ISSN 0975 – 2595

PRAJÑĀ

Volume 18, 2010

Journal of Pure and Applied Sciences

SARDAR PATEL UNIVERSITY
VALLABH VIDYANAGAR
Gujarat – 388 120, INDIA
www.spuvvn.edu

PRAJÑĀ - Journal of Pure and Applied Sciences, Vol. 18: 54 - 58 (2010)
ISSN 0975 - 2595

ANTIBACTERIAL ACTIVITY OF METHANOLIC AND ACETONE EXTRACT OF SOME MEDICINAL PLANTS USED IN INDIAN FOLKLORE MEDICINE

J. P. Patel*

Department of Biology, V.P. & R.P.T.P. Science College, Vallabh Vidyanagar-388120

ABSTRACT

Antibacterial study of methanolic and acetone extracts of crude and treated (with 50 % lead acetate) extracts of medicinal plants viz, *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 hyssopifoliu*m (Willd) (Stem, Gentianaceae) was carried out. Extractive values in methanol were found to be higher than the extractive value in acetone, for all plants. All the extracts of the plants selected for the present study were tested for their antimicrobial activity at 40-mg/ml concentrations against eight strains of bacteria, by agarwell-difusion test. Acetone extract was found to be more active as compared to that of methanol extract. The phytochemical analysis of crude and treated extracts of all the currently studied plants revealed that to contain more or less similar type of chemical constituents (except protein and carbohydrate). The eight strains of bacteria were selected for antibiotic susceptibility against standard antibiotics like Ampicillin (10µg), Tetracycline (25µg), Gentamicin (30µg), Co-Trimoxazole (25µg), Amikacin (10µg), by Octadisc.

Key words: *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 plant 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 consider the use of traditional herbal remedies in conjunction with or as an affordable alternative to orthodox medical treatment. In the present study, the methanolic and acetone extracts of five plants (traditionally used in many diseases) are studied for their antimicrobial activity in crude form and after treatment with 50 % lead acetate.

MATERIALS AND METHODS

Plant material

Authentic (powder) samples of *Alstonia scholaris* Linn. R.Br. (Apocynaceae) Stem bark; *Achyranthus aspera* Linn. (Acantheceae) whole plant; *Moringa oleifera* Lam. (Morinaceae) Leaves -, *Tinospora cordifolia* (Menispermaceae) Stem-, and *Enicostema hyssopifoliu*m (Willd) (Gentianaceae) whole plant, were collected from Bapalal Botanical Vaidya Research Center, Surat (Gujarat).

* Corresponding author: jppatel114@yahoo.co.in

Extraction

Plant material was dried at 60 ºC and made it powdered. For each species four grams air dried powdered material was placed in a mortar and pestle and macerated with 100 ml of analytic grade solvents (methanol and acetone). Transferred the extracts in to a glass stoppered conical flask and shaken frequently and then allowed it to stand for 18 hours. Filtered the extracts by Whatman No. 1 filter paper, and transferred 25 ml filtrate to a flat- bottom dish and evaporated the solvent on a water bath. Dried it at 105 ^{o}C for 6 hours and cooled it in a desiccator for 30 minutes, following then, the prepared sample was weighed without any delay and calculated the content of extractable matter in mg/g of air-dried material [7]. Crude extract (10 ml) treated with 200µl of 50 % lead acetate (Hi-media, Mumbai), mixed properly and precipitated by centrifugation (REMI, India) at 10000 rpm for 15 min. Care fully removed the supernatant in watch glass and evaporated it at room temperature, dried at 105 ^{o}C for 6 hours and cooled 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) and after treating it with 50 % lead acetate (M2) and (A2) respectively, of all the plants selected for the present study were subjected for their qualitative phytochemical screening of proteins, carbohydrates, saponins, tannins, glycosides, alkaloids, flavanoids, terpenoids, steroids and fixed oil, as per the method sited by Harborne [8].

Procured Bacterial Strain

Test organisms used in this study were collected from the Department of Biosciences, Sardar Patel University, Vallabh Vidyanagar, 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 method [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 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 measured against 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 Fig. 1. All the plants tested under study showed higher percentage of extraction in methanol than that of acetone. The yield for selected plants in acetone was found in the order of *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 in the order of *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 of methanolic extract of all plants in crude (M1) form and after treatment with 50 % lead acetate (M2) are shown in Table 1. Results for crude acetone extract (A1), and after treatment 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, 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 were 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.

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 shown 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.

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).

DISCUSSION

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

Mostly both (acetone and methanolic) types of extractions yield same type of phytochemicals like alkaloids, saponins, glycosides, flavanoids, tannins, and terpenoids as particular to different plants. And the compounds such as tannin [13, 14], glycosides [15], Saponins [16], trerpenoids and flavanoids, [17] and Alkaloids [18] were well defined as antimicrobial agents in plants.

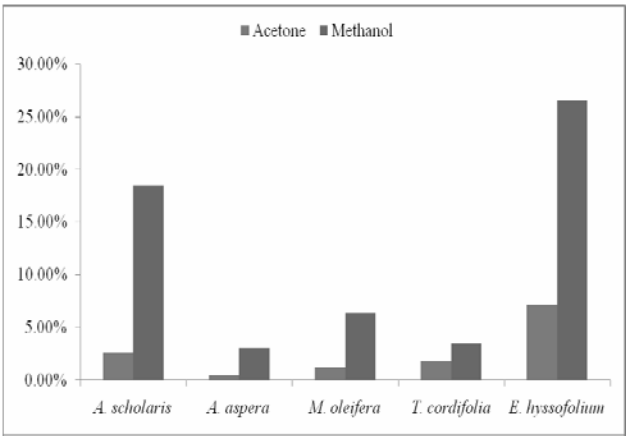

Fig. 1 Extractive value of plants.

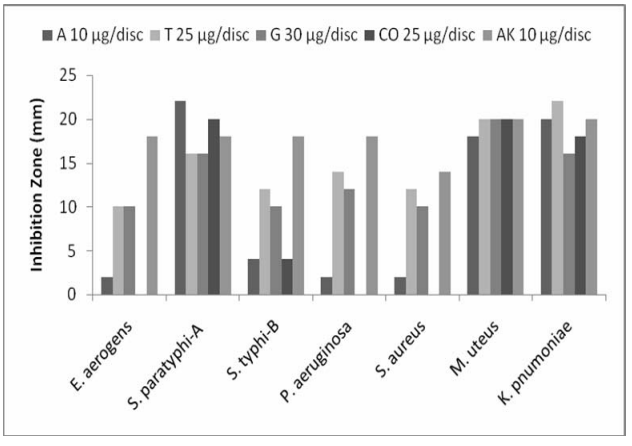

Fig. 2 Antibiotic susceptibility of bacterial strains to antibiotics

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

Table - 1 Phytochemical screening of Methanol extract.

Phytoconstituents	**Authentic powdered samples of Medicinal plants presently analyzed**									
	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.

M1: Crude extract of methanol fraction

M2: Methanol fraction after treatment with 50 % lead acetate

Table - 2 Phytochemical screening of Acetone extract.

Phytoconstituents	**Authentic powdered samples of Medicinal plants presently analyzed**									
	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.

A1: Crude extracts of Acetone fraction

A2: Acetone fraction after treatment with 50 % lead acetate

Table - 3 Antimicrobial activity of Methanol fraction at 40 mg/ml (inhibition zone in mm)

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	-

M1:-Methanol

M2:- Methanol fraction after treatment of 50 % lead acetate,

- : no inhibition zone

Table - 4 Antimicrobial activity of acetone fraction at 40 mg/ml (inhibition zone in mm)

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	-	-

A1:-Acetone,

A2:- Acetone fraction after treatment with 50 % lead acetate,

- : no inhibition zone

A1 extracts were found more active than M1 extract, which indicates that active component of plants extracted more may get extracted in very low concentration in methanol, as solubility of compound depends upon the 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) was found in the 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 in accordance with the findings of Jigna et al. [22]. Acetone extract of *A. scholaris* was more active than that of methanol extract, results thus indicate active components in *A. scholaris* were more soluble in relatively non-polar solvent, as reported by Khan et al. [23], that butanol fraction has broad spectrum of antibacterial activity. Goyal et al. [24] reported that alkaloids, sterols alkenes, are key antimicrobial agents in *A. scholaris* and their results matching with our findings. *M. oleifera* was most active plant among the plants selected for the present study. Activity of plant is attributed to the presence of saponins, tannins, alkaloids and phenols [25]. Acetone extracts of *M. oleifera* only show the antimicrobial activity against the ST and STA. Similar results were observed by Doughari et al. [26] for *Salmonella typhi*. Both bacteria are causative agents of the Typhoid fever and recent years 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 the presence of flavanoids as a major constituent in *E. hyssofolium* [30]. Singh et al. [31] reported the presence of terpenoids and glycosides in *T. cordifolia*. These components are the responsible agents for the antibacterial activity of these plants [32].

M2 and A2 extracts of all plants were more active than that of their crude extract (M1, A1), phytochemical screening revealed that carbohydrates and proteins 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 acids, proteins 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 in methanol solvents.

Antibacterial alternative for selected bacterial strains ware always in focus 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 earlier studies indicated the usefulness of plant for control of resistant strains of bacterial like *S. aureus* [35] and *P. aeruginosa* [36, 37 & 38]. Among the plants selected for the present study the acetone extract of *A. scholaris* and *M. oleifera* can be used for the new antibacterial agent, for EA, SA and ML.

REFERENCES

[1] Olalde Rangel, J.A. (2005) The systemic theory of living systems and relevance to CAM. Part I: The theory. *Evid Based Complement Alternat Med,* **2:** 13–18.

[2] Cragg, G.M., Boyd, M.R., Khanna, R., Kneller, R., Mays, T.D., Mazan, K.D., Newman, D.J. and Sausville, E.A. (1999) International collaboration in drug discovery and development: the NCI experience. *Pure Appl Chem.* **71:** 1619-1633.

[3] Olila, D., Olwa-Odyek and Opuda-Asibo, J. (2001) Antibacterial and antifungal activities of extracts of *Zanthoxylum chalybeum* and *Warburgia ugandensis,*

Ugandan medicinal plants, *African Health Sciences*, **1(2):**66-72.

[4] Fauci, A. (1998) New and reemerging diseases: The importance of biomedical research. Emerging Infectious Diseases. (www.cdc.gov/ncidod/EID/vol4no3/fauci). 4: 3.

[5] Robert, A. and Meunier, B. (1998) Is Alkylation the Main Mechanism of Action of the Antimalarial Drug Artemisinin ? *Chem. Soc. Rev*., **27:** 273-279.

[6] Setti, E.L. and Micetich, R.G. (1998) New Trends in Antimicrobial Development. *Current Medicinal Chemistry*, **5:**101-113.

[7] WHO (2002) Geneva, *Quality Control Method for Medicinal Plant Materials*, New Delhi, **51:**30.

[8] Harbone, J.B. (1984) *Phytochemical Methods – A Guide to Modern Techniques of Plant Analysis* 2nd Edition. (Chapman and Hall London. New York).

[9] Perez, C., Paulin Mand and Bazerque, P. (1990) An antibiotic assay by the well agar method. *Acta. Biological Medicine Experitimentalis,* **15:**113-115.

[10] Ibrahim, M.B., Owonubi, M.O. and Onaolapo J.A. (1997) Antimicrobial effects of extracts of leaf, stem and root-bark of *Anogiessus leicarpus* on*Staphylococcus aureus* NCTC 8190, *Escherichia coli* NCTC 10418and *Proteus vulgaris* NCTC 4636*. J. Pharmaceutical Res. Dev*. **2:**20-26.

[11] Okeke, M.I., Iroegbu, C.U., Eze, E.N., Okoli, A.S. and Esimone, C.O. (2001) Evaluation of extracts of the root of *Landolphia owerrience* for antibacterial activity. *J. Ethnopharmacol*. **78:** 119-127.

[12] Okoli, A.S., Okeke, M.I., Iroegbu, C.U. and Ebo, P.U. (2002) Antibacterial activityof *Harungana madagascariensis* leaf extracts. *Phytother. Res.* **16:**174-179.

[13] Scalbert, A. (1991) Antimicrobial properties of tannins. *Phytochemistry* **30 :** 3875-3883.

[14] Aboaba, O.O., Smith, S.I. and Olude, F. O. (2006) Antibacterial effect of edible plant extract on *Escherichia coli* 0157:H7, *Pakistan Journal of Nutrition***, 5 (4) :** 325-327.

[15] Aboaba, O. O. and Efuwape, B.M.(2001) Antibacterial properties of some Nigerian spices. *Bio. Res. Comm*., **13:** 183 - 188.

[16] Hostettman, K and Nakanishi, K. (1979) Moronic acid, a simple triterpenoid keto acid with antimicrobial activity isolated from *Ozoroa mucroanta. J. Med. Plant Res.* **31:** 358-366.

[17] Leven, M., VandenBerghe, D.A., Mertens, F., Vlictinck, A and Lammens, E. (1979) Screening of higher plants for biological activities/- antimicrobial activity. *Plant. Med.*, **36:** 311-321.

[18] Damintoti Karou1, Aly Savadogo, Antonella Canini, Saydou Yameogo, Carla Montesano, Jacques Simpore, Vittorio Colizzi and Alfred S. Traore. (2005) Antibacterial activity of alkaloids from *Sida acuta*. *African Journal of Biotechnology*. **4 (12):** 1452-1457.

[19] Eloff, J.N. (1998) Which extractan should be used for screening and isolation of antimicrobial components from plants. *J. Ethanopharmacol.*, **60:** 1-8.

[20] Cowan, M. M. (1999) Plant product as antimicrobial agents, *Clin.Microbiol.Rev*. 12: 564-582.

[21] Nikaido, H and Vaara M. (1985) Molecular basis of bacterial outer membrane permeability, Microbiological Reviews, **1:**1-32.

[22] Jigna, P., Rathish, N and Sumita, C. (2005) Priliminary screening of some folklore medicinal plants from western India for potential antimicrobial activity, *Inian J. Pharmacol*., **37(6):** 408-409.

[23] Khan, M.R., Omoloso, A.D and Kihara, M. (2003) Antibacterial activity of *Alstonia scholaris* and Leea tetramera. *Fitoterpia* **74(7-8):** 736-40.

[24] Goyal, M.M. and Varshney, A. (1995) Effects of natural products isolated from three species of *Alstonia* on some gram-positive and gram-negative bacteria. *Indian Drugs* **32(2):** 69-72.

[25] Clark, W.S. (1981) Antimicrobial activities of phenolic constituents of *Magnolia grandiflora* L. *J. Pharm. Sci.* **70:** 951-952.

[26] Doughari, J.H., Pukuma, M.S. and De N. (2007) Antibacterial effects of *Balanites aegyptiaca* L. Drel. and *Moringa oleifera* Lam. on *Salmonella typhi, African Journal of Biotechnology* , **6 (19):** 2212-2215.

[27] Chin, N.T., Perry, C.M., Ly, N.T., Ha, H.D., Thong, M and Diep, T.S. (2002) Randomized controlled comparison of azithromycin and ofloxacin for treatment of multidrug resistant or nalidixic acid resistant enteric fever. *Antimicrob. Agents Chemother*. **44:** 1855-1859.

[28] Benoit, D., Renand, L., Daniele, M., Ame, B., David, B., Michael, R.M., Elisabeth, C and Anel, C. (2003) Variant Salmonella genomic island 1 antibitotic gene resistance cluster in *Salmonella enterica* Albany. Emerg. *Infect. Dis.* **9(5):**585-591.

[29] Abdullah, W.B., Anowa, H., Doli, G., Amina, T.S., Kamrun, N., Korshed, A., Neor, A., Aliya, N., Balakrish, N., Stephen, L and Robert B. (2005) Bacteremic typhoid fever in children in an urban slum, Bangladesh. Emerg. *Infect. Dis.* **11(2):** 326-329.

[30] Ghosal, S and Jaiswal D.K. (1980) Chemical constituents of Gentianaceae XXVIII:Flavonoids of *Enicostema hyssopifolium* (Willd.) Verd. *J. Pharm Sci.* **69(1):** 53-56.

GUIDELINES FOR CONTRIBUTORS

The Editorial Board of 'PRAJNA' – Journal of Pure and Applied Sciences invites Original Research Papers in the fields of Basic and Applied Sciences (Biosciences, Chemistry, Computer Science, Electronics Science, Home Science, Materials Science, Mathematics, Physics and Statistics) for the Next Volume of PRAJNA (December 2011), *published by Sardar Patel University, Vallabh Vidyanagar, Gujarat – 388120, INDIA.*

The soft copies of regular (full-length) research papers (not exceeding 15 typed pages), prepared as per the file format shown below may be submitted for publication through e-mail to Prof. T. V. Ramana Rao, Managing Editor (spu.prajna@gmail.com) OR to a Member of the Editorial Board who represents the author's broad research area with a cc to the Managing Editor latest by August 31, 2011.

Each manuscript must be accompanied by a statement that it has not been published elsewhere and that it has not been submitted simultaneously for publication elsewhere.

Review process: Submitted papers are peer-reviewed by two to three independent reviewers after approval by the Editorial Board. Authors are encouraged to suggest three names of expert reviewers with their e-mail IDs, but selection remains the prerogative of the Editorial Board.

Articles of the following categories are also considered for publication in PRAJNA:

Short Communications are limited to a maximum of two figures and one table. They should present a complete study that is more limited in scope than is found in full-length papers. The items of manuscript preparation listed above apply to Short Communications with the following differences: (1) Abstracts are limited to 100 words; (2) instead of a separate Materials and Methods section, experimental procedures may be incorporated into Figure Legends and Table footnotes; (3) Results and Discussion should be combined into a single section.

Review Articles intended to provide concise in-depth reviews of both established and new areas and summarize recent insights in specific research areas within the scope of PRAJNA are solicited by the Editorial Board from leading researchers. The manuscript of this category should be limited to 5,000 words with an abstract of no more than 250 words, a maximum of 5 tables and figures (total), and up to 50 references. Word count includes only the main body of text (i.e., not tables, figures, abstracts or references).

Commentaries call attention to papers of particular note and are written at the invitation of the Editorial Board.

Perspectives present a viewpoint on an important area of research and are written only at the invitation of the Editorial Board. Perspectives focus on a specific field or subfield within a larger discipline and discuss current advances and future directions. Perspectives are of broad interest for non-specialists and may add personal insight to a field.

Letters are brief comments that contribute to the discussion of a research article published in the last issue of PRAJNA. Letters may not include requests to cite the letter writer's work, accusations of misconduct, or personal comments to an author. Letters are limited to 500 words and no more than five references. Letters must be submitted within 3 months of the publication date of the subject article.

Also announcement of forthcoming Seminars / Conferences / Symposia / Workshops etc. will be considered for publication in PRAJNA.

File format for soft copies:
Texts (should be of Times New Roman with 9 point for Abstract and 11 point for other matter) and Tables, if any, must be saved in *.doc (Word) or *.rtf (rich text) format, graphs in Excel and for illustrations (diagrams, maps, drawings, etc.), the TIF format (300 dpi minimal resolution) is the most appropriate (*.TIF or *.JPEG extension).

Instructions for preparation of manuscripts:

1. The paper should be written in English and neatly typed with double spacing.
2. The title of the paper and the name(s) of the author(s) be in capital letters. The name of the institution be given in small letters below the name (s) of the author(s).
3. The 'Abstract of the paper, in not more than 150 words, should be provided on a separate page along with 4-6 keywords.
4. The sub-titles, e.g. INTRODUCTION, should be written in capital letters.

5. Displayed formulae, mathematical equations and expressions should be numbered serially. Table should be with a title in addition to a serial number for it.
6. Photographs / Figures should be original with good contrast so as to be in a form suitable for direct reproduction / scanning.
7. Footnotes are not normally allowed, except to identify the author for correspondence.
8. All figures must be numbered serially as they appear in the text, and their legends / captions should necessarily be provided.
9. References should be numbered in brackets [ ] in the order of appearance in the text. All the references in the bibliographic list must correspond to in-text references and vice versa. Abbreviated periodical titles should follow standard subject Abstracts. Names which are not listed by any standard subject indexing organizations should be spelled out in full.
10. All references should be clear and follow the examples below:

Periodical articles

[2] Sadqui, M., Fushman, D. and Munoz, V. (2006) Atom – by – atom analysis of global downhill protein folding. *Nature*, **442:** 317 – 321.

Books

[16] Stebbins, G. L. (1974) *Flowering plants: Evolution above the species level*, Arnold Press, London, pp. 1 – 399.

Chapters from a book

[19] Schafer, H. and Muyzer, G. (2001) Denaturing gradient gel electrophoresis in marine microbial ecology. In *Methods in Microbiology* (Ed. Paul, J. H.), Academic Press, London, Vol. 30, pp. 425 – 468.

Thesis or other diplomas

[21] Nayaka, S. (2004) *The visionary studies on the lichen genus Lecanora sensu lato in India.* Ph. D. Thesis, Dr. R. M. L. Avadh University, Faizabad, India.

Conference proceedings

[4] Mohapatra, G. C. (1981) Environment and culture of early man in the valley of rivers Chenab and Ravi, western sub-Himalayas. In *Proceedings X Congress of IUPPS*, Mexico, pp. 90 – 123.

Online documentation

[9] Koning, R. E. (1994). Home Page for Ross Koning. Retrieved 26-6-2009 from *Plant Physiology Information Website*: http://plantphys.info/index.html.

Note:

Manuscripts prepared faithfully in accordance with the instructions will accelerate their processing towards publication; otherwise it would be delayed in view of their expected re-submission.

For and on behalf of Editorial Board, PRAJNA

Prof. T. V. Ramana Rao
Managing Editor, PRAJNA
B R Doshi School of Biosciences,
Satellite Campus, Vadtal Road,
Sardar Patel University,
VALLABH VIDYANAGAR
Gujarat – 388120
Phone: (Lab): 02692-234412 Extn. 111
Mobile: 98254 38147
Fax: 02692-237258 /236475
e-mail: spu.prajna@gmail.com
Website:www.spuvvn.edu

NOTE: This information may be kindly circulated among your colleagues.