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BIOACTIVE CONSTITUENTS FROM BARK OF PELTOPHORUM PTEROCARPUM BAKER EX K. HEYNE

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Abstract– *In vitro* antimicrobial and antioxidant activities of sequential bark extracts of *Peltophorum pterocarpum* Baker ex K. Heyne, was tested against four Gram negative, two Gram-positive bacteria and five fungal isolates. Antioxidant profile was investigated using 2, 2-Diphenyl-1-picryl-hydrazyl and reduction capability by ferric ion reducing antioxidant potentials method. Bark ethyl acetate extract showed significant activity against *B. subtilis* and *P. aruginosa* (IZ 18.33 ± 0.33 mm and 18.33 ± 0.57 mm with MIC of 62.5 and 31.25 µg/ml, respectively) similarly, higher antioxidant potentials with lower IC₅₀value (5 µg/ml; % inhibition of 97.08 at 80 µg/ml), whereas in FRAP method, methanol extract (1463.33 ± 3.33 AAE/mg dw antioxidant potential) show better activity. Further, GC-MS analysis of the ethyl acetate extract confirmed 33 compounds. The main identified constituent was 1,2-benzenedicarboxylic acid, bis(2-ethylhexyl) ester with 48.9% availability.

INTRODUCTION

Antibiotic resistance is a type of drug resistance where a microorganism develops ability to survive in exposure of an antibiotic. Genes and evolutionary stress such as exposure to antibiotics selects its resistant trait, greater the duration of exposure, greater the risk of the resistance development (D'Costa et al., 2011). Some of the common resistant pathogens are Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Bacillus subtilis and Candida albicans. Resistance of these pathogenic strains increasing worldwide causing pneumonia, bacteremia, meningitis, sinusitis, peritonitis, food poisoning and arthritis. These problem demands renewed effort to seek new antibacterial agents, effective against pathogenic microorganisms resistant to current antibiotics (Donadio et al., 2010).

Antioxidants are the substances that delay the oxidation process, inhibiting free radicals, initiated chain polymerization and other subsequent oxidizing reactions (Cespedes *et al.*, 2008), these are widely used in food, cosmetic and pharmaceutical industries (Al-Duais *et al.*, 2009). Antioxidants play an important role in biological systems because they defend against oxidative damage and participate in the major signaling pathways of cells (Kumar *et al.*,

2008; Gulcin et al., 2009; Wang et al., 2009).

Plants have limitless ability to synthesize aromatic substances, most of which are phenols or their oxygen-substituted derivatives such as tannins. These substances serve as plant defense mechanisms against predation of microorganisms, insects and herbivores. Since long time, traditional healers using plants as medicaments to cure infectious conditions (Lu *et al.*, 2007; Mbwambo *et al.*, 2007). Many of these plants have been investigated for their antimicrobial and antioxidant activities where large number of plant products significantly inhibited growth of pathogenic bacteria and having antioxidant potentials (Senatore *et al.*, 2007; Singh *et al.*, 2007).

Peltophorum pterocarpum Baker ex K. Heyne is a semi-arid tree of 50 feet in height and grows in many part of India as an ornamental tree. Bark of the plant is used in dysentery, as a constituent of gargle, tooth powder, lotions, in eye trouble, muscular pain and sores (Deshaprabhu, 1966). On phytochemical investigation flavonoids from whole plant (Rahman *et al.*, 1969; Varshney and Dubey, 1969); berginin from flowers (Sulochana *et al.*, 1970); peltophorin, leucocyanidin, quercetin from bark, leaves, fruits and wood (Sastry *et al.*, 1977); tannins from roots and essential oils were reported from pod extracts (Badami and Daulatabad, 1969; Menon *et al.*, 1982).

Pharmacologically antimicrobial (Ahmad and Sultana, 2003; Duraipandiyan *et al.*, 2006), antiinflammatory (Menon *et al.*, 1982; Swarnlakshmi *et al.*, 1984), enterohaemorrhagic effect (Voravuthikunchai *et al.*, 2004) have been documented earlier.

This paper describes bioeffecacies of *P. pterocarpum* successive extracts with its chemical investigation via GC-MS analysis.

MATERIALS AND METHODS

All the chemicals and solvents used in this study were of analytical grade and obtained from HiMedia Chemicals Mumbai, India.

Plant material

Arial parts of *P. pterocarpum* were collected at full flowering stage from the campus, University of Rajasthan, Jaipur, in the months of July-Aug 2010 and authenticated from the Herbarium of the Department itself. Voucher specimen have been deposited (Herbarium Sheet No. 20707) in the Herbarium and Laboratory for further reference. For experiment, bark of *P. pterocarpum* collected in the months of July-August, shade-dried and powdered. The powdered plant material was extracted separately in pet. ether, dichloromethane (DCM), ethyl acetate and methanol successively. These extracts were concentrated using vacuum desiccator and stored at 4 °C for further use.

Antimicrobial activity

In *In vitro* preliminary analysis, successive extracts were dissolved in DMSO (4 mg/mL concentration). Muller-Hinton agar for antimicrobial and Sabourod Dextrose agar were used for antifungal activities. Pure cultures of Gram +ve bacteria (Bacillus subtilis, MTCC 441; Staphylococcus aureus, MTCC 740), Gram -ve bacteria (Escherichia coli, MTCC 443; Pseudomonas aeruginosa, MTCC 741; Enterobacter aerogenes, MTCC 111; Raoultella planticola, MTCC 530) and fungal strains (Aspergillus flavus ATCC 16870; A. niger ATCC 322, Candida albicans ATCC 4718, Penicillium chrysogenum ATCC 5476 and Tricophyton rubrum ATCC 2327) were obtained from Institute of Microbial Technology, Chandigarh. These cultures were grown and maintained on Nutrient broth (NB) at 37 °C for 24 h in case of bacteria and Sabouraud Dextrose Broth (SDB) medium at 24 °C for 48 h in case of fungi. Agar well diffusion method (Boyanova et al., 2005) was used for antimicrobial

potentials. Briefly, the surface of the agar medium was punched using a sterile cork borer (diameter 6 mm). A 40 μ l of extract was dropped into the wells and kept in incubator for 24 h. Following incubation, the inhibition zone in all well was read and diameter expressed in mm. Gentamycin (10 μ g/ml) in case of bacteria and ketoconozole (100 units/ml) in case of fungi, were used as positive control. DMSO used as a negative control. Minimum Inhibitory Concentration (MIC) was defined as the lowest concentration of the extract resulted in complete inhibition of visible growth of tested microbes in inoculated plates. Agar well diffusion method was used for MIC determination

Antioxidant activity

1,1-Diphenyl-2-picrylhydrazyl radical scavenging assay

The scavenging effect of the different fractions on the DPPH radical was measured using a modified version of the method (Fogliano *et al.*, 1999). In brief, the extract solution in methanol at different concentrations (80, 60, 40, 20, 10 μ g/ml) were added to 2.5 ml of 0.02 % (w/v) solution of DPPH in methanol. The scavenging activities on DPPH radical were determined by measuring the absorbance at 517 nm after 30 min of incubation. The DPPH radical scavenging activity % inhibition was calculated using the following formula:

% Inhibition= (ODControl-OD Sample) /OD Control) ×100

Where OD Control is the absorbance of the blank (containing all reagents except the extract solution) and OD Sample is the absorbance of the test sample. The DPPH radical scavenging activity of quercetin was also assayed for comparison. All tests were performed in triplicate.

Reducing power assay

Reducing power was determined according to the method of Yen and Chen (1995). Each of the extract (1000, 500, 250, 125, 62.5 μ g/ml, 1ml) in ethanol was mixed with 2.5 ml of 0.2 M phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide (Sigma) and the mixture was incubated at 50 °C for 20 min. After incubation, 2.5 ml of 10% trichloroacetic acid was added. The upper layer of 2.5 mL was mixed with 2.5 ml deionized water and 0.1% ferric chloride (0.5 ml). Optical density was measured at 700 nm. A higher absorbance indicates a greater reducing

power. Ascorbic acid used aspositive control.

Statistical analysis

All data were subjected to one-way analysis of variance in a statistical analysis system. Duncan's multiple range tests was performed to compare significant differences (P < 0.05) in variables between groups. Each set of experiments were conducted in triplicate.

Gas chromatography-Mass spectrophotometry analysis

Ethyl acetate extract was used for Gas chromatography-Mass spectrophotometry (GC-MS) analysis due toits higher phenolic contents (higher intensities on FeCl₃ spray). GC-MS (Model; QP₂₀₁₀ series, Shimadzu, Tokyo, Japan) equipped with a VF-5ms fused silica capillary column of 30 cm length, 0.25 mm diameter and 0.25µm film thickness was use for the analysis. For detection, an electron ionization system with ionization energy of 70eV was used. Helium gas (99.99%) was used as a carrier gas at a constant flow rate of 1.51 ml/min. injector and mass transfer line temperature were set at 200 and 240 °C respectively. The oven temperature was programmed from 70 to 220 °C at 10 °C/min, held isothermal for 1min and finally raised to 300 °C at 10 °C/min. 2 ml of respective diluted samples was manually injected in the split less mode, with split ratio of 1:40 and with mass scan of 50-600 amu. Total running time of GC-MS is 35 min. The relative percentage of the each extract constituents was expressed as percentage with peak area normalisation.

The identity of the components in the extracts was assigned by the comparison of their retention indices and mass spectra fragmentation patterns with those stored on the computer library and also with published literatures. NIST08.LIB [9], WILEY8.LIB [10], PESTEI_3.LIB, and FA_ME.LIB library sources were used for matching the identified components from the plant material.

RESULTS AND DISCUSSION

Antimicrobial activity

Antimicrobial activity and MIC of the extracts was measured using agar well diffusion method (Table 1). Bark extract was active against most of the tested extracts. The extract exhibits higher to moderate activity towards *B. subtilis, P. aruginosa, R. planticola* with inhibition zone of 18.33 ± 0.33 , 18.33 ± 0.57 and

16.00 \pm 0.57 mm and MIC of 62.5, 31.25 µg/ml in last two cases respectively. Further dichloromethane extract also exhibited appreciable activity against *B. subtilis, E.coli* and *P. aeruginosa* (IZ of 19.33 \pm 0.88, 16.00 \pm 0.57 and 18.00 \pm 0.57 mm respectively with MIC 125, 15.62 and 125 µg/ml respectively). The result reveled that ethyl acetate extract exhibited potentials similar to positive control.

Antioxidant activity

1,1-Diphenyl-2-picrylhydrazyl radical scavenging assay

ROS are formed as natural byproducts of normal metabolism and have important role in cell signaling. The effects of ROS on the cell metabolism have been well documented in variety of species (Osawa and Namiki, 1981; Dorman *et al.*, 2003).

The DPPH radical is a stable free radical, which has been widely accepted as a tool for estimating free radical scavenging activities of antioxidants (Sanchez-Moreno, 2002). When DPPH radicals are scavenged, the color of reaction mixture changes from purple to yellow and absorbance at 517 nm decreases. Ethyl acetate extract demonstrated lower IC_{50} value i.e. 5 µg/ml with % inhibition of 97.08 at 80 µg/ml concentration followed by methanol extract (IC_{50} 5.5 µg/ml; % inhibition 96.06) (Table 2).

Reducing power assay

The reduction of Fe (III) ions is often used as a indicator of electron donating activity, which is an important mechanism of the phenolic antioxidant action, and strongly correlated with other antioxidant properties. Among the tested extracts, methanol extract showed appreciable reduction with 1463.33 \pm 3.33 AAE/mg dw antioxidant potential (Table 3). Another potential extract was ethyl acetate extract (895.33 \pm 3.78 AAE/mg dw), indicating similar reductive potentials to standard ascorbic acid.

On the basis of activities ethyl acetate extracts were analyzed for GC-MS analysis where, 49 distinct peaks were identified through the NIST08 L. database (Table 4). The major compound present in the extract was 1,2-benzenedicarboxylic acid, bis(2-ethylhexyl) ester with RT: 25.28, area 48.47 % (molecular formula: $C_{24}H_{38}O_4$, MW: 390). Other major components were β -caryophyllene (RT: 10.51, area 5.80%), cedryl methyl ester (RT: 12.85, area 5.80%) 9-octadecenoic acid (Area 6.65%), ethyl docosonate(3.81%), hexadecanoic acid (Area 3.24%),

Microorganisms				Nature of extract	
	P	et. ether	DCM*	Ethyl acetate	Methanol
B. subtilis	IZ ^a	17.66± 0.33	19.33 ± 0.88	18.33 ± 0.33	15.00 ± 0.57
	MIC ^b	125	125	62.5	125
	AIc	0.80	0.87	0.83	0.68
E. aerogenes	IZ	13.00 ± 0.00	14.33 ± 0.33	13.00 ± 0.57	13.33 ± 0.66
0	MIC	250	250	62.5	250
	AI	0.92	1.02	0.92	0.95
E. coli	IZ	15.00 ± 0.57	16.00 ± 0.57	15.66 ± 0.33	14.00 ± 0.57
	MIC	125	15.62	31.25	31.25
	AI	0.78	0.72	0.71	0.84
P. aruginosa	IZ	15.33 ± 0.33	18.00 ± 0.57	18.33 ± 0.33	15.66 ± 0.33
0	MIC	250	125	31.25	31.25
	AI	0.76	0.90	0.91	0.78
R. planticola	IZ	15.33 ± 0.66	15.00 ± 0.57	16.00 ± 0.57	15.00 ± 0.57
11 prime on	MIC	125	125	31.25	250
	AI	0.69	0.68	0.72	0.68
S. aureus	IZ	14.66 ± 0.33	15.33 ± 0.33	14.66 ± 0.70	13.33 ± 0.88
	MIC	500	125	250	125
	AI	0.69	0.73	0.69	0.63
A. flavus	IZ	14.00 ± 0.57	13.33 ± 0.67	-	12.84 ± 0.56
11. juicus	MIC	250	250	_	250
	AI	0.51	0.49	_	0.46
A. niger	IZ	10.33 ± 0.33	12.33 ± 0.33	11.00 ± 0.66	11.00 ± 1.00
	MIC	250	500	62.5	125
	AI	0.45	0.45	0.40	0.40
C. albicans	IZ	13.66 ± 0.66	14.66 ± 0.88	15.66 ± 0.88	12.66 ± 0.66
C. utoteuns	MIC	250	62.5	500	1000
	AI	0.50	0.66	0.71	0.57
P. chrysogenum	IZ	13.33 ± 0.66	13.33 ± 0.66	15.33 ± 0.33	12.66 ± 0.66
1. chrysogenum	MIC	125	250	250	62.5
	AI	0.63	0.63	0.73	0.60
T. rubrum	IZ	13.33 ± 0.33	12.66 ± 0.33	14.33 ± 0.33	12.66 ± 0.66
1. <i>ruorum</i>	MIC	13.33 ± 0.33 250	12.66 ± 0.33 250	14.33 ± 0.33 62.50	12.66 ± 0.66 500
	AI	0.45	0.45	0.49	0.43

Table 1. Antibacterial activity of *P. pterocarpum* bark.

Test samples 4 mg/well. Standard test drugs: Gentamycin for bacteria, Ketonocozole for fungi (10 mcg/disc). DCM*=Dichloromethane.

 IZ^{a} =Inhibition zone (in mm) including the diameter of well (6 mm). MIC^b= Minimum inhibitory concentration in μ g/ml. AI^c = Activity index = Inhibition zone of the sample/Inhibition zone of the standard.

Nature of	^a % Inhibition (concentration in μg/ml)						
extract	IC ₅₀	10	20	40	60	80	
Pet. ether	6.5	78.07 ± 2.80	90.62 ± 1.95	93.81 ± 0.76	94.58 ± 0.28	94.88 ± 0.68	
DCM	6	84.10 ± 0.39	85.99 ± 0.06	89.59 ± 0.10	91.13 ± 0.28	94.63 ± 0.11	
Ethyl acetate	5	96.26 ± 0.19	96.57 ± 0.08	96.93 ± 0.18	96.95 ± 0.26	97.08 ± 0.31	
Methanol	5.5	86.54 ± 0.30	93.48 ± 0.76	95.80 ± 0.09	96.01 ± 0.06	96.06 ± 0.11	
Quercetin	4	62.42	80.58	93.38	93.82	94.71	

Table 2. Antioxidant activity of *P. pterocarpum* bark by DPPH method.

 s^{a} % Inhibition = 1-(Absorbance of the sample/Absorbance of the control) × 100

Nature of	1	Antioxidant activity	in ^b AAE /mg dw (cor	ncentration in µg/ml))
extract	62.5	125	250	500	1000
Pet. ether	37.00 ± 3.00	151.00 ± 0.00	176.66 ± 13.97	408.33 ± 10.14	864.66 ± 17.72
DCM	41.66 ± 4.41	47.00 ± 3.51	121.66 ± 10.48	306.66 ± 12.02	726.00 ± 6.69
Ethyl acetate	85.00 ± 5.78	179.00 ± 10.11	301.66 ± 1.66	573.00 ± 14.23	895.45 ± 3.78
Methanol	113.33 ± 7.27	160.00 ± 15.29	268.66 ± 7.82	630.00 ± 16.09	1463.33 ± 3.33
Ascorbic acid	62.5	125	250	500	1000

Table 3. Antioxidant activity of *P. pterocarpum* bark by FRAP method.

^bAAE/g = Ascorbic acid equivalent/ mg extract Gas chromatography-Mass spectrophotometry analysis

Table 4. GC-MS spectra of bark ethyl acetate extract of P. pr	rterocarpum.
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Peak	R.Time	Area%	Name	Properties known
1.	10.517	5.80	β-caryophyllene	Anti-inflammatory
2.	10.952	2.57	α -humulene, α -caryophyllene	Antitumor, antimicrobial
3.	12.023	0.71	Elemol	Neruropharmacological properties
ł.	12.854	5.80	Cedryl methyl ester	Flavor, antitumor, insecticidal
5.	14.055	1.10	Benzene, ethylphenoxy- ar-	-
			ethylphenyl phenyl ether	
5.	14.732	0.26	2-phenyldodecane	Anti-carcinoma
7.	14.909	0.35	6-Phenyltridecane	11
3.	14.986	0.23	Heptadecane	Antimicrobial
).	15.534	0.37	Nonadecane	Antimicrobial, antiradical, spasmolytic
•	101001	0107		· · · · · · · · · · · · · · · · · · ·
10.	15.789	2.81	2-Phenyltridecane	-
11.	16.272	2.96	Hexadecanoic acid	Antioxidant, hypocholesterol
				emicnematicide, pesticide, lubricant,
				antiandrogenic, flavor
2.	16.827	0.41	(1-methylnonadecyl) benzene	-
13.	17.668	0.17	9-Octadecenoic acid	Fungicidal, reduce muscular tension
				in cardiac muscle
4.	18.730	0.68	Octadecane	Antimicrobial
5.	20.415	0.20	10-12-Pentacosadiynoic acid	Antiviral
6.	21.056	0.26	abieta-9(11),8(14),12-trien-12-ol	-
17.	22.690	1.62	2,4-bis(1-phenylethyl)phenol	Molting hormone
18.	25.287	48.47	1,2-benzenedicarboxylic acid	Antimicrobial, antitick, allelopathic
19.	26.054	0.51	Ethyl docosanoate	Antibody production
20.	28.109	0.40	Tetracontane	Antibacterial, antioxidant
21.	28.630	0.38	Tetracosanoic acid methyl ester	-
22.	29.501	1.05	Ethyl docosanoate	Antibody production
23.	30.009	0.47	Squalene, Spinacene	Antioxidant, antitumor
24.	30.823	0.27	Tetratriacontane	Antioxidant, antimicrobial
<u>-</u> 25.	30.907	0.23	1-Eicosanol	Antidiabetic, antioxidant,
_0.	50.707	0.20	1-Eleosation	anticholinesterase
26.	33.242	1.97	2,4,6-Tris-(1-phenylethyl)-phenol	Pesticide
20. 27.	33.500	2.45	Acetic acid, 7-isopropyl-3a,3b,9b-	
_/.	55.500	2.40	trimethyl-2-phenyl-dodecahydro-6-	-
10	24 420	0.82	oxa-cyclopenta[a]phenanthren-3-yl ester	Antimianabial anti influence to
28.	34.439	0.83	β-Sitosterol	Antimicrobial, anti-inflammatory,
N O	07.407	0.00		analgesic, antipyretic
29.	37.496	0.28	Stigmasta-5,22-dien-3-ol	Antitumor, anti-inflammatory,
20	00 5/1	0.10		Antioxidant
30.	38.761	0.19	Stigmast-5-en-3-ol	Insecticidal, fungicidal, antioxidant
31.	40.308	0.24	Lupeol	Antibacterial, Antioxidant, Antitumor
32.	42.209	0.23	Stigmast-4-en-3-one	Antioxidant
33.	43.463	0.40	3-Hydroxy-6-isopropenyl-4,8a-dimethyl-	
		100.00	octahydro-2-naphthalenyl acetate	

benzene, 2-phenyltridecane (RT: 15.78, area 2.81%), tricontane (1.35%), β -sitosterol (0.83%), heptadecane (0.76%), tricosane (0.68%) and octacosane (0.60%). Further, properties of identified compounds have been given in Table 4.

Several plants reported elevated antimicrobial potentials due to presence of long chain (C6 to C10) alcohols and aldehydes like hepadecane, nonadecane, hexadecanoic acid, tricosane and triacontane (30). Hydrophobicity enables these compounds to partition the lipids of the bacterial cell membrane, disturbing the cell structures and rendering them more permeable (Sikkema et al., 1994). Extensive leakage from bacterial cells or the exit of critical molecules and ion will lead to death (Denyer and Hugo, 1991). Long chain (C6 to C10) alcohols were particularly active against Grampositive bacteria (Delaquis et al., 2002), the antimicrobial properties of alcohols were known to increase with molecular weight (Morton, 1983). Further purification and bioactivities are in active progress.

New antimicrobial agents against bacterium are very valuable, especially in multidrug resistant strain. Present investigation for antibacterial, antioxidant and chemical investigation supports traditional use of bark infusion as therapeutic agent. Further, the plant can be used as antibacterial supplement in the developing countries towards the development of new drug of natural origin. Additional *in vivo* studies and clinical trials will also be needed to justify the potentials of ethyl acetate extract in topical and oral applications.

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