Research Article **Phytochemistry**

International Journal of Pharma and Bio Sciences

ISSN 0975-6299

STUDIES ON PHYTOCHEMICAL CONSTITUENTS, ANTIMICROBIAL AND ANTIOXIDANT ACTIVITIES OF SOME MEDICINAL PLANTS OF NORTH COASTAL ANDHRA PRADESH

MAHESH KUMAR MVS*, VSSL PRASAD TALLURI AND S V RAJAGOPAL

Department of Biotechnology, GITAM Institute of Science, GITAM University, Visakhapatnam -530045, India.

ABSTRACT

The phytochemical, antimicrobial activity against gram negative and positive bacteria and fungi, DPPH radical scavenging and FRAP assay antioxidant activities of aqueous and organic crude extracts of *Millingtonia hortensis, Andrographis paniculata, Aegle marmelos, Coccinia grandis, Tinospora cordifolia, Achyranthus aspera* was studied *invitro*. The phytochemical screening of crude plant extracts revealed the presence of bioactive compounds such as phenols, flavanoids, alkaloids, terpenoids, glycosides, phytosterols and proteins. All the extracts exhibited antibacterial and antioxidant activities and the activities varied from solvent to solvent. The results showed that methanlolic extracts exhibited significant antimicrobial and antioxidant activities when compared to other organic solvent and aqueous extracts. Further, the crude methanolic extract of *Millingtonia hortensis* exhibited significant activities when compared to the crude methanolic extracts of other plants.

KEYWORDS: Medicinal plant extracts, Antimicrobial activity, Antioxidant activity, Phytochemical constituents.

MAHESH KUMAR MVS
Department of Biotechnology, GITAM Institute of Science,
GITAM University, Visakhapatnam -530045, India.

***Corresponding author**

INTRODUCTION

Plants and plant products can be used as medicines from the beginning of human civilization. India is a rich source of herbal medicine[1,2]. In recent days, maximum numbers of plants are screened for their potential pharmacological value. The medicinal plants contain novel chemical substances that show distinct physiologic action on pathogenic microorganisms. The most important of these plant phytochemicals are flavonoids, alkaloids, tannins, terpenoids, glycosides, phytosterols and phenolic compounds. These compounds are responsible for multiple biological effects including antimicrobial, antioxidant, antiproliferative, anti-inflammatory and immunomodulatory activities[3]. The antioxidant compounds of higher plants protect the living organisms by scavenging the free radicals and prevent the damage caused by them[4,5]. As there is increasing occurrence of multi drug resistant pathogenic bacterial strains and emergence of new strains with less susceptibility to antibiotics, there is a need to search for new drugs and infection-fighting strategies[6]. The antimicrobial compounds produced by plants are active against plant and human pathogenic organisms[7]. There are many reports in the literature with reference to the antimicrobial activity of plant crude extracts and the bioassay guided fractionation to isolate active principles[8].

MATERIALS & METHODS

All the chemicals used were of analytical grade and purchased from Himedia and Merck, Mumbai, India.

Collection of plant materials

Fresh leaves of six medicinal plants namely *Millingtonia hortensis, Andrographis paniculata, Aegle marmelos, Coccinia grandis, Tinospora cordifolia, Achyranthus aspera* were collected in sterile bags and carried to the laboratory. These plants were abundantly found in Seethampeta Village (Latitude-18°69□N, Longitude-83°8□E), Srikakulam District, North Coastal region of Andhra Pradesh and authenticated by Prof. M.Venkaiah and Dr. S.B.Padal, Department of Botany, Andhra University, Visakhapatnam. Specimens of the same were deposited in Botany Department Herbarium and voucher numbers were as follows *Millingtonia hortensis* (BDH-22079) *Andrographis paniculata* (BDH-22087) *Aegle marmelos* (BDH-22090) *Coccinia grandis* (BDH-22089) *Tinospora cordifolia* (BDH-22091) and *Achyranthus aspera* (BDH-22088).

Preparation of plant material

The fresh leaves were washed with tap water and then thoroughly cleaned with distilled water and shade dried for a week. Then the dried leaves were grinded to a fine powder by using mortar and pestle.

Extraction of crude bioactive compounds

The extraction of crude bioactive compounds from finely powdered leaves was performed by using Soxhlet extraction method [9,10]. The powders were extracted with Soxhlet extractor using hexane, chloroform, methanol and water separately for 48 hours nearer to the solvents boiling points. The extracts were then concentrated to dryness using rotary evaporator (Superfit PBV-6).

Test microorganisms used

The test microorganisms used for antibiotic assays were *Staphylococcus aureus* (MTCC 96), *Escherichia coli* (MTCC 727), *Streptomyces griseus* (MTCC 3474), *Proteus vulgaris* (MTCC 744), *Salmonella typhi* (MTCC 734), *Klebsiella pneumonia* (MTCC 9544), *Candida albicans* (MTCC 3958) *and Aspargillus flavus* (MTCC 893). These bacterial and fungal strains were obtained from Microbial Type Culture Collection and Gene Bank (MTCC), Chandigarh. The bacterial cultures were maintained on LB nutrient agar slants and fungal cultures on Potato dextrose agar (PDA).

Screening for Antibacterial Activity

The antibacterial activity was carried out by using 24h cultures of bacterial strains. Activity of all the plant extracts was tested separately using agar well diffusion method.[11-13]. The medium was sterilized by autoclaving at 120°C. About 30 mL of the agar medium mixed with 0.02mL of respective bacterial strains and transferred aseptically into each sterilized petri plate. The plates were left at room temperature for solidification. Wells of 6 mm diameter was made using a sterile cork borer. 50µL (1mg/mL) of each plant extract was loaded in each well and plates were incubated at 37 °C for 24 hours. The standard 6 mm discs of Streptomycin & Chloramphenicol (10µg) were used as positive controls and pure solvents and distilled water as negative controls. The diameter of the zone of inhibition was measured with antibiotic zone scale in mm and the experiment was carried out in triplicates.

Screening for Antifungal Activity

The antifungal activity was carried out by agar well diffusion method[11-13]. The cultures of 48h old grown on potato dextrose agar (PDA) were used for inoculation of fungal strain on PDA plates. An aliquot (0.02mL) of inoculum was introduced into PDA medium in sterile condition and was poured into petri dishes by pour plate technique. After solidification, 6 mm wells were made on agar plate by using sterile cork borer. 50µL (1mg/mL) of each plant extract was loaded in wells and incubated at 28°C for 48 hours. The antifungal activity was evaluated by measuring zones of inhibition. The standard 6 mm discs of Fluconazole & Nystatin (10µg) were used as positive controls for antifungal activity and pure solvents and distilled water as negative controls. The diameter of the zone of inhibition was measured with antibiotic zone scale in mm and the experiment was carried out in triplicates.

Determination of Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration was determined using agar well diffusion assay[11]. The methanolic extracts were loaded into the wells at different concentrations. The lowest concentration of extract that inhibited the growth of microorganism was considered as MIC.

Screening for Antioxidant activity

a. Determination of free radical scavenging using DPPH method

DPPH scavenging activity was measured by the method of Cuendet *et al*[14-17]. To 1.0 mL of each plant extract 3.0 mL of methanolic solution of DPPH (0.1mM) was added. For control, the plant extract was replaced by absolute methanol. The reaction mixture was incubated for 30 min at 37^{0}C and absorbance was measured at 517nm using UV-Visible spectrophotometer. The samples were prepared in triplicate for each analysis. The percentage of inhibition was calculated from the following equation: A_0-A x100/A_0, where A_0 and A are the absorbance of control and test sample, respectively. BHT and Rutin were used as standards.

b. Determination of Ferric Reducing Antioxidant Power assay (FRAP)

FRAP assay was carried out according to the method of Benzie and Strain (1999)[18]. FRAP reagent was prepared with acetate buffer (0.1M, pH 3.6), 10 mM TPTZ solution in 40 mM HCl and 20 mM ferric chloride solution in proportion of 10:1:1 (v/v) respectively. The FRAP reagent was prepared fresh daily and was warmed to 37°C in oven prior to use. 100 µL of plant extract was mixed with 3 mL of working FRAP reagent and absorbance (593 nm) was measured at 0 minute after vortexing. Thereafter, samples were placed at 37°C in water bath and absorbance was again measured after 4 minutes. Ascorbic acid was taken as standard and proceeded in the same way. The samples were prepared in triplicate for each analysis. FRAP value of Sample (µM) can be calculated using the equation: (Change in absorbance of sample from 0 to 4 minute / Change in absorbance of standard from 0 to 4 minute) X FRAP value of standard.

c. Determination of total phenolic (TP) content

The concentration of total phenolics in plant extracts was determined using spectrophotometric method (Singleton *et al*.,

1999)[19]. The reaction mixture was prepared by mixing 0.5 mL of methanolic solution of each plant extract, 2.5 mL of 10% Folin-Ciocalteu's reagent dissolved in water and 2.5 mL 7.5% $NaHCO_3$. Blank was simultaneously prepared, containing 0.5 mL methanol, 2.5 mL 10% Folin-Ciocalteu's reagent dissolved in water and 2.5 mL of 7.5% of $NaHCO_3$. The samples were incubated at 45^0C for 45 min. The absorbance was determined using spectrophotometer at 765 nm. The samples were prepared in triplicate for each analysis and the mean value of absorbance was obtained. The same procedure was repeated for the standard solution of gallic acid using 0, 50,100,150,200,250 mg/L in methanol: water (50:50 v/v) and the calibration curve was constructed. Based on the measured absorbance, the concentration of total phenolics was read (mg/mL) from the calibration curve; then the content of phenolics in each plant extract was expressed in terms of gallic acid equivalent (mg of GA/g of extract).

Phytochemical analysis of different crude extracts

The plant extracts were tested for the presence of active principles such as phenolics, flavonoids, phytosterols, terpenoids, glycosides, alkaloids and proteins. Following standard procedures were used[20-22].

Test for Phenolics: The presence of phenolics was tested using ferric chloride test (Mace, 1963). To the plant extract few drops of neutral 5% ferric chloride solution was added. A dark green colour indicates the presence of phenolics.

Test for Flavonoids: The presence of flavonoids in the plant extracts was performed using alkaline reagent test. The plant extract when treated with sodium hydroxide solution, shows increase in the intensity of yellow color which would become colorless on addition of few drops of dilute HCl, indicates the presence of flavonoids.

Test for Alkaloids: The presence of alkaloids was tested using Hager's Test (Wagner *et al* 1996). The plant extract was treated with few drops of saturated picric acid solution. Formation of yellow precipitate indicates the presence of alkaloids.

Test for Phytosterols and Terpenoids: The presence of phytosterols and terpenoids in the plant extract was performed using Liebermann Burchard test (Finar, 1986). The crude extract was mixed with few drops of acetic anhydride, boiled and cooled. Conc. H_2SO_4 was then added from the sides of the test tube and observed for the formation of a brown ring at the junction of two layers. Green coloration of the upper layer and the formation of deep red color in the lower layer indicates the positive test for steroids and terpenoids respectively.

Test for Glycosides: The presence of glycosides was tested using Legal's Test (Evans, 1997). The plant extract was dissolved in pyridine, sodium nitroprusside solution was added and made alkaline using 10% NaoH. Appearance of pink colour indicates the presence of glycosides.

Test for Proteins: The presence of proteins was tested by Biuret method (Gohan *et al*). the plant extract was treated with 10% sodium hydroxide solution and two drops of 0.1% copper sulphate solution and observed for the formation of violet/pink color.

Table 1
***Antibacterial activity of some medicinal plants crude extracts against gram negative bacteria tested by agar well* diffusion assay**

	Diameter of zone of inhibition (mm)															
Plant Species	***E.coli***				***K.pneumonia***				***P.vulgaris***				***S.typhi***			
	M	**C**	**H**	**W**	**M**	**C**	**H**	**W**	**M**	**C**	**H**	**W**	**M**	**C**	**H**	**W**
Millingtonia hortensis	20.67 ± 0.47	11.67 ± 0.66	ND	10.67 ± 0.33	17.33± 0.33	11.33 ± 0.47	11.67 ±0.66	11.33± 0.47	16.67± 0.47	11.33 ± 0.66	11.33 ±0.33	10.67± 0.66	12.67 ±0.47	11.33 ± 0.66	ND	10.33 ±0.33
Andrographis paniculata	14.33 ±0.47	11.67 ± 0.33	12.33 ±0.66	ND	13.67± 0.66	ND	10.67 ±0.33	ND	12.67± 0.66	11.33 ± 0.33	11.33 ±0.47	9.33± 0.47	11.67 ±0.47	ND	ND	ND
Aegle marmelos	11.67 ±0.33	ND	10.67 ±0.47	ND	10.67± 0.47	ND	ND	10.67± 0.33	10.67± 0.33	ND	9.67 ±0.33	ND	ND	ND	ND	ND
Coccinia grandis	11.67 ±0.47	ND	10.67 ±0.47	ND	10.33± 0.33	ND	ND	ND	11.67± 0.33	10.67 ± 0.47	ND	ND	ND	ND	ND	ND
Tinospora cordifolia	16.67 ±0.47	11.67 ± 0.47	ND	ND	11.67± 0.66	ND	ND	9.67± 0.33	10.33± 0.47	ND	9.33 ±0.33	9.67± 0.47	ND	ND	ND	ND
Achyranthus aspera	11.67 ±0.47	ND	10.67 ±0.33	ND	10.67± 0.33	ND	11.33 ±0.47	ND	11.33± 0.33	ND	ND	ND	10.33 ±0.66	ND	ND	ND

M-Methanol, C-Chloroform, H-Hexane, W-Water, ND-Not Determined
Values are mean inhibition zone (mm) ± S.D of three replicates

Table 2
Antibacterial activity of some medicinal plants crude extracts against gram positive bacteria tested by agar well diffusion assay

	Diameter of zone of inhibition (mm)							
Plant Species	***S.aureus***				***S.griseus***			
	M	**C**	**H**	**W**	**M**	**C**	**H**	**W**
Millingtonia hortensis	17.67± 0.66	14.3± 0.47	10.6± 0.33	10.6± 0.66	15.6± 0.47	10.33± 0.33	10.67± 0.66	10.6± 0.33
Andrographis paniculata	12.67± 0.47	10.6± 0.33	10.6± 0.33	ND	12.6± 0.66	ND	10.33± 0.33	10.6± 0.47
Aegle marmelos	11.67± 0.33	ND	ND	ND	12.6± 0.47	ND	9.67± 0.47	ND
Coccinia grandis	11.67± 0.33	ND	11.6± 0.47	ND	10.6± 0.33	ND	10.67± 0.33	ND
Tinospora cordifolia	10.67± 0.2	ND	11.6± 0.33	9.00± 0.00	10.6± 0.33	ND	9.67± 0.33	9.67± 0.47
Achyranthus aspera	11.67± 0.47	ND	ND	ND	ND	ND	ND	ND

M-Methanol, C-Chloroform, H-Hexane, W-Water, ND-Not Determined
Values are mean inhibition zone (mm) ± S.D of three replicates

Table 3
Antifungal activity of some medicinal plants crude extracts against Fungal species tested by agar well diffusion assay

	Diameter of zone of inhibition (mm)							
Plant Species	***C.albicans***				***A.flavus***			
	M	**C**	**H**	**W**	**M**	**C**	**H**	**W**
Millingtonia hortensis	17.67± 0.47	15.3± 0.47	11.6± 0.33	10.3± 0.66	14.3± 0.47	12.67± 0.66	11.67± 0.47	10.6± 0.47
Andrographis paniculata	13.33± 0.47	12.6± 0.47	11.3± 0.33	ND	11.3± 0.47	11.67± 0.47	9.33± 0.33	10.3± 0.33
Aegle marmelos	12.33± 0.33	10.6± 0.33	ND	ND	9.33± 0.33	9.33± 0.33	10.33± 0.47	ND
Coccinia grandis	12.67± 0.66	9.67± 0.33	10.3± 0.47	ND	9.33± 0.33	9.67± 0.33	ND	ND
Tinospora cordifolia	11.67± 0.33	ND	ND	ND	10.3± 0.66	ND	ND	ND
Achyranthus aspera	12.33± 0.47	ND	ND	ND	9.67± 0.47	ND	ND	ND

M-Methanol, C-Chloroform, H-Hexane, W-Water, ND-Not Determined
Values are mean inhibition zone (mm) ± S.D of three replicates

Table 4
Diameter of zone of inhibition showed by positive controls against bacterial and fungal strains

	Diameter of zone of inhibition (mm)			
Microorganisms	**Streptomycin**	**Chloramphenicol**	**Fuconazole**	**Nystatin**
Bacteria:				
E.coli	16	15	--	--
K.pneumonia	22	21	--	--
P.vulgaris	16	16	--	--
S.typhi	14	19	--	--
S.aureus	09	15	--	--
S.griseus	20	15	--	--
Fungi:				
C.albicans	--	--	18	15
A.flavus	--	--	14	17

Table 5
Minimum Inhibitory Concentration(MIC) of methanolic plant extracts on tested bacterial and fungal strains

Plant	MIC (µg/mL)							
Species	*E.coli*	*K.pneumonia*	*P.vulgaris*	*S.typhi*	*S.aureus*	*S.griseus*	*C.albicans*	*A.flavus*
Millingtonia hortensis	62.5	125	250	500	125	250	125	125
Andrographis paniculata	125	125	250	500	500	500	125	250
Aegle marmelos	250	500	500	_ _	250	250	125	500
Coccinia grandis	250	500	500	_ _	250	250	125	500
Tinospora cordifolia	125	250	250	_ _	500	500	250	500
Achyranthus aspera	250	500	250	500	250	_ _	125	500

'_ _' Not Determined

Table 6
Different medicinal plants crude extracts showing antioxidant activity using DPPH activity, FRAP assay and Total phenolic content

Plant Species	DPPH radical scavenging activity				Determination of Ferric Reducing Antioxidant Power assay (FRAP)				Estimation of Total Phenolic [TP] Content			
	M	C	H	W	M	C	H	W	M	C	H	W
Millingtonia hortensis	66.35± 0.13	45.52± 0.10	29.14± 0.12	19.76± 0.03	6.265± 0.023	3.968± 0.042	2.593± 0.028	1.352± 0.035	242.50± 2.04	37.50± 2.04	35.83± 1.18	76.67± 1.18
Andrographis paniculata	41.20± 0.72	11.19± 0.23	20.74± 0.11	18.63± 0.02	4.949± 0.016	3.859± 0.007	1.125± 0.040	0.425± 0.024	192.50± 2.04	35.83± 1.18	28.33± 1.18	69.17± 1.18
Aegle marmelos	42.73± 0.43	13.84± 0.37	3.65± 0.16	4.76± 0.01	5.212± 0.008	2.161± 0.026	0.814± 0.033	0.328± 0.026	117.5± 2.04	23.33± 1.18	15.83± 1.18	43.33± 1.18
Coccinia grandis	56.51± 0.18	41.32± 0.14	23.33± 0.31	19.03± 0.12	3.852± 0.012	1.862± 0.036	1.264± 0.026	1.425± 0.036	175.83± 1.18	18.33± 1.18	20.83± 1.18	45.83± 1.18
Tinospora cordifolia	57.47± 0.07	43.21± 0.07	27.23± 0.08	17.39± 0.10	4.298± 0.024	1.361± 0.032	0.967± 0.016	0.726± 0.034	175.83± 1.83	20± 0.00	20.83± 1.18	50.83± 1.18
Achyranthus aspera	39.83± 0.15	25.03± 0.09	4.24± 0.21	3.91± 0.31	5.018± 0.016	3.451± 0.025	1.276± 0.033	0.508± 0.035	77.50± 2.04	24.17± 1.18	13.33± 1.18	28.33± 1.18

M-Methanol, C-Chloroform, H-Hexane, W-Water
Values are mean ± S.D of three replicates

Table 7
Screening of some medicinal plants crude extracts for phytochemical constituents

Phytochemical constituent	Plant Species																							
	Millingtonia hortensis				*Andrographis paniculata*				*Aegle marmelos*				*Coccinia grandis*				*Tinospora cordifolia*				*Achyranthus aspera*			
	M	C	H	W	M	C	H	W	M	C	H	W	M	C	H	W	M	C	H	W	M	C	H	W
Total phenols	+	+	+	+	+	+	-	+	+	+	-	-	+	-	-	-	+	+	-	-	+	+	-	-
Flavonoids	+	+	+	+	+	+	-	+	+	+	-	-	-	-	-	-	+	+	-	-	+	+	-	-
Alkaloids	+	+	-	-	-	-	-	+	+	+	-	+	+	-	-	-	+	+	-	-	+	+	-	-
Terpenoids	+	+	-	+	+	+	-	+	+	-	-	-	+	-	-	-	+	+	-	-	+	-	-	-
Phytosterols	+	-	-	-	-	+	-	-	+	+	-	-	+	+	-	+	+	+	-	+	-	-	+	+
Glycosides	+	+	+	+	+	-	+	-	+	+	-	-	+	+	-	+	+	+	-	+	-	+	-	+
Proteins	+	+	-	+	+	+	+	+	-	+	-	-	-	+	-	+	+	+	-	+	-	-	+	+

M-Methanol, C-Chloroform, H-Hexane, W-Water, "+" Positive Test & "-" Negative Test

RESULTS & DISCUSSION

The healing properties of medicinal plants are possibly due to the presence of various phytochemical constituents such as total phenolics, flavonoids, alkaloids, terpenoids and phytosterols etc. Phytochemical analysis of plant extracts revealed the presence of constituents which are known to show medicinal in addition to physiological activities[23]. The results of phytochemical screening of crude plant extracts were represented in Table-7. Amoung the various extracts, methanolic extracts of all the medicinal plants screened were known to be rich in the presence of phytochemicals such as total phenolics and flavonoids. Thus the preliminary screening analysis is helpful in the detection of bioactive compounds and lead to the discovery and development of novel drugs [24]. The antimicrobial activity of different extracts of some medicinal plants against gram negative and positive bacteria and fungi were presented in tables – 1, 2 & 3 respectively. In the present investigation, significant antibacterial and antifungal activities were observed for methanolic extracts of all the plants screened. The zone of inhibition ranged between 9 mm to 20.67±0.47mm. The maximum activity (20.67±0.47mm) was recorded from the methanolic extract of *Millingtonia hortensis* against gram negative pathogenic bacteria *E.coli. Millingtonia hortensis* was the only plant that showed significant antibacterial and antifungal activities against all the tested pathogens. This may be due to the potent bioactive components which are present in the methanolic plant extract. However some plant extracts were unable to exhibit antimicrobial activity against tested bacterial and fungal strains. The hexane extracts of all the plants tested unable to show antibacterial activity against *S.typhi*. The chloroform extracts of all the plants tested except *Millingtonia hortensis* were unable to exhibit significant antibacterial activity against *K.pneumonia, S.griseus* and *S.typhi.* There was no significant inhibitory effect from aqueous extracts against *E.coli, S.aureus, S.typhi, C.albicans and A.flavus* except for *Millingtonia hortensis.* The minimum inhibitory concentration (MIC) of methanolic plant crude extracts against the tested organisms was shown in Table-5.The MIC for the methanolic plant crude extracts ranged from 62.5 µg/mL to 500 µg/mL. The methanolic extract of *Millingtonia hortensis* exhibited least MIC against *E.coli* (62.5 µg/mL).

The antioxidant activities of various extracts of some medicinal plants were tested using DPPH radical scavenging assay and FRAP assay. Further, the total phenolic content was also determined. The results were presented in table-6. The radical scavenging activity and total antioxidant activity was recorded maximum for methanolic extracts when compared to other organic solvents and aqueous extracts especially for *Millingtonia hortensis*. Significant total phenolic content was determined from the methanolic extracts of *Millingtonia hortensis .* The maximum antimicrobial activity against tested pathogenic microorganisms and low MIC exhibited by the methanolic extract of *Millingtonia hortensis* showed that the bioactive compounds which are responsible for these activities can be used as alternative to synthetic antibiotics for the treatment of infections which are caused by these pathogenic organisms, as the majority of the microorganisms are developing resistance to the known antibiotics[25]. Further, the significant antioxidant activities recorded and high amount of total phenolic content present in the methanolic extract of *Millingtonia hortensis* are responsible for preventing the damage caused by the free radicals by scavenging them and protect the living organisms.

ACKNOWLEDGEMENT

The authors are grateful to management of GITAM University, Visakhapatnam and Maharajah's Post graduate College, Vizianagaram, India for providing facilities to carry out this work.

REFERENCES

1. Owolabi J, Omogbai EKI & Obasuyi O. Antifungal and antibacterial activities of ethanolic and aqueous extract of Kigelia african (Bignoniaceae) stem bark. *African Journal of Biotechnology* 2007; 6(14): 882-85.
2. Jain SK, *Dictionary of Indian Folk Medicines and ethobotany*. Deep Publication (New Delhi)1991.
3. Bhuvaneswari S and Manivannan S. Anti-diabetic and anti-inflammatory activity of *Caralluma adscendens var. Adscendens*; Int Journal of Pharma and Bio Sci, 2014; Vol-5 (1): P42-P49.
4. Ali BH, Blunden G, Tanira MO & Nemmar A. Some phytochemical, pharmacological and toxicological properties of ginger (*Zingiber officinale* Roscoe): a review of recent research. *Food Chem. Toxicol* 2008; 46: 409-20.
5. Ghosal S, Tripathi VK & Chauhan S. Active constituents of Emblica officinalis, Part I, The chemistry and antioxidant effects of two new hydrolysable tannins, emblicanin A and B, *Indian Journal of Chemistry* 1996: 35B: 941–48.
6. Sieradzki K, Robert RB, Haber SW & Tomasz A. The development of vancomycin resistance in a patient with methicillin-resistant Staphylococcus aureus infection, *N.Engl Med* 1999; 340(7): 517-23.
7. B.N.Devendra, N.Srinivas, VSSL Prasad Talluri and P Swarna latha. Antimicrobial activity of *moringa oleifera* lam., leaf extract, against selected bacterial and fungal strains. Int Journal of Pharma and Bio Sci 2011 ;Vol-2 (3); B13-B18.
8. Meretrk, Haticekati, Nurettinyayli, Zihnidemurbau. Antimicrobial properties of Silene multifida (Adams) Rohrb Plant extract. *Turk J. Biol.* 2006; 30: 17- 21.
9. Dung NX, Dinh T. *Extraction and Distillation of essential Oils, Processing, Analysis and Application of Essential Oils*, 1st Edition, Har Krishan Bhalla & Sons Book Company, 2005, 59.
10. Hazra P *et al*, Solvent extraction of *Artemisia annua* L. on pilot scale, *Research and Industry*, 1989; 36: 14-16.
11. Perez C, Paul M Bazerque P. Antibiotic assay by agar-well diffusion method. *Acta Biol Med Exp* 1990; 15: 113-15.
12. Murry PR, Baron EJ, Pfaller MA, Tenover FC & Yolken HR, *Manual of clinical Microbiology*, 6th edition (ASM Press, Washington, DC) 1995, 15-18.
13. Olurinola PF, *A Laboratory manual of Pharmaceutical Microbiology (*Idu,Abuja,Nigeria) 1996, 69-105.
14. Siva Kumar K & Raja Gopal SV. Radical scavenging activity of Green Algal Species, *Journal of Pharmacy Research* 2008; 4: 723-25.
15. Rajagopal SV, Rao AP, Raman BV, Rao MR, Sankar AU, Kumari AS, *et al*. Biochemical and antibacterial studies on green algae of Visakhapatnam coast, *Journal Of Pure And Applied Microbiology* 2008; 2: 573-78.
16. Raja Gopal SV, Raman BV, Bhaskar Reddy I, Siva Kumar K. Antioxidants and free radical scavenging activity of Brown Algae of Visakhapatnam Coast, *Asian Journal of Chemistry,* 2008; 20: 5293-300.
17. Cuendet M, Hostettmann K, Potterat O. Iridoid glucosides with free radical scavenging properties from Fragrea blumei, *Helv Chim Acta,* 1997; 80: 1144–151.
18. Benzie FF, Strain JJ. Ferric Reducing Antioxidant Power Assay, Direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration, *Methods in enzymology* 1999; 299: 15-23.
19. Singleton VL, Orthofer R, Lamuela-Raventos RM. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu

reagent, *Methods in enzymology* 1999; 299: 152-78.

20. Raman N. *Phytochemical Techniques* (New Indian Publishing Agencies, New Delhi) 2006; 19.
21. Harborne JB, *Phytochemical Methods* (Springer (India) Pvt.Ltd, New Delhi) 2005; 17.
22. Khandelwal KR, *Textbook of Practical Pharmacognosy* (Nirali publication, Pune) 7th edn. 2000: 149-89.
23. Sofowra A. *Medicinal Plants And traditional Medicine in Africa*, (Spectrum Books Ltd, Ibadan, Nigeria) 1993: 191-289.
24. Mallikharjuna PB, Rajanna LN, Seetharam YN, Sharanabasappa GK. Phytochemical Studies of Strychnos potatorum L.f - A Medicinal Plant, *E-Journal of Chemistry* 2009; 4(4): 510-18.
25. Singleton P, *Bacteria in Biology, Biotechnology and Medicine,* 4th Edn (John Wiley and Sons Ltd, New York) 1999.

This article can be downloaded from www.ijpbs.net