Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Biomedicine

journal homepage:www.elsevier.com/locate/apjtb

Document heading

Antibacterial activities of some plant extracts used in Indian traditional folk medicine

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ARTICLE INFO

Article history:
Received 10 August 2011
Received in revised form 2 September 2011
Accepted 24 September 2011
Available online 15 October 2011

Keywords:
Antibacterial activity
Disk diffusion method
Folk medicine
Bacteria

ABSTRACT

Objective: To evaluate the antibacterial activity of the leaf extracts of *Cestrum diurnum*, *Ocimum sanctum*, *Carcica papaya*, *Solanum villosum*, *Vitex negundo*, and *Clerodendron inerme* against two gram positive bacteria (*Staphylococcus aureus* MTCC 2940 and *Bacillus subtilis* MTCC 441) and two gram negative bacteria (*Escherichia coli* MTCC 739 and *Pseudomonas aeruginosa* MTCC 2453). **Methods:** The sensitivity of two gram positive and two gram negative pathogenic multi-drug resistant bacteria to extracts of leaves of six medicinal plants used as popular medicine in India was studied *in vitro* by the disk diffusion method and minimal inhibitory concentration (MIC). **Results:** All the bacterial strains were found to be sensitive to aqueous, n-hexane and ethanol extracts. But, it is evident that the organic extracts were comparatively more effective than aqueous extracts. **Conclusions:** It can be concluded that the leaf extracts of the six medicinal plants possess antibacterial activity against human pathogens.

1. Introduction

Resistance of microbes to available antimicrobial agents is a major global public health problem. Infective diseases account for approximately one half of all deaths in tropics. Plants have been an integral part of human society since the beginning of civilization. India is rich in its plant diversity. A number of plants have been documented for their medicinal potential, which are used by the traditional healers, herbals folklorists in Indian systems of medicine namely Ayurveda, Unani, Siddha apart from homeopathy and electropathy. These plant species play major roles in the health care of the nation's population.

Different national and international pharmaceutical companies are utilizing such plant-based formulations in treatment of various diseases and disorders around the world[1-7].

Cestrum diurnum (*C. diurnum*) L. (Solanaceae) is a shrub that is also known as day jasmine. There are several applications of the plant that have been well documented in several literatures and the toxicity of the species to humans and livestock has been frequently reported[8,9]. The leaves contain a calcinogenic glycoside called 1, 25-dihydroxycholocalciferol that leads to a vitamin D toxicity and elevated serum Ca^{2+} and deposition of calcium in soft tissues[10].

Ocimum sanctum (*O. sanctum*) L. (Lamiaceae), commonly known as tulsi, is a tropical, much branched, annual herb. Apart from the religious significance, it also has substantial medicinal meanings and is used in Ayurvedic treatment. Tulsi is reported to be anti-inflammatory due to the presence of eugenol oil in its leaves. It is useful in curing respiratory tract infections. The urosolic acid present in tulsi has anti-allergic properties. The plant can play a role in the management of immunological disorders such as allergies and asthma. The juice of the leaves is used against fever and as an antidote for snake and scorpion bites. It's antispasmodic properties can relieve abdominal pains and help in lowering the blood sugar level[11].

Carica papaya (*C. papaya*) L. (Caricaceae), commonly known as papaya, is a small, soft-wooded, fast growing, short lived laticiferous tree upto 8.0 m in height with a straight cylindric stem bearing characteristics *i.e.* leaf scars throughout and with a tuft of leaves at the top; leaves deeply lobed, palm-like with characteristically long. The

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fruits are bitter, acrid, thermogenic, anodyne, stomachic, anthelminthic, styptic, anti-inflammatory, antifungal and diuretic. They are useful in vitiated conditions of vata, cough, bronchitis, stomachalgia, dyspepsia, anorexia, intestinal worms, haemorrhoids, inflammations, splenomegaly, ringworm, skin diseases, and injuries of the urinary tract. The latex is anthelminthic and laxative[12].

Solanum villosum (*S. villosum*) L. (Solanaceae), commonly known as black nightshade, is a brittle-stemmed weed that grows up upto a meter in height. It is often used as an ointment for abscesses, sores and in a douche for leucorrhoea, eczema, nappy rash, wounds, and cold sore[8].

Vitex negundo (*V. negundo*) L. (Verbenaceae), commonly known as nirgundi, is a large shrub growing throughout India, Ceylon, Afghanistan, China, Madagascar and Philippines. The plant has a pungent bitter, acrid taste, anthelminthic and promotes the growth of hair, useful in diseases of the eye, constipation, inflammation, leucoderma, enlargement of the spleen, bronchitis, asthma, biliousness, painful teething of children[13].

Clerodendron inerme (*C. inerme*) L. (Verbenaceae), commonly known as ghentu, is an Indian medicinal shrub, whose leaves and roots are employed externally for the treatment of hypotensive effect on rabbit, tumors and certain skin diseases, chronic pyrexia and also used as tonics[14].

The present study has been designed to determine the role of leaf extracts (aqueous extract, n-hexane and ethanol extracts) of *C. diurnum*, *O. sanctum*, *C. papaya*, *S. villosum*, *V. negundo*, and *C. inerme* for potential antibacterial activity against two gram positive bacteria *i.e. Staphylococcus aureus* (*S. aureus*) MTCC 2940 and *Bacillus subtilis* (*B. subtilis*) MTCC 441 and two gram negative bacteria *i.e. Escherichia coli* (*E. coli*) MTCC 739 and *Pseudomonas aeruginosa* (*P. aeruginosa*) MTCC 2453. The observed inhibition zones were measured (in mm) and compared against standard antibiotics cefadroxil and nalidixic acid.

2. Materials and methods

2.1. Plant materials collection

The plant materials used in this study consisted of mature leaves of *C. diurnum*, *O. sanctum*, *C. papaya*, *S. villosum*, *V. negundo*, and *C. inerme*, collected from a village named Lakudi, from the outskirts of Burdwan (23°16′N, 87°54′E), West Bengal, India, during spring season (mid-March to mid-April 2007). The leaves were initially rinsed with distilled water and dried on paper towels in the laboratory at (37±1) ℃ for 24 h.

2.2. Preparation and preservation of plant extract

2.2.1. Preparation of aqueous extract

Each of the six samples, which consisted of mature leaves of *C. diurnum*, *O. sanctum*, *C. papaya*, *S. villosum*, *V. negundo*, and *C. inerme*, was weighed out (50 g) and soaked separately in 500 mL of cold water contained in conical flasks stoppered with rubber corks and left undisturbed for 24 h. They were then filtered off using sterile filter papers (Whattman No. 1) into clean conical flasks and subjected to water bath evaporation, where the aqueous water solvents were evaporated at boiling temperature of 100 ℃. The standard extracts thus obtained were then stored at 4 ℃ in a refrigerator until further use[15].

2.2.2. Preparation of n-hexane and ethanol extracts

After drying, the plant materials were ground separately in a grinding machine (MX -110 PN, Japan) in the laboratory. Exposure to sunlight was avoided to prevent the loss of active components. The n-hexane and ethanol extraction fluid (500 mL) was mixed with each of powdered plant materials (50 g). The mixtures were then kept for 24 h in tightly sealed vessels at room temperature, protected from sunlight, and stirred thoroughly several times a day with sterile glass rods. The mixtures thus obtained were filtered through Whattman No. 1 filter papers and the residues were adjusted to the required concentration (50 mL of ethanol and 50 mL of n-hexane for the residue of 50 g of powdered plant material) with the extraction fluid for further extraction. This was repeated three times, and a clear colorless supernatant extraction liquid was finally obtained. The extracted liquids were subjected to rotary evaporation in order to remove the ethanol and n-hexane. The semisolid extracts produced were kept at -80 ℃ (REVCO model No. ULT 790-3-V 32) in a freezer overnight and then subjected to freeze-drying for 24 h at -60 ℃ in a 200 mL vacuum. Then the extracts were stored in an airtight container at 4 ℃ in the refrigerator until further use. All the dried extracts were exposed to UV rays (200-400 nm) for 24 h and checked frequently for sterility by streaking on nutrient agar plates[16].

2.3. Antibacterial assay

2.3.1. Agar-well diffusion method

The assay was conducted by agar well diffusion method[17]. The bacterial strains grown on nutrient agar at 37 ℃ for 18 h were suspended in a saline solution (0.85% NaCl) and adjusted to a turbidity of 0.5 McFarland standards (10^8 CFU/mL). The suspension was used to inoculate into petri plates (90 mm in diameter) with a sterile non-toxic cotton swab on a wooden applicator. Wells (6 mm in diameter) were punched in the agar and filled with 50 μL of 2 000 μg/mL extracts. The dissolution of the organic extracts (ethanol, n-hexane) was aided by 1% (v/v) dimethylsulphoxide (DMSO) and that of the aqueous extract was aided with water, which did not affect the growth of microorganisms, in accordance with our control experiments. The surfaces of media were inoculated with bacteria from a broth culture. High-potency bio-discs were placed on the agar. After 18 h of incubation at a specific temperature [(30±1) ℃ for *B. subtilis* and 37 ℃ for *S. aureus*, *E.coli* and *P. aeruginosa*], the plates were examined and the diameters of the inhibition zones were measured to the nearest millimeter.

2.3.2. Dilution method for minimum inhibitory concentration (MIC)

Of the six plants tested, the ones that showed antibacterial activity against some of the selected pathogens were selected for further tests to calculate their MIC by dilution method. These tests were performed in sterile 96-well microplates and macroplates. The microdilution was performed in 96-well microtiter plates with U-shaped wells, while the macrodilution technique was as described by the National Committee for Clinical Laboratory Standards[18,19]. In brief, the cultures were diluted in Mueller-Hinton broth at density adjusted to 0.5 McFarland turbidity. The final inoculum was 5×10^5 CFU/mL of bacterial colony. Controls with 0.5 mL of culture medium only or others with plant extracts were used in the tests. The wells were filled with 100 μL of sterile H_2O, and 100 μL of the plant extracts were added to the wells by serial two-fold dilution from the suspension of plant extract stock solution. Each well was inoculated with 100 μL of 0.5 McFarland standard bacterial suspensions so that each well got 5×10^5 CFU/mL. The plates were covered, placed in plastic bags and incubated at 37 ℃ for 24 h. In this study, the MIC was the lowest concentration of plant extracts that exhibited the growth of the organisms in the wells by visual reading.

2.4. Statistical analysis

Since the readings of control (distilled water and DMSO) experiments in the *in vitro* antibacterial studies of those plants were zero, the data were analyzed by simple arithmetic means of the different extracts, and the standard errors were compared with the control.

3. Results

The antibacterial activities of the leaf extracts of *C. diurnum*, *O. sanctum*, *C. papaya*, *S. villosum*, *V. negundo*, and *C. inerme* in different solvents (aqueous, n-hexane and ethanol) against *E. coli*, *P. aeruginosa*, *B. subtilis* and *S. aureus* were shown in Table 1. In Table 1, all the bacterial strains were found to be sensitive to aqueous, n-hexane and ethanol extracts. But, it is evident that the organic extracts were comparatively more effective than aqueous extracts. The organic and aqueous extracts of *C. diurnum* were comparatively more effective than other plants. MIC value of the tested plant extracts against the tested microorganisms was shown in Table 2.

Table 1

Antibacterial activities of specific concentrations (30 mg/disc) of aqueous, n-hexane and ethanol extracts (arranged chronologically) of six medicinal plants compared with control (distilled water and DMSO) and standard antibiotics (cefadroxil and nalidixic acid-30 μg/disc) (mean±SE).

Medicinal plants	Extraction solvent	Antibacterial activity			
		S. aureus	*B. subtilis*	*E. coli*	*P. aeruginosa*
C. diurnum	Aqueous	16.67±0.88	4.00±0.58	10.33±0.88	15.67±0.88
	n-hexane	20.00±0.58	4.00±0.58	2.00±0.58	22.33±0.88
	Ethanol	17.67±1.20	9.67±0.88	9.00±0.58	22.00±0.58
O. sanctum	Aqueous	14.67±0.88	6.33±0.33	11.00±0.78	12.00±0.58
	n-hexane	15.66±0.88	8.00±0.58	12.33±0.88	15.00±0.58
	Ethanol	18.00±0.58	10.33±0.33	16.00±0.58	16.00±0.58
C. papaya	Aqueous	9.00±0.58	3.66±0.33	2.33±0.33	2.00±0.58
	n-hexane	17.33±0.88	8.67±0.88	7.33±0.88	4.00±0.58
	Ethanol	15.67±0.88	10.00±0.00	6.00±0.58	1.67±0.33
S. villosum	Aqueous	11.00±0.58	10.00±0.58	5.67±0.33	4.00±0.58
	n-hexane	10.33±0.33	8.00±0.58	11.67±1.20	4.00±0.58
	Ethanol	13.00±1.15	8.67±0.88	11.00±0.58	8.33±0.33
V. negundo	Aqueous	11.00±0.58	6.00±0.58	5.67±0.33	4.00±0.58
	n-hexane	9.00±0.58	11.00±0.58	4.67±0.88	2.00±0.58
	Ethanol	11.00±0.58	10.00±0.58	3.00±0.58	2.67±0.88
C. inerme	Aqueous	6.00±0.58	8.67±0.33	4.33±0.88	5.33±0.88
	n-hexane	4.33±0.33	11.00±0.58	6.00±0.00	4.00±0.58
	Ethanol	11.67±0.33	14.00±0.58	11.00±0.58	2.00±0.58
Distilled water		0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
DMSO		0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Cefadroxil		28.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Nalidixic acid		0.00±0.00	0.00±0.00	11.00±0.00	20.00±0.00

Table 2
MIC of different extracts by dilution method.

Solvents	Concentrations (mg/mL)	*C. diurnum*				*O. sanctum*				*C. papaya*				*S. villosum*				*V. negundo*				*C. inerme*			
		1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
Aqueous	0[a]	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	5	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	10	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	15	+	+	+	−	−	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	−	+	+
	20	−	−	−	−	−	+	−	−	−	+	+	+	−	−	+	+	−	+	+	+	−	−	+	−
	25	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
n-hexane	0[a]	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	5	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	10	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	15	−	+	+	−	−	+	+	−	−	+	+	+	+	+	+	+	+	−	+	+	+	−	+	+
	20	−	+	+	−	−	−	−	−	−	−	+	+	−	+	−	+	−	−	+	+	+	−	−	+
	25	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Ethanol	0[a]	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	5	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	10	+	+	+	−	−	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	15	−	+	+	−	−	+	−	−	−	+	+	+	−	+	+	+	+	+	+	+	+	−	+	+
	20	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	+	−	−	+	+	−	−	−	+
	25	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−

1: *S. aureus*; 2: *B. subtilis*; 3: *E. coli*; 4: *P. aeruginosa*; 0[a]: Control without any extract; +: Growth; −: No growth.

4. Discussion

The antibacterial compounds extracted from these plants might inhibit bacteria by a different mechanism to that of currently used antibiotics and have therapeutic values as antibacterial agents.

All types of extracts of all the plants showed varied antibacterial efficacies against all the reference bacteria. The ethanol extracts showed the best result followed by n-hexane and aqueous extracts. Aqueous extracts showed less activity than n-hexane and ethanol extracts possibly because of the presence of similar active substances in aqueous extracts, in low concentrations or active substances were soluble in organic solvents and, therefore, not present in water extracts as also suggested by Sharma *et al*[20]. In general, the plant antibiotic substances appear to be more inhibitory to gram-positive organisms than gram-negative type. Unlike gram-positive bacteria, the lipopolysaccharide layer along with proteins and phosholipids are the major components in the outer surface of gram-negative bacteria[21-36]. The outer lipopolysaccharide layer hinders access of most compounds to the peptidoglycan layer of the cell wall. This explains the resistance of gram negative strains to the lytic action of most extracts.

The MIC of crude extracts of individual plants varies against different test strains. The relationship between zone of inhibition and MIC value may or may not be related. The crude extracts have mixture of phytoconstituents, which may influence the diffusion power of the active constituents. Several workers have made similar observations by using essential oils or complex mixture from higher plants[37-40]. For example some water-soluble compounds may have a higher diffusion power and lower antimicrobial activity[41]. Therefore, direct relationship of zone inhibition size with MIC value is expected with pure compounds not with crude extracts. On the other hand, these test strains may have different levels of intrinsic tolerance to antimicrobials and thus the MIC values differ from isolate to isolate. However, in such cases, the potency of crude extract on the basis of mean MIC values may be helpful in defining the relative potency of the extracts.

The activity against both the types of bacteria may be indicative of the presence of broad spectrum antibiotic compounds or simply general metabolic toxins. Although this study investigated the *in vitro* antimicrobial activity, the results substantiate the ethnobotanical use of the six studied species for the treatment of various bacteria related diseases. In conclusion, it is suggested that these plants may be used to discover natural bioactive products that might lead to the development of new drugs.

Conflict of interest statement

We declare that we have no conflict of interest.

References

[1] Gur S, Turgut-Balik D, Gur N. Antimicrobial activities and some fatty acids of turmeric, ginger root and linseed used in the treatment of infectious diseases. *World J Agri Sci* 2006; **2**(4): 439-442.

[2] Bhattacharjee I, Chatterjee SK, Chatterjee SN, Chandra G. Antibacterial potentiality of *Argemone mexicana* solvent extracts against some pathogenic bacteria. *Mem Ins Oswaldo Cruz* 2006;

101(6): 645–648.

[3] Chatterjee SK, Bhattacharjee I, Chandra G. Bactericidal activities of some common herbs in India. *Pharm Biol* 2007; **45**(5): 350–354.

[4] Shyur LF, Yang NS. Metabolomics for phytomedicine research and drug development. *Curr Opin Chem Biol* 2008; **12**: 66–71.

[5] Baquero E, Giraldo D, Molina C, Bermudez AJ. Current situation of medicinal plants commerce in Venezuela: risks and opportunities. *Bol Latinoam Caribe Plant Med Aromat* 2009; **8**: 24–32.

[6] Bhattacharjee I, Chattterjee SK, Chandra G. Isolation and identification of antibacterial components in seed extracts of *Argemone mexicana* L. (Papaveraceae). *Asian Pac J Trop Med* 2010; **3**(7): 547–551.

[7] Chattterjee SK, Bhattacharjee I, Chandra G. Isolation and identification of bioactive antibacterial components in leaf extracts of *Vangueria spinosa* (Rubiaceae). *Asian Pac J Trop Med* 2011; **4**(1): 35–40.

[8] Begum SA, Goyal M. Research and medicinal potential of the genus *Cestrum* (Solanaceae)–a review. *Pharmacogn Rev* 2007; **1**(2): 320–332.

[9] Ghosh A, Chowdhury N, Chandra G. Laboratory evaluation of a phytosteroid compoundof mature leaves of day jasmine (Solanaceae: Solanales) against larvae of *Culex quinquefasciatus* (Diptera: Culicidae) and non–target organisms. *Parasitol Res* 2008; **103**: 271–277.

[10] Ghosh A, Chandra G. Biocontrol efficacy of *Cestrum diurnum* L. (Solanaceae: Solanales) against the larval forms of *Anopheles stephensi*. *Nat Prod Res* 2006; **20**(4): 371–379.

[11] Joshi B, Lekhak S, Sharma A. Antibacterial property of different medicinal plants: *Ocimum sanctum*, *Cinnamomum zeylanicum*, *Xanthoxylum armatum* and *Origanum majorana*. *Kathmandu Univ J Sci Eng Technol* 2009; **5**(1): 143–150.

[12] Tawo EN. Antibacterial activity and medicinal properties of *Carica papaya*. *Glob J Environ Sci* 2010; **9**(1&2): 81–83.

[13] Vishwanathan AS, Basavaraju R. A review on *Vitex negundo* L.–a medicinally important plant. *EJBS* 2010; **3**(1): 30–42.

[14] Guessan KN, Zirihi GN, Mea A. Hypotensive effect of aqueous extract of *Clerodendrum inerme* leaves on the arterial pressure of rabbits. *Int J Pharm Biomed Res* 2010; **1**(2): 73–77.

[15] Wang Y, Tang J, Pan Y, Yang T. Aqueous extract of *Panax notoginseng* affecting the blood TC, TG and ET–1 levels in post operative rabbits. *J Med Plant Res* 2011; **5**(8): 1425–1428.

[16] Chessbrough M. *Medical laboratory manual for tropical countries*. Oxford: Linacre House; 2000, p. 260.

[17] Perez C, Paul M, Bazerque P. An antibiotic assay by the agar–well diffusion method. *Acta Biol Med Exp* 1990; **15**: 113–115.

[18] National Committee for Clinical Laboratory Standards. *Performance standards for antimicrobial disc susceptibility tests*. PA: NCCLS Publications; 1993, p. M2–A5.

[19] Paiva SR, Figueiredo MR, Aragao TV, Kaplan MA. Antimicrobial activity *in vitro* of plumbagin isolated from *Plumbago* species. *Mem Inst Oswaldo Cruz* 2003; **98**: 959–961.

[20] Sharma C, Aneja KR, Kasera R. Screening of *Berberis aristata* DC. for antimicrobial potential against the pathogens causing ear infection. *Int J Pharmacol* 2011; **7**(4): 536–541.

[21] Kumar VP, Chauhan NS, Padh H, Rajani M. Search for antibacterial and antifungal agents from selected Indian medicinal plants. *J Ethopharamacol* 2006; **107**: 182–188.

[22] Raja RDA, Jeeva S, Prakash JW, Antonisamy JM, Irudayaraj V. Antibacterial activity of selected ethnomedicinal plants from South India. *Asian Pac J Trop Med* 2011; **4**(5): 375–378.

[23] Madhumitha G, Saral AM. Preliminary phytochemical analysis, antibacterial, antifungal and anticandidal activities of successive extracts of *Crossandra infundibuliformis*. *Asian Pac J Trop Med* 2011; **4**(3): 192–195.

[24] Johnson M, Wesely EG, Kavitha MS, Uma V. Antibacterial activity of leaves and inter–nodal callus extracts of *Mentha arvensis* L. *Asian Pac J Trop Med* 2011; **4**(3): 196–200.

[25] Peixoto JRO, Silva GC, Costa RA, de Sousa Fontenelle JL, Vieira GHF, Filho AAF, et al. *In vitro* antibacterial effect of aqueous and ethanolic *Moringa* leaf extracts. *Asian Pac J Trop Med* 2011; **4**(3): 201–204.

[26] Bhattacharjee I, Chatterjee SK, Chandra G. Isolation and identification of antibacterial components in seed extracts of Argemone mexicana L. (Papaveraceae). *Asian Pac J Trop Med* 2010; **3**(7): 547–551.

[27] A Mansour, K Enayat, Mohammad–Saeid Neda, A Behzad. Antibacterial effect and physicochemical properties of essential oil of *Zataria multiflora* Boiss. *Asian Pac J Trop Med* 2010; **3**(6): 439–442.

[28] SM Seyyednejad, H Koochak, E Darabpour, H Motamedi. A survey on *Hibiscus rosa–sinensis*, *Alcea rosea* L. and *Malva neglecta* Wallr as antibacterial agents. *Asian Pac J Trop Med* 2010; **3**(5): 351–355.

[29] MS Moghadam, S Maleki, E Darabpour, H Motamedi, SMS Nejad. Antibacterial activity of eight Iranian plant extracts against methicillin and cefixime restistant *Staphylococcous aureus* strains. *Asian Pac J Trop Med* 2010; **3**(4): 262–265.

[30] H Koochak, SM Seyyednejad, H Motamedi. Preliminary study on the antibacterial activity of some medicinal plants of Khuzestan (Iran). *Asian Pac J Trop Med* 2010; **3**(3): 180–184.

[31] Jarrar N, Abu–Hijleh A, Adwan K. Antibacterial activity of *Rosmarinus officinalis* L. alone and in combination with cefuroxime against methicillin–resistant *Staphylococcus aureus*. *Asian Pac J Trop Med* 2010; **3**(2): 121–123.

[32] Kannathasan K, Senthilkumar A, Venkatesalu V. *In vitro* antibacterial potential of some *Vitex* species against human pathogenic bacteria. *Asian Pac J Trop Med* 2010; **4**(8): 645–648.

[33] Kumar SC, Bhattacharjee I, Chandra G. Isolation and identification of bioactive antibacterial components in leaf extracts of *Vangueria spinosa* (Rubiaceae). *Asian Pac J Trop Med* 2011; **4**(1): 35–40.

[34] Mandal S, DebMandal M, Kumar NP, Saha K. Antibacterial activity of honey against clinical isolates of *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella enterica* serovar Typhi. *Asian Pac J Trop Med* 2010; **3**(12): 961–964.

[35] Kannan RRR, Arumugam R, Anantharaman P. Antibacterial potential of three seagrasses against human pathogens. *Asian Pac J Trop Med* 2010; **3**(11): 890–893.

[36] Johnson M, Wesely EG, Hussain MZ, Selvan N. *In vivo* and *in vitro* phytochemical and antibacterial efficacy of *Baliospermum montanum*(Willd.) Muell. Arg. *Asian Pac J Trop Med* 2010; **3**(11): 894–897.

[37] Ahmad I, Aqil F. *In vitro* efficacy of bioactive extracts of 15 medicinal plants against ESβL–producing multi drug–resistant enteric bacteria. *Microbiol Res* 2007; **162**(3): 264–275.

[38] Arjunan I, Gopinath D, Murthy NS. Role of informal care providers in home based long term care in diabetes mellitus at Kaiwara Primary Health Center area, Karnataka, India. *Asian Pac J Trop Dis* 2011; **1**(2): 127–130.

[39] Tewari V, Tewari A, Bhardwaj N. Histological and histochemical changes in placenta of diabetic pregnant females and its comparision with normal placenta. *Asian Pac J Trop Dis* 2011; **1**(1): 1–4.

[40] Idogun ES, Kasia BE. Assessment of microalbuminuria and glycated hemoglobin in type 2 diabetes mellitus complications. *Asian Pac J Trop Dis* 2011; **1**(3): 203–206.

[38] Nang HLL, May CY, Ngan MA, Hock CC. Extraction and identification of water soluble compounds in palm pressed fiber by SC–CO2 and GC–MS. *Am J Environ Sci* 2007; **3**(2): 54–59.