PRAJÑĀ - Journal of Pure and Applied Sciences Vol. 19: 14 - 18 (2011)
ISSN 0975 - 2595

SCREENING OF CRUDE ROOT EXTRACTS OF SOME INDIAN PLANTS FOR THEIR ANTIBACTERIAL ACTIVITY

Purvesh B. Bharvad, Ashish R. Nayak, Naynika K. Patel and J.S.S. Mohan*

Department of Biosciences, Sardar Patel University, Vallabh Vidyanagar - 388120, Gujarat, India

ABSTRACT

The hexane, ethyl acetate, methanol and distilled water root extracts of twenty one plants were screened for their potential antibacterial activity against six gram positive and six gram negative bacterial strains using agar well diffusion method. The minimum inhibitory concentration (MIC) values were evaluated by serial broth dilution method for the plant extracts showing more than 7 mm zone of inhibition. Root extracts were prepared by infusion extraction method using solvents. The hexane and methanolic root extracts inhibited by 33.33% and 38.09% against *Staphylococcus epidermidis* respectively, where as methanolic root extract inhibited by 57.14% against *Micrococcus luteus.* Least to no activity was found in distilled water extracts of all plants. The MIC values were observed in the range of >8 mg/ml to 0.25 mg/ml for the tested plant extracts. This study revealed that plant extracts have greater potential as antimicrobial compound/s against microorganisms and may be used in the treatment of diseases.

Key worlds : *Indian medicinal plants, antimicrobial activity, crude root extracts, MIC value*

INTRODUCTION

Medicinal plants are a source of great economic value in the Indian subcontinent. Nature has bestowed on us a very rich botanical wealth and a large number of diverse types of plant grow in different parts of the country. In India, thousands of species are known to have medicinal value to cure specific ailments since ancient times. Many works aimed to know the efficacy of different antimicrobial and phytochemical constituents of medicinal plants as possible alternatives to chemical synthetic drugs to which many infectious microorganisms have become resistant. In recent years, antimicrobial properties of Indian medicinal plants have been reported [1]. Incidents of epidemic due to drug resistant microorganisms are now a common global problem posing enormous public health concerns [2]. There has been an increasing incidence of multiple resistances in human pathogenic microorganisms in recent years, largely due to indiscriminate use of commercial antimicrobial drugs commonly employed in the treatment of infectious diseases.

Plants have been known to synthesize a variety of chemical substances, such as phenolic compounds, terpenes, steroids, alkaloids, glycosides, fats and others. However these have been found to have profound effects on chemical systems with therapeutic properties. Over 50% of all modern clinical drugs are of natural products origin and play an important role in drug development in the pharmaceutical industry. Indian medicinal plants are regularly used in various system of medicine because of minimal side effect and cost effectiveness [3].

However, a majority of traditionally used medicinal plants have not yet been systematically screened against various microbial pathogens [4]. In the last few years, a number of studies have been conducted by researchers to prove efficacy of 23 medicinal plant species against four bacteria [5]**,** *Ceiba pentandra* and *Loranthus bengwensis* [6], *Prosopis africana* [7]**,** *Callistemon citrinus, Cymbopogon citratus* and *Albizia lebbeck* [8]. At present, a majority of botanical drugs under development are derived from ethanobotanical sources and traditional medicinal uses to combat the treatment paused by the microorganisms, which became resistance to antimicrobial agents. In this context, the present investigation is aimed to screen crude root extracts of 21 medicinal plants belonging to 16 different families against six gram positive and six gram negative bacteria for antibacterial activity.

*Corresponding author: jssmohan@hotmail.com

MATERIALS AND METHODS

Plant materials

Roots of twenty-one plant species belonging to 16 families were collected from different areas of Anand and Vallabh Vidyanagar (Table - 1). All the specimens were identified by referring "Flora of Gujarat state" [9] and with the help of Dr. A. S. Reddy, Plant Taxonomist, Department of Biosciences, Sardar Patel University, Vallabh Vidyanagar, Anand, Gujarat.

Preparation of extract

Collected roots were washed thoroughly with running tap water and dried at room temperature and powdered with grinder mixer. Extracts were prepared by sequential extraction method described by Houghton and Raman [10]. In this method, 100 gm of dry powdered material of each sample was soaked in 500 ml hexane for 24 hours at room temperature and shaken occasionally. Extracts were filtered by Whatman filter paper no.1 and filtrates were centrifuged at 3000 rpm for 10 minutes to remove any solid debris. The supernatant was collected and concentrated by solvent recovering assembly and dried completely at room temperature. The residue was dried and resuspended in to each of 500 ml ethyl acetate, methanol and distilled water sequentially for 24 hours at room temperature. The extract was filtered and filtrate was centrifuged at 3000 rpm for 10 minutes and the supernatants were collected and dried. All the fractions were stored in a refrigerator until further use.

Selected microorganisms

In this study, the six Gram-positive bacteria i.e. *Bacillus cereus* (ATCC 11778), *Bacillus subtilis* (ATCC 6051), *Staphylococcus aureus* (isolated), *Staphylococcus epidermidis* (ATCC 155), *Micrococcus luteus* (ATCC 4698), *Enterococcus faecalis* (isolated) and six Gram-negative bacteria i.e. *Escherichia coli* (ATCC 25922), *Salmonella typhi* (NTCC8394), *Salmonella paratyphi (*MTCC 735*), Pseudomonas aeruginosa* (ATCC 25668), *Klebsiella pneumoniae* (ATCC 15380), *Serratia marcescens* (isolated) were selected.

Table - 1 Antibacterial activity of different (hexane, ethyl acetate, methanol and distilled water) crude root extracts of selected plant species.

Plant name (Family)	Extracts	Zone of Inhibition (mm)											
		Gram Positive						Gram Negative					
		BC	BS	SA	SE	ML	EN	EC	ST	SP	PS	SM	KP
Ailanthus excelsa Roxb. (Simaroubaceae)	H	0	5	1	0	4	0	0	0	0	0	2	0
	E	0	6	2	2	4	1	0	0	0	0	3	1
	M	3	4	0	0	5	2	0	2	0	4	6	0
	W	3	0	2	2	1	0	0	0	0	0	1	0
Artocarpus integrifolia auct.non L. f. (Moraceae)	H	7	6	0	0	7	2	3	0	0	0	6	2
	E	0	2	0	4	3	0	0	0	0	0	0	0
	M	2	4	0	8	0	0	0	0	0	0	6	0
	W	0	0	3	0	2	0	0	1	0	2	1	0
Averrhoa carambola L. (Averrhoaceae)	H	2	4	0	7	0	0	0	0	0	0	0	3
	E	0	2	3	0	1	0	0	0	0	0	0	0
	M	0	0	0	0	0	0	0	0	0	0	0	0
	W	0	0	0	1	0	2	0	1	3	0	0	1
Bauhinia variegata L. (Fabaceae)	H	5	0	0	0	3	0	0	0	0	0	4	0
	E	0	5	0	4	5	0	0	0	0	0	0	0
	M	0	0	0	4	0	0	0	0	0	0	0	0
	W	0	0	2	0	1	0	0	0	0	0	1	0
Cordia dichotoma Forst. (Boraginaceae)	H	7	4	0	0	0	7	0	0	0	0	0	3
	E	0	3	0	13	0	0	0	0	0	0	0	4
	M	0	5	3	6	2	4	0	7	3	8	4	4
	W	4	0	0	0	2	0	0	0	2	0	0	1
Delonix regia (Boj.) Raf. (Fabaceae)	H	0	0	0	6	2	0	0	0	0	0	0	0
	E	0	6	0	0	0	0	0	0	0	0	4	0
	M	3	7	2	7	0	0	2	0	3	6	0	0
	W	2	0	3	7	0	0	1	0	0	1	0	1
Ficus racemosa L. (Moraceae)	H	7	4	0	5	7	6	0	0	0	0	5	4
	E	0	2	1	3	0	0	0	0	0	0	2	0
	M	0	0	0	4	0	0	0	0	4	0	0	0
	W	0	0	0	2	1	0	1	0	0	0	3	0
Gmelina arborea L. (Verbenaceae)	H	0	6	0	0	4	0	0	0	0	0	0	0
	E	0	4	4	0	3	0	0	0	0	0	0	0
	M	0	6	4	3	6	0	0	5	4	0	0	4
	W	0	1	0	0	5	0	0	1	0	0	2	0
Madhuca indica J.F.Gmel (Sapotaceae)	H	6	11	0	5	3	4	0	0	0	0	3	0
	E	0	4	7	4	4	0	0	0	0	0	0	3
	M	0	7	5	6	7	9	0	9	7	8	4	6
	W	0	0	3	1	1	0	0	0	2	0	0	1
Mangifera indica L. (Anacardiaceae)	H	0	1	0	5	4	3	3	0	0	0	0	4
	E	0	2	0	0	0	5	0	0	0	4	1	0
	M	8	0	0	4	10	0	0	0	5	0	9	0
	W	2	0	0	2	2	0	0	1	0	1	0	0
Manilkara hexandra (Roxb.) Dub. (Sapotaceae)	H	4	3	2	0	3	0	0	0	0	0	2	0
	E	3	4	0	4	5	0	5	2	0	4	0	2
	M	10	10	12	7	11	10	0	10	12	10	12	12
	W	1	3	0	4	2	3	2	0	0	0	0	1
Mitragyna parviflora (Roxb.) Korth. (Rubiaceae)	H	11	16	0	12	11	3	4	7	0	3	10	13
	E	0	1	5	4	0	4	0	0	7	6	10	0
	M	4	0	0	3	9	0	0	0	6	0	7	0
	W	2	2	1	4	5	0	2	3	0	1	5	0
Murraya paniculata (L.) Jack. (Rutaceae)	H	0	0	0	10	3	0	0	0	0	0	4	0
	E	0	0	3	4	0	0	0	0	0	0	1	0
	M	0	6	3	2	0	10	3	0	3	4	3	0
	W	2	0	3	0	5	0	0	0	0	0	1	0
Pithecellobium dulce (Roxb.) Bth. (Fabaceae)	H	6	1	0	2	5	5	4	0	0	0	0	5
	E	0	2	5	0	5	3	0	0	0	6	0	0
	M	5	0	0	0	7	0	0	0	4	0	7	0
	W	1	0	2	0	2	0	0	4	0	6	0	0
Psidium guajava L. (Mytraceae)	H	6	2	1	4	6	0	3	0	0	0	5	2
	E	0	4	0	0	2	0	0	0	0	0	7	4
	M	9	0	8	10	10	0	9	0	10	17	10	0
	W	3	0	5	0	7	0	0	0	1	0	1	1

Salvadora persica L. (Salvadoraceae)	H	1	5	0	1	0	1	1	0	0	0	0	3
	E	2	1	4	0	0	5	0	0	0	3	4	0
	M	7	0	0	0	6	0	0	0	0	0	7	0
	W	2	1	3	5	3	0	2	1	0	0	3	0
Saraca indica auct. non. L. (Fabaceae)	H	2	3	1	0	4	0	5	0	0	0	0	0
	E	2	2	0	0	3	0	0	0	0	1	1	0
	M	6	0	0	3	7	0	0	0	0	0	8	0
	W	1	0	0	0	0	0	2	0	0	1	3	1
Sterculia urens Roxb. (Sterculiaceae)	H	0	6	0	4	5	0	0	0	0	0	0	0
	E	0	0	2	4	0	3	0	0	0	0	4	0
	M	8	6	7	9	9	7	10	0	11	8	12	0
	W	3	0	3	0	7	4	0	1	0	0	0	0
Tabebuia argente a Brill. (Bignoniaceae)	H	2	4	1	3	0	0	0	0	0	0	0	0
	E	1	2	0	0	4	2	0	0	0	0	0	0
	M	3	0	13	6	0	3	0	0	0	0	0	0
	W	3	0	0	0	0	3	0	0	0	1	0	0
Terminalia bellirica (Gaertn.) Roxb. (Combretaceae)	H	4	3	0	0	3	4	5	0	0	0	0	0
	E	6	2	0	0	5	0	2	0	0	0	6	0
	M	0	0	0	0	5	0	0	0	0	0	0	0
	W	0	0	0	2	0	0	1	0	0	0	0	0
Thespesia populnea (L.) Sol. Ex Corr. (Malvaceae)	H	3	1	2	2	0	0	4	0	0	0	0	0
	E	1	2	0	0	3	0	0	0	0	0	0	0
	M	4	0	0	0	4	0	0	0	0	0	5	0
	W	1	0	0	2	2	0	0	0	0	0	3	0
Ciprofloxacin (20µg/ml)		12	11	11	9	11	11	12	19	10	7	8	16

Table - 2 Minimum inhibitory concentration of effective plant extracts.

Plant name	Extracts	MIC (mg/ml)											
		BC	BS	SA	SE	ML	EN	EC	ST	SP	PS	SM	KP
Artocarpus integrifolia	M	-	-	-	0.25	-	-	-	-	-	-	-	-
Cordia dichotoma	M	-	-	-	-	-	-	-	-	-	>8	-	-
Madhuca indica	H	-	0.5	-	-	-	-	-	-	-	-	-	-
	M	-	-	-	-	-	8	-	0.5	-	>8	-	-
Mangifera indica	M	4	-	-	-	2	-	-	-	-	-	>8	-
Manilkara hexandra	M	4	0.5	0.5	-	>8	4	-	0.5	>8	>8	>8	4
Mitragyna parvifolia	H	4	2	-	>8	8	-	-	-	-	-	>8	>8
	M	-	-	-	-	0.25	-	-	-	-	-	-	-
Murraya paniculata	H	-	-	-	2	-	-	-	-	-	-	-	-
	M	-	-	-	-	-	0.25	-	-	-	-	-	-
Psidium guajava	M	8	-	0.5	0.25	4	-	2	-	-	>8	>8	-
Saraca indica	M	>8	-	-	-	-	-	-	-	-	-	-	-
Sterculia urens	M	8	-	0.25	-	-	1	8	-	-	>8	>8	-
Tabebuia argentia	M	-	-	-	-	4	-	-	-	-	-	-	-

ABBREVATIONS

BC-*Bacillus cereus*
BS-*Bacillus subtilis*
EC-*Escherichia coli*
EN -*Enterococcus faecalis*
KP -*Klebsiella pneumoniae*
ML - *Micrococcus luteus*
PS-*Pseudomonas aeruginosa*
SE-*Staphylococcus epidermidis*
SA-*Staphylococcus aureus*
ST-*Salmonella typhi*
SM -*Serratia marcescens*
SP-*Salmonella paratyphi*
H - Hexane
E - Ethyl acetate
M - Methanol
W- Distilled water

Microbial pure cultures were obtained from MTCC (Institute of Microbial Technology, Chandigrah, India), ATCC (American type culture collection, Manassas, Virginia, USA) and NTCC (Health Protection Agency Culture Collection, UK). The bacterial cultures were grown on nutrient agar medium (Hi-Media, pH 7.4) at 37ºC and all the cultures were maintained at 4°C.

Antimicrobial screening of plant extracts

In the present study, the antimicrobial activities of root crude extracts prepared in different solvents were screened by agar well diffusion method [11]. An inoculum size of 1×10^8 CFU/ml of bacteria which compared with 0.5 McFarland turbidity standards was used [12]. Each plant extract of 100 µl (stock solution 100 mg/ml) was added in a previously marked sterile nutrient agar petriplates and the wells were punched with sterile cork borer and filled with each plant extract. Plates were placed in a refrigerator for 30 minutes for pre-diffusion of plant extract and then incubated at 37ºC for 24 hours. After incubation all the plates were examined and zone of inhibition (excluding well diameter in mm) was measured as a property of antimicrobial activity. Antibiotic such as ciprofloxacin (20µg/ml) as a positive control and 100% DMSO and solvents i.e. hexane, ethyl acetate and methanol as a negative controls

were used. Minimum inhibitory concentration was evaluated in the range of 4.0 to 0.25 mg/ml by two fold serial broth dilution method [13] for the plant extracts showing more than 7mm inhibition zone. Bioassay was carried out in duplicate and experiments were repeated twice.

RESULTS AND DISCUSSION

The antibacterial activity of crude root extracts extracted in hexane, ethyl acetate, methanol and distilled water of selected plant species against twelve bacterial strains and their potency were comparatively assessed by the presence or absence of zone inhibition (in mm). Successful prediction of botanical compounds from plant material is largely dependent on the type of solvent used in the extraction procedure. This study focused on plant extracts prepared in organic solvents (hexane, ethyl acetate and methanol) to obtain antimicrobial activity compared to aqueous extracts.

Crude root extracts of *Ailanthus excela, Artocarpus integrifolia, Averroha carambola, Bauhinia variegata, Cordia dichotoma, Delonix regia, Ficus racemosa, Gmelina arborea, Pithecellobium dulce, Salvadora persica, Tabebuia argentia, Terminalia bellarica, Thespesia populnea* prepared in hexane, ethyl acetate, methanol and distilled water exhibited only moderate to least activity as well as no activity against tested gram-positive and gram-negative bacteria. *Madhuca indica* methanolic root extract exhibited moderate activity against most of the tested organisms. Only hexane root extract showed good antibacterial activity against BS (Table - 1). Other extracts of *Mangifera indica* displayed moderate to least activity against most of the selected bacteria (Table - 1). Broad spectrum antibacterial activity was reported with methanol bark extract of *Mangifera indica* [14]. Methanolic root extracts of *Manilkara hexandra* exhibited good activity against SA, ML, SP, SM, KP, moderate activity against BC, BS, SE, EN, ST, PS and no activity was seen against EC (Table - 1). These results are mostly comparable with positive control ciprofloxin (20µg/ml). However, least to no activity was observed in hexane, ethyl acetate and distilled water extracts of *M. hexandra* against most of the tested bacteria (Table - 1). Methanolic extracts of *Allium vineale, Chaerophyllum macropodum* and *Prangos ferulaea* showed higher antibacterial activity compare to ethanol and hexane extracts against gram-positive bacteria (BC, BS, ML, SA) [15].

The hexane root extracts of *Mitragyna parvifolia* displayed good activity against BC, BS, SE, ML, KP as well as ethyl acetate extract exhibited moderate activity against SP, PS and SM, least to no activity seen against other tested organisms (Table - 1). This plant extract appear to be more effective against gram-positive bacteria than gram-negative bacteria except *Klebsiella pneumoniae* and *Pseudomonas aerouginosa***.** The results of hexane extracts of *Mitragyna parvifolia* are comparable with positive control. Similar results in Hexane and ethyl acetate root extracts of *Piqueria trinervia*- a Mexican plant were observed against eleven strains of bacteria [16]. In this study, broad spectrum antibacterial activity was seen in hexane and ethyl acetate root extracts of *Mitragyna parvifolia*. Methanolic root extracts of *Psidium guajava* demonstrated good activity against PS, moderate activity against BC, SA, SE, ML, EC, SP and SM (Table - 1). *Sterculia urens* methanolic root extract exhibited good activity against SP and SM, moderate activity against BC, BS, SA, SE, ML, EN, EC and PS and no activity was seen against other bacteria (Table - 1). *Senna siamae* ethanolic leaf extracts showed antibacterial activity against *Salmonella typhii* [17]. In this study, methanolic root extract of *Sterculia urens* showed good antibacterial activity against *Salmonella paratyphi.*

This study demonstrated that methanolic root extracts of most of the selected plants showed more antibacterial activity than other solvent extracts. Similar observations have been reported by Parekh and Chanda [18] in methanolic extracts of twelve medicinal plants belonging to different families provide more consistent antimicrobial activity compared to those extracted in water against *Bacillus cereus, Staphylococcus epidermidis, Enterococcus aerogenes, Pseudomonas vulgaris* and *Salmonella typhimurium.* Methanolic leaf extract of *Kirganelia reticulata* showed better activity compared to its chloroform and hexane extracts against *Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi* and *Staphylococcus aureus* [19]. Crude methanolic extracts of leaves and roots of *Clerodendrum inerme* also showed more pronounced antimicrobial activity than benzene and aqueous extracts [20]. Acetone and ethanol extracts of *Madhuca longifolia, Parkia biglandulosa, Pterospermum acerifolium* displayed more significant antibacterial activity as compared to water extracts [21]. However, in this study a large number of extracts were active against tested bacterial strains and no activity was observed in all negative controls.

Out of 12 selected bacteria, gram-positive organisms *i.e. Micrococcus luteus* and *Staphylococcus epidermidis* were found to be the most susceptible and gram-negative bacteria i.e. *Escherichia coli, Salmonella paratyphi* and *Pseudomonas aeruginosa* appeared to be the most resistant organisms against all extracts of the selected plants. In this study, the hexane, ethyl acetate and methanol extracts of all plants showed more antibacterial activity as compared to water extracts. Hexane root extracts inhibited *Staphylococcus epidermidis* by 33.33%, *Micrococcus luteus* was found to be most susceptible organism and inhibited by 19.04% ethyl acetate root extracts and methanolic root extracts by 57.14% of the selected plants. Most of the plant extracts exhibited inhibitory activity against one or more tested bacteria but plant extracts displaying more than 5 mm inhibition zone were taken in to consideration for the preparation of sensitivity sequence of organisms. Sensitivity of test strains was in decreasing order: in hexane extract - SE > BC = BS > ML > SM > EN = EC = KP > ST > SA = SP = PS; in ethyl acetate extract: ML > BS = SA = SM > EC > PS = EN > BC = SE =SP > ST =KP; in methanol extract: ML > SM > SE > BC = BS > PS >SP > ST = EN > EC = KP > SA.

The observed minimum inhibitory concentration (MIC) of effective plant extracts varied in the range of 0.25 - 8 mg/ml for most of the tested bacterial strains (Table - 2). The methanolic root extracts of *Artocarpus integrifolia, Cordia dichotoma, Madhuca indica, Mangifera indica, Manilkara hexandra, Psidium guajava and Tabebuia argentia*, while hexane and methanolic root extracts of *Madhuca indica* and *Mitragyna parvifolia* demonstrated minimum inhibitory concentration in the range of 0.25 to >8 mg/ml against tested microorganisms (Table - 2). *Psidium guajava* methanolic root extract MIC values were observed at 0.25 mg/ml against SE, 0.5 mg/ml against SA, 2 mg/ml against EC, 4 mg/ml against ML, and 8 mg/ml against BC and >8 mg/ml against PS and SM (Table - 2). The MIC values of *Psidium guajava* bark extract against *Staphylococcus aureus* coincides with the MIC value (0.5mg/ml) obtained for methanol root extract in this study [14]. The MIC value for *Madhuca indica* methanolic root extracts showed 0.5 mg/ml against ST, 8 mg/ml against EN and <8 mg/ml against PS (Table - 2). The MIC values of *Sterculia urens* were found as >8 mg/ml against PS and SM, 8 mg/ml against BC and EC, 1 mg/ml against EN and 0.25 mg/ml against SA (Table - 2).

The present investigation concludes that among different plant extracts *Mitragyna parvifolia* (hexane extract), *Sterculia urens* and *Manilkara hexandra* (methanol extract) show promising antibacterial properties and could be used externally against bacterial infections. Alternatively, the active principles of these plant extracts may be characterized and tested for their safety and efficacy to uncover their therapeutic potential in modern and traditional medicine against infectious diseases.

ACKNOWLEDGEMENT

Mr. Purvesh Bharvad thanks University Grant Commission, New Delhi for awarding "UGC research fellowship for meritorious students scheme".

REFRENCES

[1] Ahmed, I. and Beg, A. Z. (2001) Antimicrobial and phytochemical studies on 45 medicinal plants against multi-drug resistant human pathogens. *Journal of Ethnopharmacology,* **74:** 113 – 123.

[2] Iwu, M. W., Duncan, A. R. and Okunji, C. O. (1999) New antimicrobials of plant origin. *In Prespectives on new crops and new uses.* (Ed. Janick, J.) pp. 457 – 462.

[3] Jagtap, N. S. , Khadabadi, S. S., Ghorpade, D. S., Banarase, N. B. and Naphade, S. S. (2009) Antimicrobial and antifungal activity of *Centella asiatica* (L.) Urban, Umbeliferae. *Research Journal of Pharmacy and Technology,* 2**:** 328 – 330.

[4] Aqil, F. and Ahmed, I. (2003) Broad-spectrum antibacterial and antifungal properties of certain traditionally used Indian medicinal plants. *World Journal of Microbiology and Biotechnology,* **19:** 653 – 657.

[5] Mahida, Y. and Mohan, J. S. S. (2007) Screening of plants for their potential antibacterial activity against *Staphylococcus* and *Salmonella spp. Natural Product Radiance,* **6** (4): 301- 305.

[6] Nwachukwu, I. N., Allison, L. N.-0., Chinakwe, E. C. and Nwadiaro, P. (2008) Studies on the effects *Cymbopogon citratus, Ceiba pentandra* and *Loranthus bengwelensis* extracts on species of dermatophytes. *The Journal of American Science,* **4** (4): 52 – 63.

[7] Kolapo, A. L., Okunade, M. B., Adejumobi, J. A. and Ogundiya, M. O. (2009) Phytochemical composition and antimicrobial activity of *Prosopis africana* against some selected oral pathogens. *World Journal of Agricultural Sciences,* **5** (1): 90 – 93.

[8] Seyydnejad, S. M., Niknejad, M., Darabpoor, I. and Motamedi, H. (2010) Antibacterial activity of hydroalcoholic extracts of *Callistemon citrinus* and *Albizia lebbeck. American Journal of Applied Sciences,* **7** (1): 13- 16.

[9] Shah, G. L. (1978) *Flora of Gujarat State,* Vol I-IV, Sardar Patel University Press, Vallabh Vidyanagar, Gujarat, India.

[10] Houghton, P. J. and Raman A. (1998) Laboratory handbook for the fractionation of natural extracts. Chapman and Hall. London, pp. 1- 28.

[11] Perez, C., Pauli, M. and Bazaerque, P. (1990) An antibiotic assay by the agar well method. *Acta Biologica et Medica Experimentalis,* **15:**113 -115.

[12] Perilla, M. J. (2003) *Manual for the laboratory identification ad antimicrobial susceptibility testing of bacterial pathogens of public health importance in the developing world. WHO (World Health Organization), pp:* 209- 214.

[13] Chattopadhyay D., Mukherjee T., Pal P., Shah B. and Bhadra R. (1998) Altered membrane permeability as the basis of bactericidal action of methdilazine. *The Journal of Antimicrobial Chemotherapy,* **42:** 49- 55.

[14] Akinpelu, D. A. and Onakoya, T. M. (2006) Antimicrobial activities of medicinal plants used in folklore remedies In south-western Nigeria. *African Journal of Biotechnology,* **5** (11): 1078 -1081.

[15] Durmaz, H., Sagun E., Tarakci Z. and Ozgokce, F. (2006) Antibacterial activities of *Allium vineale, Chaerophyllum macropodum* and *Prangos ferulacea*. *African Journal of Biotechnology,* **5** (19): 1795-1798.

[16] Esparza, R. R. D., Bye, R., Meckes, M., Lopez, J. T. and Estrada, M. J. (2007) Antibacterial activity of *Piqueria trinervia,* a Mexican medicinal plant used to treat diarrhea. *Pharmaceutical Biology,* **45** (6): 446- 452.

[17] Doughari, J. H. and Okafor, N. B. (2008) Antibacterial activity of *Senna siamae* leaf extracts on *Salmonella typhi*. *African Journal of Microbiology Research,* **2:** 42 – 46.

[18] Parekh, J. and Chanda, S. (2007) Antibacterial and phytochemical studies on twelve species of Indian medicinal plants. *African Journal of Biomedical Research,* **10:**175- 181.

[19] Shruthi, S. D., Ramachandra, Y. L. Rai, P. S. and Shetty, V. A. (2010). Antibacterial potential of leaf extracts from *Kirganelia reticulata* Baill. *International Journal of Pharmaceutical Researches and Developments-Online (IJPRD),* **2** (6): 1–7.

[20] Chahal, J. K., Sarin, R. and Malwal, M. (2010). Efficacy of *Clerodendrum inerme* L. (Garden quinine) against some human pathogenic strains. *International Journal of Pharma and Biosciences,* **1** (4): 219 – 223.

[21] Khond, M., Bhosale, J. D., Arif, T., Mandal, T. K., Padhi, M. M. and Dabur, R. (2009) Screening of some selected medicinal plants extracts for *in vitro* antimicrobial activity. *Middle-East Journal of Scientific Research,* **4** (4): 271 – 278.