View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by Advanced Research Journals

International Journal of Phytomedicine 3 (2011) 261-269

http://www.arjournals.org/index.php/ijpm/index

Original Research Article

ISSN: 0975-0185

Antibacterial activity of methanolic and acetone extract of some medicinal plants used in indian folklore

J. P. Patel, Bharat Gami, Kanti Patel and Raksha Solanki

*Corresponding author:

J. P. Patel
Department of Biology,
V.P. and R.P.T.P. Science
College, Vallabh
Vidyanagar-388120,
Gujarat, India.
Email: jppatel14@yahoo.co.in

Abstract

Antibacterial study of methanolic and acetone extract in crude and treated (with 50 % lead acetate) form of medicinal plants Alstonia scholaris Linn. R.Br. (Stem bark, Apocynaceae), Achyranthus aspera Linn. (Whole plant, acantheceae), Moringa oleifera Lam. (Leaves, Morinaceae), Tinospora cordifolia (Stem, Menispermaceae), and Enicostema hyssopifolium (Willd) (Stem, Gentianaceae). Extractive values in methanol were found higher then the extractive value in acetone, for all plants. All extract of plants were tried at 40-mg/ml concentrations against eight strains of bacteria, by agarwell-difusion test. Acetone extract was found more active as compared to methanol extract. Phytochemical investigation revealed crude and treated extracts of all plants were containing more or less same type of chemical constituents (except protein and carbohydrate). Selected eight strains of bacteria were study for antibiotic susceptibility against standard antibiotics like Ampicillin (10µg), Tetracycline (25µg), Gentamicin (30µg), Co-Trimoxazole (25µg), Amikacin (10µg), by Octadisc. Treated extract of M. oleifera and A. scholaris were count as to new source of antimicrobial agent for the infectious diseases (Typhoid).

Keywords: Antibacterial activity, Medicinal Plants, infectious diseases.

Introduction

Plants are invaluable sources of pharmaceutical products [1] and plants are recognized for their ability to produce a wealth of secondary metabolites and mankind has used many species for centuries to treat a variety of diseases [2]. Despite the wide availability of clinically useful antibiotics and semisynthetic analogues, a continuing search for new anti-infective agents remains indispensable because some of the major antibacterial agents have considerable drawbacks in terms of limited antimicrobial spectrum or serious side effects [3]. The negative health trends call for a renewed interest in infectious disease in the medical and public health communities and renewed strategies on treatment and prevention. Proposed solutions are outlined as a multi-pronged approach that includes: prevention, (such as vaccination); improved monitoring; and the development of new treatments. It is this last solution that would encompass the development of new antimicrobials [4]. There is an urgent need to discover new antimicrobial agents for human and veterinary therapeutic uses, as resistance to current drugs increases in severity and extent [5, 6]. The identification of new natural products with antimicrobial activity, extraction methods, and hopefully new modes of action, is one of the ways of tackling this problem. Lack of scientific knowledge has often constituted a major constraint to consideration of the use of

This work is licensed under a Creative Commons Attribution 3.0 License.

traditional herbal remedies in conjunction with or as an affordable alternative to orthodox medical treatment. In present study methanolic and acetone extract of five plants (traditionally used in many diseases) are studied for their antimicrobial activity in crude form and after treated with 50 % lead acetate treatment.

Materials and methods:

Plant material

Authentic sample (powder) of Alstonia scholaris Linn. R.Br. (Stem bark, Apocynaceae), Achyranthus aspera Linn. (Whole plant, acantheceae), Moringa oleifera Lam. (Leaves - Morinaceae), Tinospora cordifolia (Stem-Menispermaceae), and Enicostema hyssopifolium (Willd) (Gentianaceae), collected from Bapalal Botanical Vaidya Research Center Surat (Gujarat).

Extraction

Coarsely powdered air-dried material 4 g was placed in a glass stoppered conical flask and macerated with 100 ml of analytic grade solvents (methanol, and acetone) shaking frequently, and then allowing it to stand for 18 hours. Filter it rapidly through Whatman No. 1 filter paper, taking care not to lose any solvent. Transfer 25 ml filtrate to flat- bottom dish and evaporate solvent on a water bath. Dry at 105 ^{o}C for 6 hours, cool in a desiccator for 30 minutes and weigh without delay, calculate the content of extractable matter in mg/g of air-dried material [7]. Crud extract (10 ml) treated with 200µl of 50 % lead acetate (Hi-media, Mumbai), mix it properly and precipitated by centrifugation (REMI, India) at 10000 rpm for 15 min. Care fully remove the supernatant in watch glass, evaporate at room temperature, dry at 105 ^{o}C for 6 hours, cool in a desiccator for 30 minute. Extracts were dissolved in 90 % DMSO to concentration 40 mg/ml were used for antimicrobial activity.

Phytochemical Screening

Crude extract in methanol (M1), and acetone (A1) along with after treatment of 50 % lead acetate (M2) and (A2) respectively, of all plants were subjected to qualitative phytochemical screening for protein, carbohydrate, saponin, tannin, glycoside, alkaloids, flavanoids, terpenoids, steroids and fixed oil according to the method [8].

Procured Bacterial Strain

Test organisms used in this study were collected from the Microbiology Department of Sardar Patel University, V.V.Nagar, Gujarat. The Gram-positive bacteria are Staphylococcus aureus (ATCC9144) (SA), Micrococcus luteus (ATCC4698) (ML), Klebsiella pneumoniae (ATCC15380) (KP), Bacillus subtilis (ATCC 6051) (BC), and Gram-negative bacteria are Pseudomonas aeruginosa (ATCC25668) (PA), Enterobacter aerogens (ATCC13048) (EA), Salmonella typhi (NCTC 8394) (ST) and Salmonella paratyphi- A (SPA). Strains were maintained on nutrient agar.

Agar diffusion Assay

Antimicrobial screening was done using agar well diffusion methods [9]. For this 25 ml of sterile Mueller –Hinton Agar No.2 (Hi-media), was poured in sterile autoclaved petri plates, before pouring 100µl of activated culture of microorganism was added, and then allowed to stand for solidify completely. The wells were prepared with the help of sterile 10 mm diameter cork-borer. Then 100 µl of prepared plant extract (40 mg/ml) solution were poured into the wells. Then the plates were sealed with plasticine and transferred to the refrigerator to diffuse out for 30 min. The plates were then incubated in the incubator at 37 ^{o}C for 24 hrs. Triplicate plates were prepared for each treatment and the average zone of inhibition excluding well, were recorded. 90% DMSO was used as negative control, and 0.01mg/ml tetracycline was used as positive control. Inoculum turbidity was maintained constant throughout the experiment to 0.8 OD at

660 nm. Level of turbidity is equivalent to approximately 1×10^8 CFU/ml.

Antibiotic susceptibility of selected bacterial strains

Susceptibility of selected bacterial strain was done against the standard antibiotics viz. Ampicillin(10µg), Tetracycline(25µg), Gentamicin(30µg), Co-Trimoxazole(25µg), Amikacin(10µg) by octadisc (Hi-media, Mumbai). Twenty ml of sterilized nutrient agar seeded with activated bacterial culture was poured in petri dish, allowed to solidify and octadisc was placed gently on surface by pointed forceps. Seal the plates with plasticine and incubate in the incubators at 37 ^{o}C for 48 hrs.

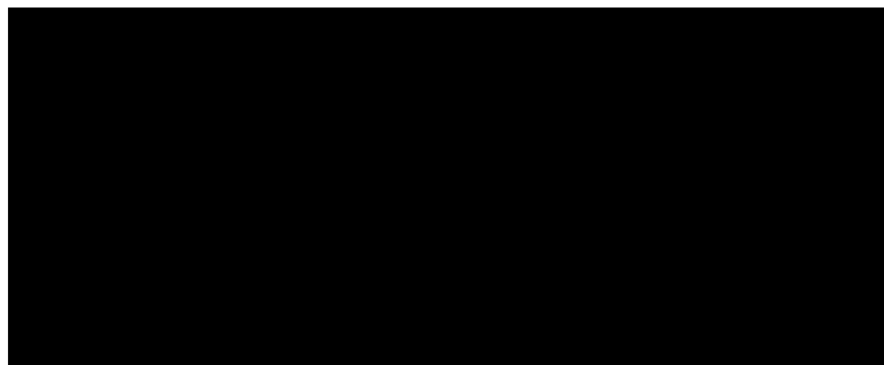
Results

Extractive value

The results of extractive value in acetone and methanol are shown in graph 1. All plants showed higher percentage of extraction in methanol then the acetone. The yield for selected plants in acetone was found in order to E.hyssofolia (7.11 %)> A.scholaris (2.50 %)> T.cordifolia (1.82%)> M. oleifera (1.22 %) > A.aspera (0.46 %), while in methanol yield was E.hyssofolia (26.5 %)> A.scholaris (18.5 %)> M.oleifera (6.36%)> T.cordifolia (3.47 %) > A.aspera (3.05 %).

Phytochemical screening

Results of phytochemical screening for methanolic extract of all plants in crude (M1) form and after treated with 50 % lead acetate (M2) are shown in Table 1. And results for crude acetone extract (A1), and after treated with 50 % lead acetate (A2) are shown in Table 2. Alkaloids were present in M1 extract of E.hyssofolia, A.scholaris, T.cordifolia, M. oleifera, A.aspera (Table 1), while absent in A1 extracts of E.hyssofolia, A.scholaris, T.cordifolia (Table 2). Primary metabolites like carbohydrate and protein were detected in both M1 and A1 extract of all selected plants, and absent in M2 and A2 extract. Steroids were present only in both crude extract (M1 and A1) of A.scholaris (Table 1 and 2). M1 extract of A.scholaris, M. oleifera and E.hyssofolia were showed positive result for glycoside (Table 1), while glycoside was present in all A1 extract of all plants (Table 2). Both M1 and A1 extract of all five plants were showed presence of flavanoids (Table 1 & 2). But M2 and A2 extracts of M. oleifera and E.hyssopifolium were only showed presence of flavanoids (Table 1 & 2). Saponin was detected in M1, M2, A1, A2 extracts of all plants (Table 1 & 2). Terpenoids was absent in M1 extract of A.aspera (Table 1), while positive for the A1 extract (Table 2). Other all plants were positive for the presence of terpenoids in crude (M1, A1) as well as treated (M2, A2) extracts (Table 1 & 2). Crude extract (A1) of A.scholaris and M. oleifera were only positive for the tannin (Table 2). Fixed oil was detected in any types of extracts in all selected plants.



Graph 1. Extractive value of plants.

Table 1: Phytochemical screening of Methanol extract.

Phytoconstituents	A.scholaris		A.aspera		M.oleifera		T.cordifollia		E. hyssofolium	
	M1	M2	M1	M2	M1	M2	M1	M2	M1	M2
1. Alkaloids	+	+	+	+	+	+	+	+	+	+
2. Carbohydrates	+	-	+	-	+	-	+	-	+	-
3. Protein	+	-	+	-	+	-	+	-	+	-
4. Steroids	+	+	-	-	-	-	-	-	-	-
5. Glycosides	+	+	-	-	+	+	-	-	+	+
6. Saponin	+	+	+	+	+	+	+	+	+	+
7. Flavanoids	+	-	+	-	+	+	+	-	+	+
8. Tannins	+	+	-	-	+	+	+	+	+	+
9. Triterpenoids	+	+	-	-	+	+	+	+	+	+
10.Fixed oils	-	-	-	-	-	-	-	-	-	-

+ = Present, - = absent.

Table 2. Phytochemical screening of Acetone extract.

Phytoconstituents	A.scholaris		A.aspera		M.oleifera		T.cordifollia		E. hyssofolium	
	A1	A2	A1	A2	A1	A2	A1	A2	A1	A2
1. Alkaloids	-	-	+	+	+	+	-	-	-	-
2. Carbohydrates	+	-	+	-	+	-	+	-	+	-
3. Protein	+	-	+	-	+	-	+	-	+	-
4. Steroids	+	+	-	-	-	-	-	-	-	-
5. Glycosides	+	+	+	+	+	+	+	+	+	+
6. Saponin	+	+	+	+	+	+	+	+	+	+
7. Flavanoids	+	-	+	-	+	+	+	-	+	+
8. Tannins	+	+	-	-	+	+	-	-	-	-
9. Triterpenoids	+	+	+	+	+	+	+	+	+	+
10.Fixed oils	-	-	-	-	-	-	-	-	-	-

+ = Present, - = absent.

Antimicrobial activity

Results of comparative antimicrobial activity of M1 extract and M2 extracts of all plants are recorded in Table 3, and results of A1 and A2 are shown in Table 4. M1and M2 extracts of all plants were completely inactive against BS (Table 3). M1, M2 extracts of M. oleifera was found most active extracts as only BS and KP only were not inhibited by extract (Table 3). In methanolic extract highest inhibition (10mm) was found by A.scholaris against ML (Table 3). Highest zone of inhibition (22mm) was observed by A2 extracts of A.scholaris against EA. KP was completely resistant towards acetone extracts (A1, A2) of E.hyssofolia, A.scholaris, T.cordifolia, M. oleifera, A.aspera (Table 4). BS, EA, and SA were found sensitive to A1 and A2 extracts of all plant (Table 4). Moderate kind of sensitivity was observed in ML and SPA, and least activity found in PA and ST, against A1 and A2 extract (Table 4). One strong observation was observed in present study was that in all plants A2 and M2 extract showed higher antibacterial activity than the A1 and M1 at same concentration (40mg/ml) respectively. All strain of bacteria was susceptible to positive control, and DMSO 90 % as negative control was not inhibiting any bacterial strain.

Table 3. Antimicrobial activity of Methanol at 40 mg/ml

Plants		BS	EA	KP	ML	PA	SA	ST	SPA
A.scholaris	M1	-	-	2	6	2	-	-	-
	M2	-	-	4	10	5	-	-	-
A. aspera	M1	-	-	-	2	-	4	2	-
	M2	-	-	-	5	-	8	6	-
M.oleifera	M1	-	4	-	2	4	2	2	2
	M2	-	8	-	5	9	7	6	6
T. cordifolia	M1	-	2	-	-	-	-	-	-
	M2	-	7	-	-	-	-	-	-
E. hyssofolium	M1	-	-	-	-	-	-	2	-
	M2	-	-	-	-	-	-	6	-

A-Methanol, B- Methanol fraction after treatment of 50 % lead acetate, - : no inhibition zone.

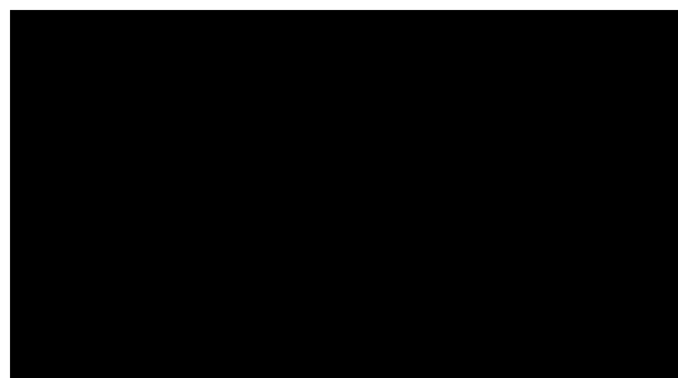
Table 4. Antimicrobial activity of acetone fraction at 40 mg/ml.

Plants		BS	EA	KP	ML	PA	SA	ST	SPA
A. scholaris	A1	6	20	-	10	-	4	-	-
	A2	8	22	-	14	-	7	-	-
A. aspera	A1	4	4	-	2	-	3	-	-
	A2	7	8	-	4	-	4	-	-
M.oleifera	A1	7	8	-	10	6	10	6	2
	A2	9	12	-	13	9	13	10	5
T. cordifolia	A1	2	5	-	-	-	5	-	8
	A2	6	9	-	-	-	10	-	12
E. hyssofolium	A1	4	6	-	-	-	7	-	-
	A2	8	9	-	-	-	10	-	-

A-Acetone, B- Acetone fraction after treatment of 50 % lead acetate, - : no inhibition zone

Antibiotic susceptibility of bacterial strains

Results of antibiotic susceptibility of the selected bacterial strains are shown in (Graph.2). ML was more susceptible to all antibiotics. EA, PA, and SA were completely resistant to co-trimoxazole (25 µg/disc). STB was also showed negligible inhibition (4mm) against co-trimoxazole (Graph 2). Highest susceptibility (22mm) was found in KP against tetracycleine (25 µg/disc). Amphicillin at 10µg/disc was showed negligible activity against EA (2mm), STB (4mm), PA (2mm), and SA (2mm).



Graph 2. Antibiotic susceptibility of bacterial strain to antibiotics.

A- Ampicillin, T- Tetracycline, G- Gentamicin, Co- Co-Trimoxazole, AK- Amikacin

Discussion

The higher yield of the methanol extracts compared with the acetone extracts suggested that secondary metabolite of plants were more soluble in methanol than the acetone. In present study cold maceration was used to extract secondary metabolite, are the probably reason for low yield of extracts, as maceration [10] and cold extraction [11, 12] generally been reported to give lower yield of plant extracts compared to hot and soxhlet extraction.

Mostly both types of extraction (acetone and methanolic) extract same type of phytochemical like alkaloids, saponin, glycoside, flavanoids, tannins, and terpenoids as particular to different plants. And these compounds were well defined as antimicrobial agents, like tannin [13, 14], glycosides [15], Saponins [16], trerpenoids and flavanoids, [17] and Alkaloids [18] in plants. A1 extracts found more active than M1 extract, which indicate that, active component of plants extract in acetone or may extract in very low concentration in methanol, as solubility of compound depend on polarity of extraction solvent [19] and [20]. High sensitivity of Gram-positive bacteria (except KP) than the Gram-negative bacteria, for selected plant extracts (M1, M2, A1, A2) were found in present study. The high resistance of gram-negative bacteria could be because of the phospholipid membrane in addition to the inner peptidoglycan layer, which makes the cell more impermeable for exogenous molecules [21].

Methanolic extract of A.aspera was inactive against BS, EA, KP, PA, and SPA, while acetone extract is active against BS and KP, these findings are corroborate with findings of [22]. Acetone extract of A.scholaris was more active than the methanol extract, results indicate active components in A.scholaris were more soluble in relatively non-polar solvent, findings are agree with [23]), as they reported butanol fraction has broad spectrum of antibacterial activity. Goyal et al. [24] reported alkaloids, sterols alkenes, are key antimicrobial agent in A.scholaris, result matching with our finding. M. oleifera was most active plant among other selected plants. Activity of plant is attribute to the presence of saponin, tannin alkaloids and phenols [25]. Acetone extracts of M. oleifera only show the antimicrobial activity against the ST and STA, same results observed by Doughari et al. [26] for Salmonella typhi. Both bacteria are causative agent for the Typhoid fever, and recent year there has been a rapid rise in multidrug resistance by ST all over the world [27, 28, 29]. A2 extract of M. oleifera can be used to developed antityphoid agent. Activity of methanolic and acetone extract of E.hyssofolium was attributed to presence of flavanoids a major constitute in E.hyssofolium [30]. Singh et al. [31] reported presence of terpenoids and glycoside in T.cordifolia. These components are the responsible for the antibacterial activity of these plant [32].

M2 and A2 extracts of all plants were more active than their crude extract (M1, A1), phytochemical screening revealed that, carbohydrate and protein were absent in M2 and A2, while these present in higher concentration in crude extract, which may be interfered in diffusion of active component. Moreover the differences of antimicrobial activity of extracts were difficult to speculate; however, many antibacterial agents may exhibit their action through inhibition of nucleic acid, protein and membrane phospholipids biosynthesis [33]. Extract with analytical grade acetone gave a relatively wide spectrum of antimicrobial activity (37.5% – 87.5%) against the test bacterial strains compared to methanol extract (12.5 % - 75 %). The relatively wider spectrum of activity of the acetone extracts over the methanol extracts is difficult to explain since all the extracts contained the metabolites, though not in the same proportions. Perhaps, the active principles were more soluble in analytical acetone than the methanol solvents.

Antibacterial alternative for selected bacterial strains ware always in focused because of its infectious nature and bacteria has ability to develop the genetic ability to transmit and acquire

resistance to drugs, which are utilized as therapeutic agents [34]. Several studied indicate the usefulness of plant for control of resistant strain of bacterial like S.aureus [35] and P.aeruginosa [36, 37, 38]. Among selected plants acetone extract of A.scholaris and M. oleifera can be used for the new antibacterial agent, for EA, SA and ML.

Conclusion

Acetone extract after treatment of 50 % lead acetate was found to be best as antimicrobial agent. It is suggested if large amount of extract needed, cold maceration should be avoided. M.oleifera and A.scholaris can be further investigation to developed new weapon to combat infectious diseases.

Referenses

1. Sadqui M, Fushman D and Munoz V. Atom–by–atom analysis of global downhill protein folding. Nature. 2006;**442:**317–321.
2. Olalde Rangel JA. The systemic theory of living systems and relevance to CAM. Part I: the theory. Evid Based Complement Alternat Med. 2005;**2:**13–18.
3. Cragg GM, Boyd MR, Khanna R, Kneller R, Mays TD, Mazan KD, Newman DJ and Sausville EA. International collaboration in drug discovery and development: the NCI experience. Pure Appl Chem. 1999;**71:**1619-1633.
4. Olila D, Olwa-Odyek and Opuda-Asibo J. Antibacterial and antifungal activities of extracts of Zanthoxylum chalybeum and Warburgia ugandensis, Ugandan medicinal plants, African Health Sciences. 2001;**1(2):**66-72.
5. Fauci A. New and reemerging diseases: The importance of biomedical research. Emerging Infectious Diseases. (www.cdc.gov/ncidod/EID/vol4no3/fauci). 1998;4:3.
6. Robert A and Meunier B. Is Alkylation the Main Mechanism of Action of the Antimalarial Drug Artemisinin. Chem. Soc. Rev. 1998;**27:**273-279.
7. Setti EL, Micetich RG. New Trends in Antimicrobial Development. Current Medicinal Chemistry. 1998;**5:**101-113.
8. WHO Geneva, Quality Control Method for Medicinal Plant Materials, New Delhi. 2002;**51:**30.
9. Harbone JB. Phytochemical Methods – A Guide to Modern Techniques of plant analysis 2nd edition. (Chapman and Hall London. New York) 1984.
10. Perez C, Paulin Mand and Bazerque P. An antibiotic assay by the well agar method. Acta. Biological Medicine Experitimentalis. 1990;**15:**113-115.
11. Ibrahim MB, Owonubi MO and Onaolapo JA. Antimicrobial effectsof extracts of leaf, stem, and root-bark of Anogiessus leicarpus onStaphylococcus aureus NCTC 8190, Escherichia coli NCTC 10418and Proteus vulgaris NCTC 4636. J. Pharmaceutical Res. Dev. 1997;**2:**20-26.
12. Okeke MI, Iroegbu CU, Eze EN, Okoli AS and Esimone CO. Evaluation of extracts of the root of Landolphia owerrience for antibacterial activity. J. Ethnopharmacol. 2001;**78:**119-127.
13. Okoli AS, Okeke MI, Iroegbu CU and Ebo PU. Antibacterial activityof Harungana madagascariensis leaf extracts. Phytother. Res. 2002;**16:**174-179.
14. Scalbert A. Antimicrobial properties of tannins. Phytochemistry. 1991;**30:**3875-3883.
15. Aboaba OO, Smith SI and Olude FO. Antibacterial Effect of Edible Plant Extract on Escherichia coli 0157:H7, Pakistan Journal of Nutrition. 2006;**5(4):**325-327.
16. Aboaba OO and Efuwape BM. Antibacterial properties of some Nigerian spices. Bio. Res. Comm. 2001;**13:**183-188.
17. Hostettman K and Nakanishi K. Moronic acid, a simple triterpenoid keto acid with antimicrobial activity isolated from Ozoroa Mucroanta. J. Med. Plant Res. 1979;**31:**358-366.

18. Leven M, VandenBerghe DA, Mertens F, Vlictinck A and Lammens E. Screening of higher plants for biological activities/-antimicrobial activity. Plant. Med. 1979;**36:**311-321.
19. Damintoti Karou1, Aly Savadogo, Antonella Canini, Saydou Yameogo, Carla Montesano, Jacques Simpore, Vittorio Colizzi and Alfred S. Traore. Antibacterial activity of alkaloids from Sida acuta. African Journal of Biotechnology. 2005;**4(12):**1452-1457.
20. Eloff JN. Which extractan should be used for screening and isolation of antimicrobial components from plants. J. Ethanopharmacol. 1998;**60:**1-8.
21. Cowan MM. Plant product as antimicrobial agents, Clin.Microbiol.Rev. 1999;12:564-582.
22. Nikaido H and Vaara M. "Molecular Basis of Bacterial Outer Membrane Permeability", Microbiological Reviews. 1985;**1:**1-32.
23. Jigna P, Rathish N and Sumita C. Priliminary screening of some folklore medicinal plants from western India for potential antimicrobial activity, Inian J. Pharmacol. 2005;**37(6):**408-409.
24. Khan MR, Omoloso AD and Kihara M. Antibacterial activity of Alstonia scholaris and Leea tetramera, Fitoterpia. 2003;**74(7-8):**736-40.
25. Goyal MM and Varshney A. Effects of natural products isolated from three species of Alstonia on some gram-positive and gram-negative bacteria. Indian Drugs 1995;**32(2):**69-72.
26. Clark WS. Antimicrobial activities of phenolic constituents of Magnolia grandiflora L J. Pharm. Sci. 1981;**70:**951-952.
27. Doughari JH, Pukuma MS and De N. Antibacterial effects of Balanites aegyptiaca L. Drel. and Moringa oleifera Lam. on Salmonella typhi, African Journal of Biotechnology. 2007;**6(19):**2212-2215.
28. Chin NT, Perry CM, Ly NT, Ha HD, Thong M and Diep TS. Randomized controlled comparison of azithromycin and ofloxacin for treatment of multidrug resistant or nalidixic acid resistant enteric fever. Antimicrob. Agents Chemother. 2002;**44:**1855-1859.
29. Benoit D, Renand L, Daniele M, Ame B, David B, Michael RM, Elisabeth C and Anel C. Variant Salmonella genomic island 1 antibitotic gene resistance cluster in Salmonella enterica Albany. Emerg. Infect. Dis. 2003;**9(5):**585-591.
30. Abdullah WB, Anowa H, Doli G, Amina TS, Kamrun N, Korshed A, Neor A, Aliya N, Balakrish N, Stephen L and Robert B. Bacteremic typhoid fever in children in an urban slum, Bangladesh. Emerg. Infect. Dis. 2005;**11(2):**326-329.
31. Ghosal S and Jaiswal DK. Chemical constituents of gentianaceae XXVIII:flavonoids of Enicostema hyssopifolium (Willd.) Verd. J.Pharm Sci. 1980;**69(1):**53-56.
32. Singh SS, Pandey SC, Srivastava S, Gupta VS, Patro B and Ghosh AC. Chemistry and Medicinal Properties of Tinospora cordifolia. Indian Journal of Pharmacology. 2003;**35:**83-91.
33. Rege NN, Nazareth HM, Bapat RD and Dahanukar SA. Modulation of immunosuppression in obstructive jaundice by Tinospora cordifolia. Indian J Med Res. 1989;**90:**478-83.
34. Franklin TJ, Snow GA, Barrett-Bee KJ and Nolan RD Biochemistry of antimicrobial action. Fourth edition. Chapman and Hall London New York. 1987;112:pp. 71-73.
35. Cohen ML. Epidemiology of drug resistance: implications for a postantimicrobial era. Science. 1992;**257:**1050-1055.
36. Anesini E and Perez C. Screening of plants used in Argentine folk medicine for antimicrobial activity. J. Ethnopharmacol. 1993;**39:**119-128.

37. TLG Monte FJQ, Matos FJA, Alencar JW, Craveiro AA, Barbosa RCSB and Lima ED. Chemical composition and antimicrobial activity of essencial oils from Brazilian plants. Fitoterapia. 1992;**63:**266-268.
38. Martinez MJ, Betancourt J, Alonso-Gonzalez N and Jauregui A. Screening of some Cuban medicinal plants for antimicrobial activity. J. Ethnopharmacol. 1996;**52:**171-174.
39. Martinez MJ, Vasquez SM, Espinosa-Perez C, Dias M and Herrera- Sanchez M. Antimicrobial properties of argentatine A isolated from Parthenium argentatum. Fitoterapia. 1994;65:371-372.