Hance	Lesser galangal	FR	12	15		20	14	7.5				
Zingiberaceae	*Amomum compactum* Sol. Ex Maton	Round cardamom	SE	12	15	15	7.5	12	15				
Zingiberaceae	*Zingiber officinale* Roscoe Syn. *Amomum zingiber*; *Z. zingiber*	Ginger	RH	12	15	17	3.75	14	7.5				

OT: Organs tested, as FL: Flower, FR: Fruit, LE: Leaves, RH: Rhizome, RO: Roots, RS: Ripen Seed, SB: Stem Bark, SE: Seeds, SG: Stem Gum, ST: Stem, US: Unripe Seed and WP: Whole Plant. a: *Bacillus subtilis* (PTCC No. 1023), b: *B. cereus* (PTCC No. 1015), c: *B. pumilis* (PTCC No. 1319), d: *Pseudomonas aeruginosa* (PTCC No. 1074), e: *P. fluorescens* (PTCC No. 1181), DIZ: (Diameter of Inhibition Zones , mm), MIC: (Minimum Inhibitory Concentration, mg/ml). Blank DIZs= 0, blank MICs= not tested since the corresponding DIZs were 0.

Determination of Minimum Inhibitory Concentration

After screening the activity of plants, the active plants were re-tested to determine their minimum inhibitory concentrations (MIC). The concentrations used were two fold dilution series of 0.46-15 mg/ml in DM solvent.

RESULTS AND DISCUSSION

As it is shown in Table 1, from 180 plant species in 72 families, 78 species (43.3%) in 42 families (58.3%) showed antibacterial activities. The table also contains MIC values of the active plants measured in two fold dilution-series of 0.46-15 mg/ml. Activities of the plant extracts were as follows: *B. cereus* (88.4%), *B. subtilis* (39.7%), *B. pumilis* (37.1%), *P. fluorescens* (37.1%) and *P. aeruginosa* (10.2%). Plant families of Apiaceae with 9, Compositae with 8 and Labiatae with 7 had the highest number of active species respectively. *Dianthus caryophyllus* (the whole plant) was the most active plant against all tested bacterial species, with the MIC of 1.87, 7.5, 3.72, 3.75 and 0.46 mg/ml against *B. subtilis*, *B. cereus*, *B. pumilis*, *P. aeruginosa* and *P. fluorescens* respectively. Many plants were found to be active against Bacilli and the lowest level of MIC for *B. subtilis*, *B. cereus* and *B. pumilis* were 1.87, 0.93 and 1.87 mg/ml respectively. The most active plants against Bacilli were *Myrtus communis*, *Cinnamomum zeylanicum*, *Rheum ribes*, *Rhus coriaria*, *D. caryophyllus*, *Ephedra intermedia*, *Terminalia chebula*, *Trachyspermum copticum* and *Zingiber officinale*, *T. chebula*, *D. caryophyllus* and *M. communis* were active against all tested bacteria. Highest level of activity against *P. aeruginosa* belongs to *T. chebula* (MIC= 1.87 mg/ml) and *D. caryophyllus* (MIC= 3.75 mg/ml). For *P. fluorescens* the most actives were *M. communis* (MIC= 1.87 mg/ml), *D. caryophyllus* (MIC= 0.46 mg/ml), *T. chebula* (MIC= 0.93 mg/ml) and *Lawsonia inermis* (MIC= 0.46 mg/ml). Since *T. chebula*, *D. caryophyllus*, and *M. communis* extracts were active against both Gram positive and Gram negative bacteria especially *P. aeruginosa*, further work is required to determine their activities on more bacterial strains isolated from clinical samples. However, search for new antibacterial agents should be continued by screening many other plant families. Iran is a big country with a vast number of medicinal plants (5) and the 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.

ACKNOWLEDGMENT

Financial support of Research Affairs Office of Bahonar Univ. of Kerman is appreciated. The spiritual result of this research is dedicated to Mr. A. Afzalipour the founder of Universities at Kerman, God may bless him.

REFERENCES

1. Evans, C.E., Banso, A., Samuel, O.A. (2002) Efficacy of some nupe medicinal plants against *Salmonella typhi*: an in vitro study. J. Ethnopharmacol. 80: 21–24.
2. Tshibangu, J.N., Chifundera, K., Kaminsky, R., Wright, A.D., Konig, G.M. (2002) Screening of African medicinal plants for antimicrobial and enzyme inhibitory activity. J. Ethnopharmacol. 80: 25–35.
3. Recio, M.C. (1989) A review of some antimicrobial compounds isolated from medicinal plants reported in the literature 1978- 1988. Phytother. Res. 3: 117-125.
4. Cragg, G.M., Newman, D.J., Snader, K.M. (1997) Natural products in drug discovery and development. J. Nat. Prod. 60: 52-60.
5. Ghahraman, A., Attar, F. (1998) Biological diversity of Iranian plant species. Tehran Univ. Press, Tehran, pp 24-25.
6. Lory, S. (1990) Pseudomonas and other Nonfermenting Bacilli. In: Davis, B.D., Dulbecco, R., Eisen, H.N., Ginsberg, H.S. (eds) Microbiology, 4th ed. Lippincott Co., Philadelphia, pp: 595-600.

7. Murray, P.R., Drew, W.L., Kobayashi, G.S. Thompson, J.H. (1990) Medical Microbiology, Mosby Co., Philadelphia, pp: 119-126.
8. Senda, K. Arakawa, Y., Nakashima, K., Ito, H., Ichiyama, S., Shimoka, K., Kato, N., Ohta, M. (1996) Multifocal outbreaks of metallo-beta-lactamase-producing *Pseudomonas aeruginosa* resistant to broad-spectrum beta-lactams, including carbapenems. Antimicrob. Agents Chemother. 40: 349-353.
9. Giamarellos-Bourboulis, E.J., Grecka, P., Dionyssiou-Asteriou, A., Giamarellou, H. (1999) *In Vitro* interactions of gamma-linolenic acid and arachidonic acid with ceftazoline on multiresistant *Pseudomonas aeruginosa*. Lipids 34 (Suppl): 151-152.
10. Boyd, R.F. (1995) Basic Medical Microbiology, 5th ed., Little, Brown and Company, Boston, pp: 310-314.
11. Kaneko, K., Hayashidani, H., Ohtomo, Y., Kosuge, J., Kato, M., Takahashi, K., Shiraki, Y., Ogawa, M. (1999) Bacterial contamination of ready-to-eat foods and fresh products in retail shops and food factories. J. Food Prod. 62: 644-649.
12. Turnbull, P.C.B. (1991) Bacillus. In: Baron, S., Jennings, P.M. (eds) Medical Microbiology, 3rd ed. Churchill Livingstone, New York, pp: 249-262.
13. McCutcheon, A.R., Ellis, S.M., Hancock, R.E., Towers, G.H. (1992) Antibiotic screening of medicinal plants of the British Colombian native people. J. Ethnopharmacol. 37: 212-223.
14. Pederson, O., Gurib-fakim, A., Subratty, H., Andersen, A. (1999) Pharmaceutical properties of seven medicinal plants of Rubiaceae from Mauritius. Pharmaceutical Biol. 37: 202-207.
15. Mansouri, S., Foroumadi, A., Ghanei, T., Gholamhosseinian Najar, A. (2001) Antibacterial activity of the crude extracts and fractionated constituents of *Myrtus communis*, Pharmaceutical Biol. 39: 399-401.
16. Mandal, S.C., Saha, B.P., Pal, M. (2000) Studies on antimicrobial activity of *Ficus racemosa* Linn. leaf extract. Phytoter. Res. 14: 278-280.
17. Ashik Mosaddik, M., Kabir, K.E., Hassan, P. (2000) Antibacterial activity of *Alangium salviifolium* folwers. Fitoterapia 71: 447-449.
18. Ebi, G.C. (2001) Antimicrobial activities of *Alchornea cordifolia*. Fitoterapia 72: 69-72.
19. Forbes, B.A., Sahm, D.F., Weissfeld, A.S., Trevino, E.A. (1990) Methods for testing antimicrobial effectiveness. In: Baron, E.J., Peterson, L.R., Finegold, S.M. (eds) Bailey & Scott's Diagnostic Microbiology, 8th ed. Mosby Co, St Louis, Missouri, pp: 171-194.