IJPSR (2011), Vol. 2, Issue 3 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 19 May, 2010; received in revised form 27 January, 2011; accepted 12 February, 2011

ANTIBACTERIAL ACTIVITY FROM BARK EXTRACTS OF *PTEROSPERMUM ACERIFOLIUM* (L.) WILLD.

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Keywords:

Similipal Biosphere Reserve,

Pterospermum acerifolium,

MIC,

Sterculiaceae

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ABSTRACT

Although there are many studies on antimicrobial activities of different plants, there is no report on antibacterial activity of *Pterospermum acerifolium* (Sterculiaceae). Therefore, a microbiological study was conducted to detect antibacterial activity of *P. acerifolium*. Successive ethanolic extract of bark of *P. acerifolium* were fractionated with hexane, butanol, methanol and water. *In vitro* antibacterial activity was tested by using agar cup and minimum inhibitory concentration method against *S. aureus*, *B. lichenoformis, B. subtilis*, *E. coli*, *P. florescence*, *P. aeruginosa*, and *S. typhimurium.* Although all five extracts showed promising antibacterial activity against test bacterial species, yet maximum activity was observed in butanol extract. *S. aureus* was the most sensitive strain. MIC values for most of the extracts ranged from 0.312 to 5.0 mg/ml; while the least MBC value was observed at 2.5 mg/ml. Phytochemical analysis exhibited the presence of alkaloids, tannin and phenolic compounds, flavonoids, glycosides, saponins, steroid & sterols and triterpenoids in different extracts. This could justify their use in treatment of microbial infections in man and livestock.

INTRODUCTION: Antimicrobial resistance has become a global problem. 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. Strategies to improve the current situation include research in finding new and innovative antimicrobials [1]. This has forced scientists to search for new antimicrobial substances from different sources especially medicinal plants [2].

The screening of plant extracts and plant products for antimicrobial activity has shown that higher plants represent a potential source of novel antibiotic prototypes [3]. Numerous studies have identified compounds within plants that are effective antibiotics [4]. Traditional healing systems around the world that utilize herbal remedies are an important source for the discovery of new antibiotics [5]. In 1997, the 30th World Health Assembly adopted a resolution urging interested governments to utilize their traditional systems of medicine with regulations suited to their national health care systems.

Some traditional remedies have already produced compounds that are effective against antibiotic-resistant strains of bacteria [6]. Infectious diseases account for approximately one-half of all deaths in tropical countries. In industrialized nations, despite the progress made in the understanding of microorganisms and their control, incidence of the epidemics due to drug-resistant microorganisms and the emergence of unknown disease causing microorganisms, pose enormous public health concerns [7].

Antibiotics and the chemotherapeutic agents have been of value in controlling many infections but they depend on judicious use to minimize the incidence of resistant forms. A considerable percentage of the peoples in both developed and developing nations use medicinal plant remedies. In the industrialised countries, consumers are seeking visible alternatives to modern medicine where there is already a problem of over-medication; accompanied by development of resistance to same [8].

Pterospermum acerifolium L. Willd (Sterculiaceae) commonly known as 'Muchukunda' is a large decidious tree distributed in tropical Asia. It has been traditionally used for blood troubles, inflammation, ulcer, tumors, leprosy and for small pox eruptions [9]. Flowers are used as a general tonic, antitumor agent, analgesic and for the treatement of diabetes, gastrointestinal disorder, leprosy, blood troubles, bronchitis, cough, cephalic pain, migraine and inflammation [10]. The leaves are used as haemostatics and antimicrobial agent [11]. Flowers have anti-inflammatory and antinociceptive activity [11].

Bark extracts has effects on oxidative damages in the gastric tissue during alcohol induced ulceration in mice [12]. However, relevent experimental work on the antimicrobial activity of the plant has not been explored. Here we report the finding of a study that was designed to test the antibacterial activity of different extracts from *Pterospermum acerifolium* bark.

MATERIALS AND METHODS:

Study area: Similipal Biosphere Reserve is located in the district of Mayurbhanj in the northern region of Orissa, between 21°08″- 21°27″ N latitude and 86°04″- 86°15″ E longitudes, unique habitat of mixed tropical forest (**Figure 1**). The ecosystem is enriched with more than 500 medicinal plants [13].

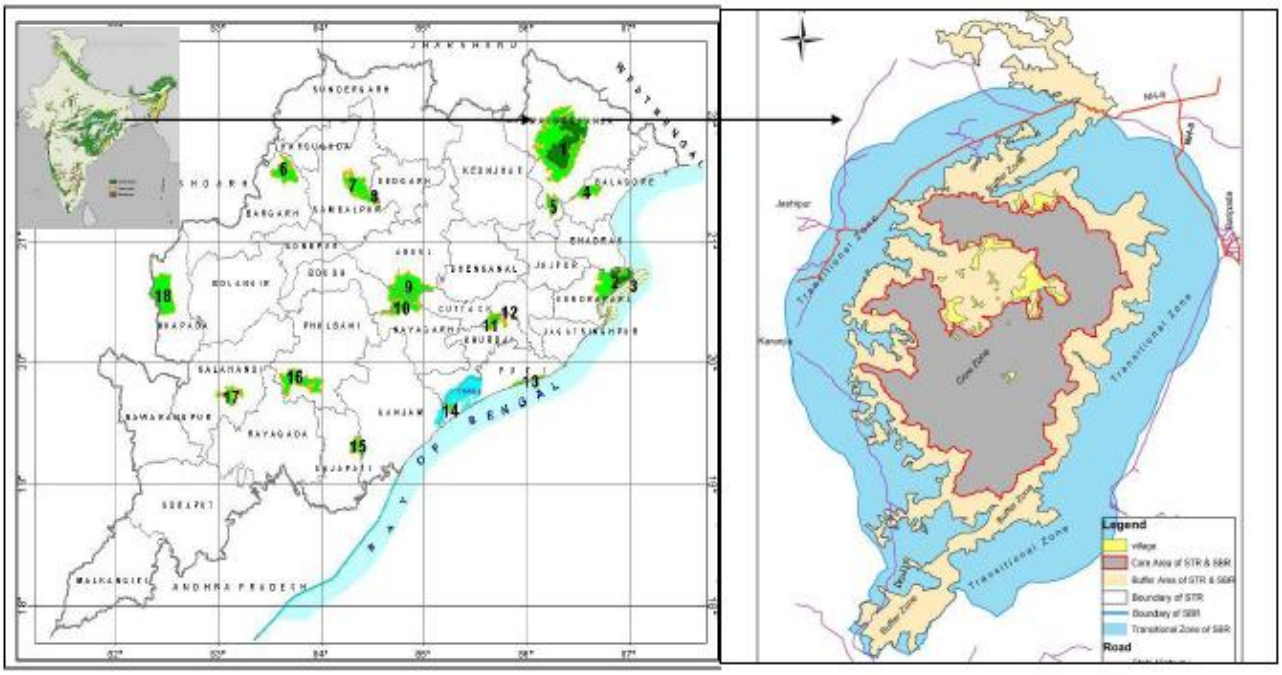

FIG. 1- MAP OF SIMILIPAL BIOSPHERE RESERVE

Plant material: Bark of *P. acerifolium* was collected from Similipal Biosphere Reserve in July 2007 and authenticated by comparison with a voucher specimen in the Post-Graduate Department of Botany, North Orissa University, Baripada, India.

Extraction procedures: *P. acerifolium* bark powder (250g) was soaked in 1500ml of 60-80% petroleum ether for two weeks and finally placed on a shaker for two hours. This was repeated twice, concentrated and the resulting material (PE) was stored at room temperature for later microbiological assay. The residual plant material was than soaked in 1500 ml 95% ethanol for 10 days (repeated twice). The final ethanol extract was filtered and evaporated to give a gummy material that was further fractionated as in figure 1.

Growth media and preparation of bacteria: Mueller Hinton agar (35g) (Hi-media, India) medium was mixed with one litre of distilled water, enclosed in a screw cap container and autoclaved at 121°C for 15min. The medium was later dispensed into 90mm sterile Petri plates and left to set. The agar plates were incubated for 24 hours at 37°C to confirm their sterility. When no growth occurred after 24 hours, the plates were considered sterile. The bacteria employed in this study consisted three Gram-positive and four Gram-negative bacteria (**Table 1**). The multiple antibiotic resistance index percentage was determined by using the formula-

$$\%\ \text{MAR index} = \frac{\text{No. of antibiotics to which the organism shared resistance}}{\text{No. of antibiotics used}} \times 100$$

Determination of antibacterial activity: The antibacterial activity screening was done as described before Panda *et al*., [14]. Briefly, cultures of the bacteria from culture plates were scooped using a wire loop and separately mixed with normal saline and agitated with vortex mixer. A loop full was withdrawn and uniformly distributed on the surface of the agar plate by streaking using a sterile swab. Wells of approximately 6mm in diameter and 2.5mm deep were made on the surface of the solid

medium using a sterile borer. The plates were turned upside down and the wells labeled with a marker. The extracts were reconstituted by dissolving 1gm of each in 1ml of dimethylsulfoxide (DMSO). Each well was filled with test sample. Sterile DMSO was used as negative control and disk of 10 microgram equivalent Gentamicin and Ciprofloxacin was used as positive control. The plates were incubated at 37°C for 24hours. After 24hours the plates were removed and zones of inhibition measured with Himedia antibiotic scale and the results were tabulated. Extracts with zones of inhibition greater or equal to 8mm diameter were regarded as positive. The mean ± SD of the inhibition zone was taken for evaluating the antibacterial activity of the extracts.

Determination of MIC: In the present experiment, extracts which showed positive result were further evaluated for determination of MIC. A broth micro-dilution technique was adopted using 96 well micro-titer plates and tetrazolium salt, 2, 3, 5-Triphenyltetrazolium Chloride (TTC) was carried out to determine the MIC following the methods with few modifications as described by Eloff [15]. In the plate, A_1 to H_1 was the blank and consisted of MH broth only. A_3 to H_3 was having the stock solution of the test extract(s) and A_4 to H_4 till A_9 to H_9 were the wells in which the test extracts were serially diluted using MH broth.

Wells A_{12} to D_{12} were control having 20µl of DMSO and E_{12} to H_{12} served as control over control. All wells were dispensed with 100µl of MH broth. 20µl of the herbal extract was transferred from stock test solution to the first well i.e. from A_4 to H_4 containing 100µl of MH broth. 20µl of the MH broth containing herbal extract was then transferred to the next well to create serial dilutions. 100µl of the 0.5 McFarland adjusted activated culture in MH broth was then added to all the wells except the blank. 5µl of 0.5 % TTC was further added to all the dilutions, blank, control and control over control. The final volume of all the wells was 205µl. The microplate was sealed and incubated at 37°C at 130 rpm. 10µl of the broth from each culture tube exhibiting MIC and control tubes were taken aseptically and were plated on one day old MH agar plate as a point inoculum and allowed to dry for 10 min under the laminar air hood. The micro-plate was sealed and incubated at 37°C at 130 rpm and observed for growth of the microorganism.

Determination of MBC and total activity (TA): 10µl of the broth from each well of 96 microtiter plate exhibiting MIC and control wells were taken aseptically and plated on one day old MH agar plate as a point inoculum and allowed to dry for 10 min under the laminar air hood. These plates were then sealed and incubated at 37°C for 24 hours and observed for growth of the bacteria. Absence of the growth of the bacteria showed the MBC result of the respective bacteria.

Total activity is a measure of the amount of material extracted from a plant in relation to the MIC of the extract, fraction or isolated compound. Reasons for screening studies abound to find new lead biologically active compounds and to confirm the ethnomedicinal use of plants to develop phytomedicines for use as herbal medicine. In many screening studies, activities are reported un-quantitatively. In mathematical terms it can be expressed as:

$$\text{Total activity (ml/g)} = \frac{\text{Amount extracted from 1 gram (mg) or amount present in fraction (mg)}}{\text{MIC (mg/ml)}}$$

The units are expressed in mg/g and indicate the degree to which the active extracts, fractions or compounds present in one gram can be diluted and still inhibit the growth of the test organisms [16].

Phytochemical analysis: Qualitative phytochemical analysis was carried out using method described by Trease and Evans [17] and Kokate *et al*., [18].

Test for alkaloids: Two grams of ground plant material was treated in a test tube with 10 ml of 1% HCl for 30 minutes in a water bath. The suspension was filtered through cotton into a test tube and was divided into two parts. Five drops of each of Dragendorff's, Wagner's reagent, Mayer's and Hager's reagent were added to the respective parts of the solution and the formation of different color indicated the presence of alkaloids.

Tests for carbohydrates: Molisch's, Fehling's and Benidict's tests were done to know the presence of carbohydrate-

Molisch's test: Aqueous or alcoholic solution of the extracted substance was added to 10% alcoholic solution of α-naphthol. Equal volume of concentrated H_2SO_4 was added along the side of the tube. A violet ring at the junction of two liquids confirms presence of carbohydrates.

Fehling's test: 2ml of Fehling's solution A and 2 ml of Fehling's solution B was added to 2ml of extracts. After boiling, if bricked precipitation appears, then reducing sugars are present.

Benedict's test: 5ml of Benedict's reagent and 3ml of test solution boiled in the water bath. If brick red precipitate appears at the bottom of the test tube then monosaccharides are present.

Test for cardiac glycosides:

Keller-Killiani test: To an extract of the drug in glacial acetic acid, few drops of ferric chloride and conc. H_2SO_4 were added. If a reddish brown color is formed at the junction of two layer and upper layer turns bluish green, confirms the presence of cardiac glycosides.

Legal Test: To a solution of glycoside in pyridine, sodium nitroprusside solution and sodium hydroxide solution were added. A pink to red colour, confirms presence of cardiac glycosides.

Modified Borntrager's test: 0.1 g of the extract was added with 5ml of dilute HCl and 5ml of 5% solution of ferric chloride and boiled for 5 minutes, cooled and filtered. This filtrate was shaken with benzene. The benzene layer was separated and an equal volume of dilute solution of ammonia was added. Formation of pink color with the ammonical layer signifies presence of anthraquinone glycosides.

Test for proteins and amino acids:

Biuret test: 2ml of extract was mixed with 2 ml of 10% NaOH solution and 2 to 3 drops of 1 % Cupper sulphate solution was added. Appearance of violet or purple color indicates the presence of proteins.

Ninhydrin test: 2 ml of extract was added with 0.5 ml of Ninhydrin solution. The mixture was boiled for 2 minutes, and then the solution was cooled. Appearance of blue color shows the presence of amino acid.

Xanthoproteic test: 2 ml. of extract was added with 1 ml of conc. HNO_3, boiled and cooled. Then 40% NaOH was added drop by drop. Appearance of colored solution indicates the presence of proteins.

Test for saponins:

Foam test: 1ml of alcoholic and aqueous extract was diluted separately with distilled water to 10 ml and was shaken in a graduated cylinder for 15 minutes and kept aside. One cm layer of foam after standing for 30 minutes indicates the presence of saponin.

Formation of honeycomb foam: Ground plant material (about 3g) was heated for 5 minutes in a test tube with 30ml of distilled water. The solution

was filtered through a filter paper at room temperature. About 10 ml of the filtered solution was shaken in a large test tube; the formation of honeycomb froth that persisted for half an hour indicated the presence of saponin.

Test for tannins and phenolic compounds:

With Ferric chloride: 5% w/v solution of ferric chloride was added in 90% alcohol. Appearance of blue color is the indication of presence of phenols.

With lead acetate: Extracts were mixed with lead acetate. Occurrence of precipitate is the indication of presence of tannins.

With gelatin solution: To a solution of tannins (0.5-1%) aqueous solution of gelatin (1%) and Sodium chloride (10%) are added. Appearance of white buff colored precipitated is the indication of presence of phenols.

Test for steroid and sterols:

Liberman Burchard reagent: To a dry test tube, 2 ml of extract solution was mixed with 2ml of acetic anhydride and 2-3 drops of conc. H_2SO_4 was added. The solution was mixed thoroughly. An emerald green color development is the indication of presence of steroids or sterols.

Salkowski's test: 5ml of solution of extract was taken in a dry test tube with chloroform. Equal volume of conc. H_2SO_4 was added gently along the sides of the test tube. The acid layer develops a yellow color with a green fluorescence and the chloroform layer will give a play of colors first from bluish red to gradually violet red.

Test for flavonoids:

With NaOH: The extract were dissolved in water, and then filtered. The filtrate was treated with sodium hydroxide. Yellow color is observed if flavonoids are present.

With H_2SO_4: A drop of Conc. H_2SO_4 acid was added to the above. Yellow color disappears.

With Mg/HCl: The extracts were dissolved in water, and then filtered. The filtrate was treated with Magnesium and a drop of conc. HCl was added. A pink color development is the indication of presence of flavonoids.

Test for gums and mucilage: By adding with 95% alcohol if precipitation results shows the presence of gums and mucilage.

RESULTS: Five different extracts viz. petroleum ether, hexane, butanol, methanol and aqueous (Figure 2) were screened against three Gram-positive and four Gram-negative bacteria (**Table 1**) during this investigation. Agar cup method was adopted and result presented in **Table 2**. Butanol extract was found to be the most active extract.

Petroleum ether and hexane extracts showed good zone of inhibition but were less effective than butanol extract. *S. aureus* was the most sensitive strain with highest zone of inhibition (16.7mm for butanol extract). A more accurate method is MIC test and result was presented in **Table 3**. The growth inhibition of the test bacteria ranged from 0.312 mg/ml (w/v) to 5.0 mg/ml (w/v).

The lowest MIC value was recorded against *S. aureus, P. fluorescence* and *Salmonella typhimurium* at 0.187 mg/ml (w/v). With concentration 1.25 mg/ml, 60% of extracts were proficient to inhibit the growth of different bacterial strains. The results of MBC showed that at concentration of 10.0 mg/ml (w/v), 50% of the test strains were killed.

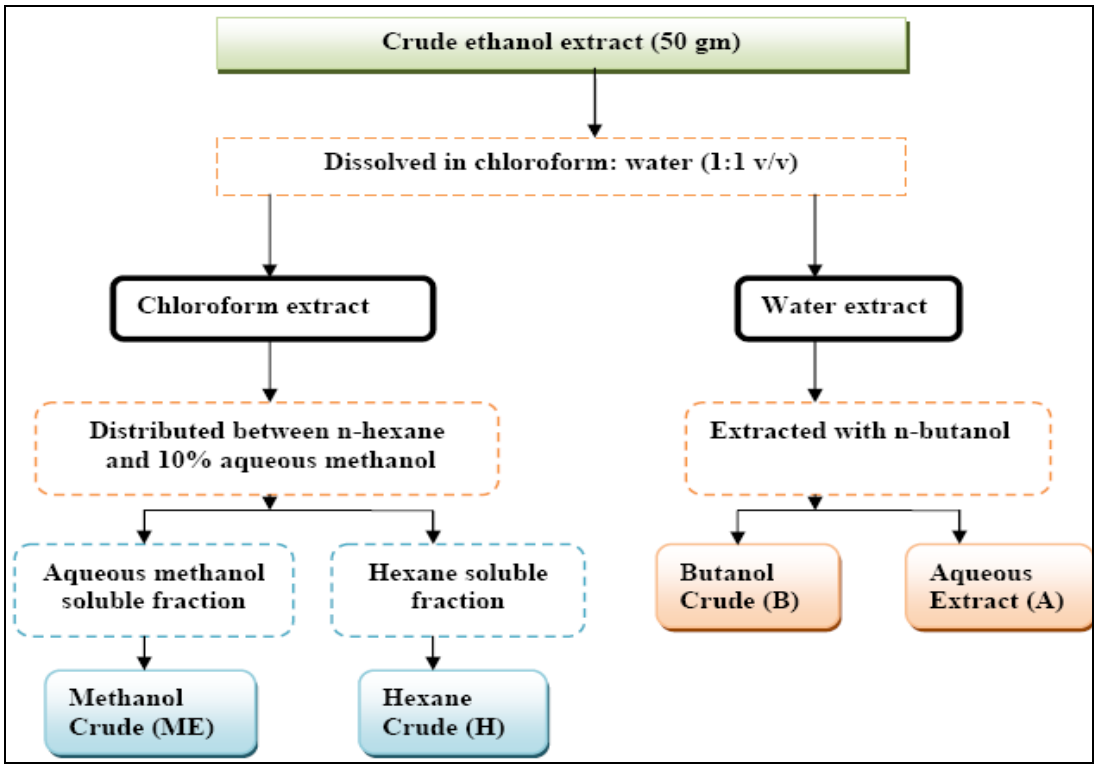

FIGURE 2-FRACTIONIZATION OF ETHANOL EXTRACTS OF *PTEROPSERMUM ACERIFOLIUM* BARK

TABLE 1: LIST OF THE BACTERIA USED TO ASSESS THE ANTIBACTERIAL ACTIVITY

Name of the organism	Relevant properties		MAR %	Sources
	Resistant to	Resistant to		
Bacillus subtilis (Gram-positive bacteria)	A, Ak, Ap, Aug, B, Ctn, Ce, Nal	C, Cez, Ch, Olf, Pb,Te, Lvx, E, Gt, G, St, Vn	40	MTCC 7164, Chandigarh
Bacillus lichenoformis (Gram-positive bacteria)	A, Ak, Ap, B, Aug, Ctn, Ce, Nal	C, Cez, Ch, Olf, Pb, Te, Lvx, E, Gt, G, St, Vn	40	MTCC 7425, Chandigarh
Staphylococcus aureus (Gram-positive bacteria)	Ak, Ap, B, Aug, Ctn, G, Ce, Nal, Pb	A, C, Cez, Ch, Olf, Te, Lvx, E, Gt, St, Vn	45	MTCC 1144, Chandigarh
Escherichia coli (Gram-negative bacteria)	Ak, Ap, B, Ctn, E, Aug, Ce, Nal	A, C, Ch, Caz, G, Gf, Lvx, Ofl, Pb, St, Te, Vn	40	MTCC 1098, Chandigarh
Pseudomonas aeruginosa (Gram-negative bacteria)	A, Ak, Ap, B, Ctn, E, Aug, Ce, Nal, Pb	C, Ch, Caz, G, Gf, Lvx, Ofl, St, Te, Vn	50	MTCC 1034, Chandigarh
Pseudomonas florescence (Gram-negative bacteria)	A, Ak, Ap, B, Ctn, E, Aug, Ce, Nal, Pb	C, Ch, Caz, G, Gf, Lvx, Ofl, St, Te, Vn	50	MTCC 1748, Chandigarh
Salmonella typhimurium (Gram-negative bacteria)	Ak, Ap, B, Ctn, E, Aug, Ce, Nal, Pb	A, C, Ch, Caz, G, Gf, Lvx, Ofl, St, Te, Vn	45	MTCC 3216, Chandigarh

Amikacin-Ak (30µg); Amoxicillin-Aug (10µg); Amphotericin-Ap (100unit); Ampicillin-A (10µg); Bacitracin-B (10units); Cefoxitin-Ctn (10µg); Ceftriaxone-Cez (10µg); Cephotaxime-Ce (30µg); Chloroamphinecol-Ch (10µg); Ciprofloxacin-C (10µg); Erythromycin-E (15µg); Gatifloxacin-Gf (30µg); Gentamycin-G (10µg); Levofloxacin-Lvx (5µg) ; Naladixic acid-Nal (30µg); Ofloacin-Ofl (5µg); Polymyxin-B-Pb (300 unit); Streptomycin-St (10µg); Tetracycline-Te (10µg) Vancomycin-Vn (30 µg) (Hi media Pvt. Ltd., Mumbai, India)

TABLE 2 : SCREENING OF ANTIBACTERIAL ACTIVITY OF *PTEROSPERMUM ACERIFOLIUM* BARK

Strain no.	Zone of inhibition of different extracts (25 mg/ml)					
	PE	H	B	ME	A	Ciprofloxacin
1	13.0±1.0	13.3±2.08	10.6±0.57	13.6±0.57	12.7±2.52	16.6±1.52
2	14.7±0.58	13.3±2.08	13.0±1.73	12.3±1.52	-	15.6±0.57
3	15.3±1.15	13.0±1.73	16.7±2.08	12.7±0.58	11.7±1.53	29.0±2.0
4	13.3±1.15	13.3±1.15	13.3±0.57	13.3±1.15	11.0±1.73	19.6±2.08
5	10.0±2.0	-	12.3±0.58	11.7±1.53	-	17.1±1.15
6	14.0±1.0	14.0±1.0	15.0±1.0	11.3±1.58	11.7±1.15	19.0±2.64
7	14.7±0.58	12.3±1.52	13.0±1.73	12.3±0.7	12.7±0.58	24.6±1.53

All values are mean zone of inhibition ± SD; (-) No zone of inhibition; Zone of inhibition including 6 mm borer

TABLE 3 : RESULTS OF MIC AND MBC OF ALL EXTRACTS OF *PTEROSPERMUM ACERIFOLIUM* BARK

Plant extract	Strain no.	Extract concentration in mg/ml				Antibiotic-Ciprofloxacin		
		MIC	MBC	MBC/MIC	TA	MIC	MBC	MBC/MIC
PE	1	1.25	<10.0	-	19.6	0.001	0.001	1.0
	2	0.625	10.0	16.0	39.2	0.001	0.001	1.0
	3	0.312	5.0	16.0	78.4	0.002	0.002	1.0
	4	2.50	10.0	4.0	9.8	0.001	0.001	1.0
	5	2.50	<10.0	-	9.8	0.001	0.001	1.0
	6	1.25	10.0	8.0	19.6	0.001	0.001	1.0
	7	1.25	10.0	8.0	19.6	0.001	0.001	1.0
H	1	0.625	10.0	16.0	20.3	0.001	0.001	1.0
	2	1.25	5.0	8.0	10.1	0.001	0.001	1.0
	3	0.625	2.5	8.0	20.3	0.002	0.002	1.0
	4	0.625	5.0	16.0	20.3	0.001	0.001	1.0
	5	2.50	<10.0	-	5.1	0.001	0.001	1.0
	6	0.625	5.0	8.0	20.3	0.001	0.001	1.0
	7	0.312	5.0	16.0	40.6	0.001	0.001	1.0
B	1	1.25	5.0	4.0	34.7	0.001	0.001	1.0
	2	0.625	2.5	4.0	69.4	0.001	0.001	1.0
	3	0.312	2.5	8.0	139.1	0.002	0.002	1.0
	4	1.25	5.0	4.0	34.7	0.001	0.001	1.0
	5	1.25	10.0	8.0	34.7	0.001	0.001	1.0
	6	0.312	5.0	16.0	139.1	0.001	0.001	1.0
	7	0.625	5.0	8.0	69.4	0.001	0.001	1.0
ME	1	2.50	>10.0	-	4.28	0.001	0.001	1.0
	2	2.50	10.0	4.0	4.28	0.001	0.001	1.0
	3	2.50	>10.0	-	4.28	0.002	0.002	1.0
	4	1.25	10.0	8.0	8.56	0.001	0.001	1.0
	5	1.25	10.0	8.0	8.56	0.001	0.001	1.0
	6	1.25	10.0	8.0	8.56	0.001	0.001	1.0
	7	1.25	10.0	8.0	8.56	0.001	0.001	1.0
A	1	2.50	>10.0	-	27.3	0.001	0.001	1.0
	2	5.0	>10.0	-	13.7	0.001	0.001	1.0
	3	2.50	10.0	4.0	27.3	0.002	0.002	1.0
	4	2.50	10.0	4.0	27.3	0.001	0.001	1.0
	5	5.0	>10.0	-	13.7	0.001	0.001	1.0
	6	2.50	10.0	4.0	27.3	0.001	0.001	1.0
	7	1.25	10.0	8.0	54.6	0.001	0.001	1.0

Extract concentration in mg/ml

All lowest MICs exhibited by extracts with MBC value four times of MIC, in corresponding microorganisms, highlighting their interesting antimicrobial potency. Phytochemical screening of all extracts of bark is presented in **Table 4**. All extracts produced positive result for phytochemicals as follows: petroleum ether (flavonoids, saponins, tannin and phenolic compounds, flavonoids, steroids and sterols, oils and fats); hexane (saponins, tannin and phenolic compounds, steroids and sterols, triterpenoids, oils and fat); butanol (alkaloids, flavonoids, saponins, tannin and phenolic compounds, steroids and sterols, triterpenoids, oils and fat and glycosides); methanol (alkaloids, saponins, flavonoids, oils and fat); aqueous (alkaloids, steroids and sterols, glycosides and flavonoids).

TABLE 4 : SCREENING OF PHYTOCHEMICALS OF *PTEROSPERMUM ACERIFOLIUM* BARK

Name of the phytochemicals	Qualitative test	PE	H	B	ME	A
Alkaloids	Mayer's reagent	+	-	+	+	+
	Dragendroff's	+	-	+	+	+
	Hager's reagent	+	-	-	-	-
	Wagner's reagent	-	-	-	-	-
Carbohydrates	Molisch's test	-	-	-	-	-
	Fehling's test	-	-	-	-	-
	Benedict's test	-	-	-	-	-
Tannin and phenolic compounds	With Ferric chloride	+	+	+	-	-
	With lead acetate	+	+	-	-	-
	With gelatin solution	-	-	-	-	-
Glycosides	Keller-Killiani test	-	-	+	-	+
	Legal Test	-	-	-	-	-
	Borntrager's test	-	-	-	-	-
Proteins and amino acids	Biuret test	-	-	-	-	-
	Ninhydrin test	-	-	-	-	-
	Xanthoproteic test	-	-	-	-	-
Gum & mucilages	Millon's test	-	-	-	-	-
	Molisch's test	-	-	-	-	-
Flavonoids	With NaOH	+	-	+	+	-
	With H_2SO_4	-	-	-	-	-
	With Mg/HCl	-	-	-	-	-
Saponins	Honeycomb foam	+	+	+	+	-
	Foam test	+	+	+	+	+
Steroids and sterol	Salkowski's test	-	-	-	-	-
	Liberman Burchard	+	+	+	-	+
Triterpenoids	Thionlylchloride test	-	+	+	-	-
Oils and fats	With filter paper	+	+	+	+	-
	With alkaline KOH	-	-	+	+	-
Vitamin C	With Indophenol's	-	-	-	-	-
	Sod. nitroprusside	-	-	-	-	-

(+) Present; (-) Absent

DISCUSSION: Several techniques have been described and used in searching for new antimicrobials from natural products, i.e. for successfully detecting their active components against human pathogenic bacterial strains. One of these assays is agar diffusion, a method that has been used while screening plant extracts for their antimicrobial effects. All extracts studied showed antibacterial activity. This could justify their use in treatment of microbial infections in man and livestock. Petroleum ether, hexane and butanol extracts showed higher activity compared to methanol and aqueous extracts on test bacteria. This may be as a result of absence of soluble active constituents in methanol and aqueous extract as compared to nonpolar extracts.

The result shows that this plant could be effective and suitable for general use against Gram-negative, Gram-positive and opportunistic pathogens of humans. *P. acerifoilum* extracts have measurable activity against *B. lichenoformis, S. aureus, P. florescence* and *Salmonella typhimurium.* This justifies its use in treatment of UTI in animals as reported by Katende *et al*., [19]. *S. aureus* was the most susceptible strain to all the test extracts. This could support the use of these plants in treatment of *Staphylococcal* infections. From Table 2 it is clear that Gram-positives are more susceptable in compare to Gram-negatives.

In general the plant antibiotic substances appear to be more inhibiting to Gram-positive organisms than to the Gram-negatives [20]. It is reported that Pencillin and some of the other prominent antibiotic agents of fungal origin are also rather selective in their inhibitory action, most of them being inhibited to Gram-positive bacteria. Unlike Gram-positive bacteria, the lipopolysaccharide layer along with proteins and phospholipids are the major components in the outer surface of Gram-negative bacteria [21]. Access of the most compounds to the peptidoglycan layer of the cell wall is hindered by the outer lipopolysaccharide layer. This explains the resistance of Gram-negative bacteria to the lytic action of most extracts exhibiting the activity. It can be assumed that antibacterial extracts from the test plants can be useful in warding of infectious diseases and is therefore a compelling reason to suppose that, as anti-infective agents from test plants are active against human pathogens [22].

However agar diffusion method has some limitations, such as low throughput. A more generally accurate method of assessment is the micro-dilution techniques in 96 well microplates. The bacterial growth and antimicrobial effects can be evaluated by turbidometry measuring optical density of bacterial suspension or by using indicator dyes [15]. A variety of dyes have also been used for measuring MIC values for new compounds, and especially with non-aqueous plant extracts. Tetrazolium salts, such as 2, 3, 5-triphenyltetrazolium chloride (TTC), can also be used as a dye to indicate bacterial growth.

Tetrazolium salts act as electron acceptors and are reduced by biologically active bacteria from a colorless compound to red in case of TTC [15]. The MIC results showed that extracts such as petroleum ether, hexane, butanol, methanol and aqueous were able to prevent the growth of most bacterial strains with selective activities. The rate of growth inhibition of the test bacteria ranged from 0.312mg/ml (w/v) to 5.0mg/ml (w/v). The lowest MICs exhibited by extracts with MBC values four or eight time that of MIC, in corresponding microorganisms, highlighting their interesting antibacterial potency. From these results, it can be observed that, most of the test samples exerted a lethal effect on the test organisms. In addition to these MBC/MIC ratios lower than 4 was obtained with most of the samples, suggesting killing effects

[23]. Some extracts were found to have broad spectrum activity viz. butanol, petroleum ether, methanol and aqueous where MBC was found 4 time higher value of MIC. Antimicrobial resistance has been reported for most of the principal pathogenic microorganisms like *Staphylococcus aureus*, *Escherchia coli* and *Salmonella typhimurium* [24, 25].The test organisms in the present case showed different MAR index with *Salmonella typhimurium* and *Staphylococcus aureus* (45%), *Escherchia coli*, *Bacillus subtilis* and *B. lichenoformis* (40%), *Pseudomonas aeruginosa* and *P. florescence* (50%).

The lack of susceptibility of *Pseudomonas aeruginosa* to the extracts could be attributed to the fact that this bacteria are naturally resistant to many antibiotics due to the permeability barrier afforded by its outermembrane [26]. Also its tendency to colonise in a biofilm form makes the cells impervious to therapeutic concentrations of antibiotics. Since its natural habitat is the soil, living in association with bacilli, actinomycetes and molds, it has developed resistance to a variety of their naturally occurring antibiotics [26]. For the same reason the plant is not also active against *Bacillus subtilis.*

The lack of susceptibility of the bacteria to the plant extracts against *Eschirichia coli* could be attributed to the fact that, unlike conventional pharmaceutical products which are usually prepared from synthetic materials by means of reproducible manufacturing techniques and procedures, herbal medicinal products are prepared from materials of plant origin which may be subjected to contamination and deterioration [27]. The storage of extracts may require special condition of humidity or temperature or protection from light. The plant extracts might contain little of the active ingredient. The extracts which were inactive *in-vitro* may have properties similar to pro-drugs which are administered in an inactive form; their metabolites could be active *in-vivo* [28].

The present study suggested a wide range of positive reactions for alkaloids, flavonoids, saponins, tannin and phenolic compounds, steroids and sterols, triterpenoids, oils and fat and glycosides in this plant. However majority of the extracts give positive results for saponins, steroids and sterols and alkaloids. Evaluation of phytochemicals such as tannin and phenolic compounds, steroid and sterols revealed the presence of most of constituents in nonpolar extracts (petroleum ether, hexane and butanol) compared to polar extracts (methanol and aqueous).

Percentage yield of different extracts were in the order aqueous >butanol >petroleum ether >hexane > methanol. In many screening studies, activities are reported un-quantitatively. Even if extract data are expressed in quantitative terms such as MIC, it is usually not possible to compare different plants or different fractions with the results presented. By calculating total activity result obtained that butanol and aqueous extracts can act as a superior extract compared to other extracts though aqueous extract showed less activity. This study confirms the potential antibacterial activity of butanol extracts of *Pterospermum acerifolium* bark. This credit to maximum activity of butanolic extract was supposed to butanol being an organic solvent and will dissolve organic compounds better, hence liberate the active component required for antibacterial activity.

CONCLUSION: Further studies are required to isolate the active compounds from aqueous and butanol extract of *Pterospermum acerifolium* bark, responsible for the antimicrobial affect which might be a lead compound in antibacterial field.

ACKNOWLEDGEMENTS: The present research has been funded by the Department of Science and Technology, Government of Orissa (Grant No. 2818/28.06.2006). We are also grateful to the authorities of North Orissa University for providing necessary facilities to carry out this research.

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