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Antimicrobial, free radical scavenging activities and chemical composition of *Peltophorum pterocarpum* Baker ex K. Heyne stem extract

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ABSTRACT

Stem infusion of *Peltophorum pterocarpum* Baker ex K. Heyne used in dysentery, for gargles, tooth powder and muscular pain. In present paper antimicrobial, antioxidant along with their phytochemical evaluation will be performed. Pet. ether, dichloromethane, ethyl acetate and methanol fractions were tested for their antimicrobial (using agar well diffusion method) and antioxidant activities (DPPH and FARP methods). Bioactive fraction was further subjected to GC-MS analysis for evaluation of its chemical composition. Maximum antimicrobial inhibition was demonstrated by ethyl acetate extract against *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Satphylococcus aureus* (IZ 17.33 ± 0.33 , 17.00 ± 0.00 , with MIC of 31.25 and 125 $\mu\text{g/mL}$ respectively). In antioxidant potentials, similar fraction showed significant activity i.e. 6 $\mu\text{g/mL}$ with IC_{50} value of 96.70 ± 0.22 at 80 $\mu\text{g/mL}$ concentrations, coinciding with FRAP results (1310.00 ± 0.00 mg AAE/g dw antioxidant potentials). Based on the results, ethyl acetate bioactive fraction was further subjected to GC-MS studies where 58 distinct peaks were observed. Valeranone (RT: 13.67 of Total: 28.00 %) identified as the major peak with β -sitosterol (area 5.51%), isosativene (RT: 10.70, area 2.77%), hexadecanoic acid (area 3.71%) and octadecanoic acid (area 2.39%).

Key words: *Peltophorum pterocarpum*, Antimicrobial activity, Antioxidant activity, Gas chromatography-mass spectrometry.

INTRODUCTION

Plants provide an important source of natural products, many of which formed basis for the development of medicinally important drugs. Unfortunately, nature often provides such compounds in lower yields and the difficulties associated with their isolation from other less interesting and co-occurring constituent's present problems particularly when large quantities of the biologically active compounds are required. However, bioactivity-guided fractionation proved a useful method in order to structurally characterize these active constituents.

Peltophorum pterocarpum Baker ex K. Heyne is a deciduous tree usually reaching a height of 15-24 m and 50-100 cm diameter. The bark of the plant is used in dysentery, for gargles and tooth powder, eye diseases, muscular pain and in sores as lotions. Heartwood is red, hard and strong, used for coach building and cabinet work [1]. Phytochemically, peltophorin, campesterol, stigmasterol, β -sitosterol, lupeol and naringenin-7-glucoside were isolated from flowers and bark [2-4] (-)-epicatechol, (+)-leucocynidin, berginin, tannins were isolated [5] from sapwood. Antimicrobial activity of benzene and methanol fractions of flowers was conducted against several Gram

-ve and Gram +ve bacteria [6-8]. Likewise, whole plant extracts were tested against other pathogenic microbes [9-13].

In the present study, metabolites-rich fractionation of stem extract of *P. pterocarpum* was carried out in order to isolate antimicrobial and antioxidant potent fractions along with the characterization of compounds using GC-MS analysis.

MATERIALS AND METHODS

Plant materials: *P. pterocarpum* was collected from the campus, in the month of July, 2009 authenticated from the Herbarium, Department of Botany, University of Rajasthan, Jaipur, India and voucher specimen was deposited (Herbarium Sheet No. 20707). For experiment, stem of *P. pterocarpum* were collected in the months of July-August, shade-dried, weighed and powdered.

Extraction procedure: The dried plant material (200 g) was extracted in 500 ml of ethanol (3× 18 h), the extract was filtered through Whatman No. 1 filter paper, dried *in vacuo* and weighed. In order to get bioactive-rich fractions, alcoholic extract was further sub-fractionated to pet. ether (60-80 °C), dichloromethane (DCM), ethyl acetate and methanol. Each of these extracts was filtered, evaporated to dryness and weighed to calculate the extractive values (%).

Antimicrobial activity: Pure cultures of Gram +ve bacteria (*Bacillus subtilis*, MTCC 441; *Staphylococcus aureus*, MTCC 740) and Gram -ve bacteria (*Escherichia coli*, MTCC 443; *Pseudomonas aeruginosa*, MTCC 741; *Enterobacter aerogenes*, MTCC 111 and *Raoultella planticola*, MTCC 530) were obtained from IMTECH, Chandigarh. These cultures were grown and maintained on Nutrient Broth (NB) at 27 °C for 48 h. For antifungal screening, *Aspergillus flavus* (ATCC 16870), *A. niger* (ATCC 322), *Candida albicans* (ATCC 4718), *Penicillium chrysogenum* (ATCC 5476) and *Tricophyton rubrum* (ATCC 2327) obtained from IARI, New Delhi, were cultured on Sabouraud Dextrose Agar (SDA) medium at 37 °C for 48 h.

Antimicrobial assay was performed by agar well diffusion method [14]. Müller-Hinton medium for antibacterial and SDA medium were used for antifungal activity. Microbial cell suspension of about 1.5×10^6 CFU/mL obtained from a McFarland turbidity standard No. 0.5 was used. This was used to inoculate by flooding the surface of respective media plates (200 µL inoculum). Excess liquid was air-dried under a sterile hood and then wells of 6 mm diameter were made using sterile cork borer. 40 µL of test solution (4 mg/mL in DMSO) was poured inside the wells and incubated at 5–8 °C for 2–3 h to permit good diffusion. Plates were then transferred to incubator of 28 °C for 24 h. After incubation, diameter of the clear inhibition zone was measured by Inhibition zone recorder (HiMedia). A parallel negative control (solvent DMSO), and positive control gentamycin (10 µg/mL) in case of bacteria and ketoconazole (100 units/mL) in case of fungi were used.

Activity of the extract was referred in terms of activity index (AI = Inhibition zone of test sample/ Inhibition zone of standard) with comparison to standard reference drug.

Minimum inhibitory concentration (MIC): Minimum inhibitory concentration (MIC) was determined using agar well diffusion method. Serial dilutions of all the extracts were prepared ranging from 2000 µg to 20 µg and used tested as mentioned above. The diameter of the clear inhibition zone was measured by inhibition zone recorder (HiMedia) for each sample. The MIC was regarded as the lowest concentration that did not permit any visible growth after 7 d of inoculation.

Antioxidant activity using 2, 2-Diphenyl-1-picryl-hydrazyl radical (DPPH) method: The effect on DPPH radical was determined using the method of [15]. Different concentrations of extract (0.8, 0.6, 0.4, 0.2, 0.1 mg/mL) were prepared in methanol and mixed with 2.5 ml of DPPH (2 mg/10 mL methanol). After 30 min of incubation time, optical density (OD) was measured at 517 nm using a UV-Vis spectrophotometer (Varian type Cary PCB 150 Water Peltier System with Standard Cuvetts). The concentrations that cause 50% reduction in absorbance (IC₅₀) were calculated. Percent inhibition of DPPH was calculated by following equation.

$$\% \text{ Inhibition} = 1 - (\text{OD}_{\text{Sample}} / \text{OD}_{\text{Control}}) \times 100$$

Where, OD_{Sample} is the absorbance of the test sample and OD_{Control} as the absorbance of the test control.

Antioxidant activity using Ferric ion reducing antioxidant potential (FRAP) assay: Total reducing power of extracts was determined according to FRAP method [16]. Specific concentration of standard (ascorbic acid) and extracts (62.5-1000 µg/mL) were prepared in 1 mL ethanol separately, mixed with 2.5 mL each of phosphate buffer (0.2 M, pH 6.6) and potassium ferricyanide (1%). After incubation at 50°C (20 min), 2.5 mL of 10% trichloroacetic acid was added to the mixture, which was then centrifuged 1000×g for 10 min. The upper layer of solution (2.5 mL) was taken, mixed with 2.5 mL of distilled water and 0.5 mL 1% ferric chloride, incubated for 30 min and OD was measured at 700 nm. Standard calibration curve of ascorbic acid was prepared and total antioxidant potentials calculated as mg of ascorbic acid equivalents (mg AAE/g of extract). Higher absorbance of the reaction mixture indicated greater reducing power.

Gas Chromatography and Mass Spectroscopy (GC-MS): The active extract was analyzed by GC-MS (Hewlett-Packard 6890/5973 operating at 1000 eV ionization energy, equipped with a HP-5), capillary column (phenyl methyl siloxane, 25 m×0.25 mm i.d) with Helium (He) as the carrier gas (0.9 ml/min) with split ratio 1:5; oven temperature 100° C (3 min) to 280° C at 1 to 40°C per min; detector temperature, 250 to 280° C. Retention indices were determined by using retention times of samples that were injected under the same chromatographic conditions. The components of the standard and plant samples were identified by comparison of their mass spectra and retention time with those given in literature and by comparison with the mass spectra of the Wiley library or with the published mass spectra.

Statistical analysis: All determinations were carried out in triplicate and analyzed using one-way ANOVA followed by Student's t-test, where *p* values < 0.05 were considered significant.

RESULTS AND DISCUSSION

In present study the antimicrobial activity of plant extract ethyl acetate fraction was most effective against *P. aeruginosa* and *S. aureus* (IZ 17.33 ± 0.33 and 17.00 ± 0.00 mm with MIC of 31.25 and 125 µg/mL; Table 1, Table 2), in case of antifungal activity *T. rubrum* inhibited effectively by methanolic fraction (IZ 17.00 ± 1.00 mm; MIC 500 µg/mL). Methanol extract also proved effective against *E. coli*, *P. aeruginosa*, *R. planticola* and *S. aureus* (IZ 16.00 ± 0.57, 17.00 ± 1.00, 16.33 ± 0.70 mm in last two cases). Their MIC ranges 31.25-125 µg/mL. In case of antimicrobial activity, *P. pterocarpum* stem extracts exhibited higher degree of antimicrobial activity against both Gram +ve and Gram-ve bacteria. Stem ethyl acetate extracts significantly inhibited the growth of *B. subtilis* and *P. aeruginosa* with lower MIC and higher inhibition zone.

Table 1. Antimicrobial activity of *P. pterocarpum* stem.

Test microorganisms		Nature of extract			
		Pet. ether	DCM	Ethyl acetate	Methanol
<i>B. subtilis</i>	IZ ^a	14.00 ± 0.57	11.00 ± 0.00	16.00 ± 1.15	15.00 ± 0.57
	AI ^b	0.80	0.50	0.72	0.68
<i>E. aerogenes</i>	IZ	13.66 ± 0.33	11.00 ± 0.57	14.66 ± 0.66	14.66 ± 0.88
	AI	0.62	0.50	0.66	0.66
<i>E. coli</i>	IZ	13.00 ± 0.00	10.00 ± 0.00	14.66 ± 0.33	16.00 ± 0.57
	AI	0.68	0.52	0.77	0.84
<i>P. aeruginosa</i>	IZ	14.33 ± 0.33	12.33 ± 0.33	17.33 ± 0.33	17.00 ± 1.00
	AI	0.71	0.61	0.86	0.78
<i>R. planticola</i>	IZ	13.00 ± 0.57	10.66 ± 0.82	15.33 ± 0.66	16.33 ± 0.66
	AI	0.69	0.48	0.69	0.74
<i>S. aureus</i>	IZ	13.66 ± 0.60	11.33 ± 0.81	17.00 ± 0.00	16.33 ± 0.70
	AI	0.65	0.53	0.80	0.77
<i>A. flavus</i>	IZ	-	-	-	-
	AI	-	-	-	-
<i>A. niger</i>	IZ	12.33 ± 1.20	14.66 ± 0.32	10.66 ± 0.66	12.00 ± 1.00
	AI	0.68	0.81	0.59	0.68
<i>C. albicans</i>	IZ	13.33 ± 0.33	10.33 ± 0.33	15.00 ± 0.57	15.00 ± 0.00
	AI	0.60	0.57	0.68	0.68
<i>P. chrysogenum</i>	IZ	12.66 ± 0.33	10.33 ± 0.33	12.66 ± 0.33	10.00 ± 0.57
	AI	0.60	0.49	0.60	0.61
<i>T. rubrum</i>	IZ	12.66 ± 0.33	10.00 ± 1.00	14.66 ± 0.33	17.00 ± 1.00
	AI	0.49	0.39	0.54	0.62

Test samples 4 mg/well; ^aIZ = Inhibition zone (in mm); ^bAI = Activity index;
DCM = dichloromethane.

Table 2. MIC of *P. pterocarpum* stem extracts.

Test microorganisms	Nature of extract			
	Pet. ether	DCM	Ethyl acetate	Methanol
<i>B. subtilis</i>	125	NT	125	62.5
<i>E. aerogenes</i>	125	NT	31.25	31.25
<i>E. coli</i>	125	NT	31.25	62.5
<i>P. aeruginosa</i>	125	NT	31.25	31.25
<i>R. planticola</i>	250	NT	250	125
<i>S. aureus</i>	31.25	NT	125	125
<i>A. flavus</i>	-	-	-	-
<i>A. niger</i>	125	NT	125	62.5
<i>C. albicans</i>	1000	NT	250	125
<i>P. chrysogenum</i>	125	NT	125	125
<i>T. rubrum</i>	500	NT	250	500

NT = MIC not performed due to poor yield. ^aIZ = Inhibition zone (in mm); ^bMIC = Minimum inhibitory concentration in µg/ml.

DPPH radical-scavenging method was employed to evaluate the antioxidant activities of test extracts/fractions due to its simplicity, sensitivity and reproducibility [17]. In DPPH activity, ethyl acetate extract showed significant activity i.e. 6 µg/mL IC₅₀ value with % inhibition of 96.70 ± 0.22 at 80 µg/mL concentration followed by its methanolic extract with IC₅₀ 7 µg/mL (% inhibition 94.25 ± 0.59). Similar observations were recorded on FRAP method where ethyl acetate extract demonstrated 1310.00 ± 0.00 AAE/mg dw antioxidant potential followed by its methanolic extract (1240.00 ± 5.79 AAE/mg dw antioxidant potential; Table 3). Likewise, stem extracts demonstrated higher levels of antioxidant potentials. In the present study, the IC₅₀ of stem ethyl acetate extracts (6 µg/mL) indicated similar antioxidant activity to quercetin (4 µg/mL), and more effective than *Ginkgo biloba* extract whose IC₅₀ is 40.72 µg/mL [18, 19]. The standardized extract of *G. biloba* has been widely employed for its significant benefit in neurodegenerative disorders [20].

Table 3. *In vitro* antioxidant activity of *P. pterocarpum* by DPPH and FRAP method

Nature of extract	DPPH method [^a % Inhibition (concentration in µg/ml)]					
	IC ₅₀	10	20	40	60	80
Pet. ether	7.5	68.23 ± 0.51	83.95 ± 0.62	88.34 ± 0.88	89.36 ± 0.04	92.58 ± 0.17
DCM	NT	NT	NT	NT	NT	NT
Ethyl acetate	6	83.95 ± 0.58	90.54 ± 1.12	96.39 ± 0.22	96.52 ± 0.21	96.70 ± 0.22
Methanol	7	70.81 ± 0.78	86.17 ± 0.37	93.09 ± 0.42	93.73 ± 0.78	94.25 ± 0.59
Quercetin	4	62.42	80.58	93.38	93.82	94.71
Nature of extract	FRAP method [activity in mg AAE/g dw ^b (concentration in µg/ml)]					
	62.5	125	250	500	1000	
Pet. ether	51.66 ± 3.33	81.66 ± 6.67	191.66 ± 6.01	358.33 ± 10.94	785.65 ± 10.42	
DCM	NT	NT	NT	NT	NT	
Ethyl acetate	76.66 ± 6.12	88.33 ± 3.33	213.00 ± 7.24	728.33 ± 3.33	1310.00 ± 0.00	
Methanol	56.66 ± 1.66	98.33 ± 3.33	275.23 ± 8.68	573.33 ± 9.36	1240.00 ± 5.79	
Ascorbic acid	62.5	125	250	500	1000	

NT = not performed due to poor yield; ^a% Inhibition = 1 - (Absorbance of the sample / Absorbance of the control) × 100; ^bmg AAE/g = mg Ascorbic acid equivalent/g extract.

In our studies we are able to identify and quantify several monoterpenes and sesquiterpenes components of ethyl acetate extract. Most of the identified compounds exhibited valuable pharmacological activities. Major compound jatamansone/valeranone has a well known anticonvulsant property, whereas epiglobulol have antiseptic and cytotoxic behavior. Other isolated compounds such as valeranal, isosativen and mgastigma-4,6e, 8e triene were also well known for their antimicrobial and antioxidant properties. As compounds were identified using Gas Chromatography-Mass Spectroscopy (GC-MS) equipped with NIST05 library databases. These databases were compiled using more than 80,000 electron compact (EI) mass spectra. Only matching of large degree of certainty using reverse fit mode were accepted.

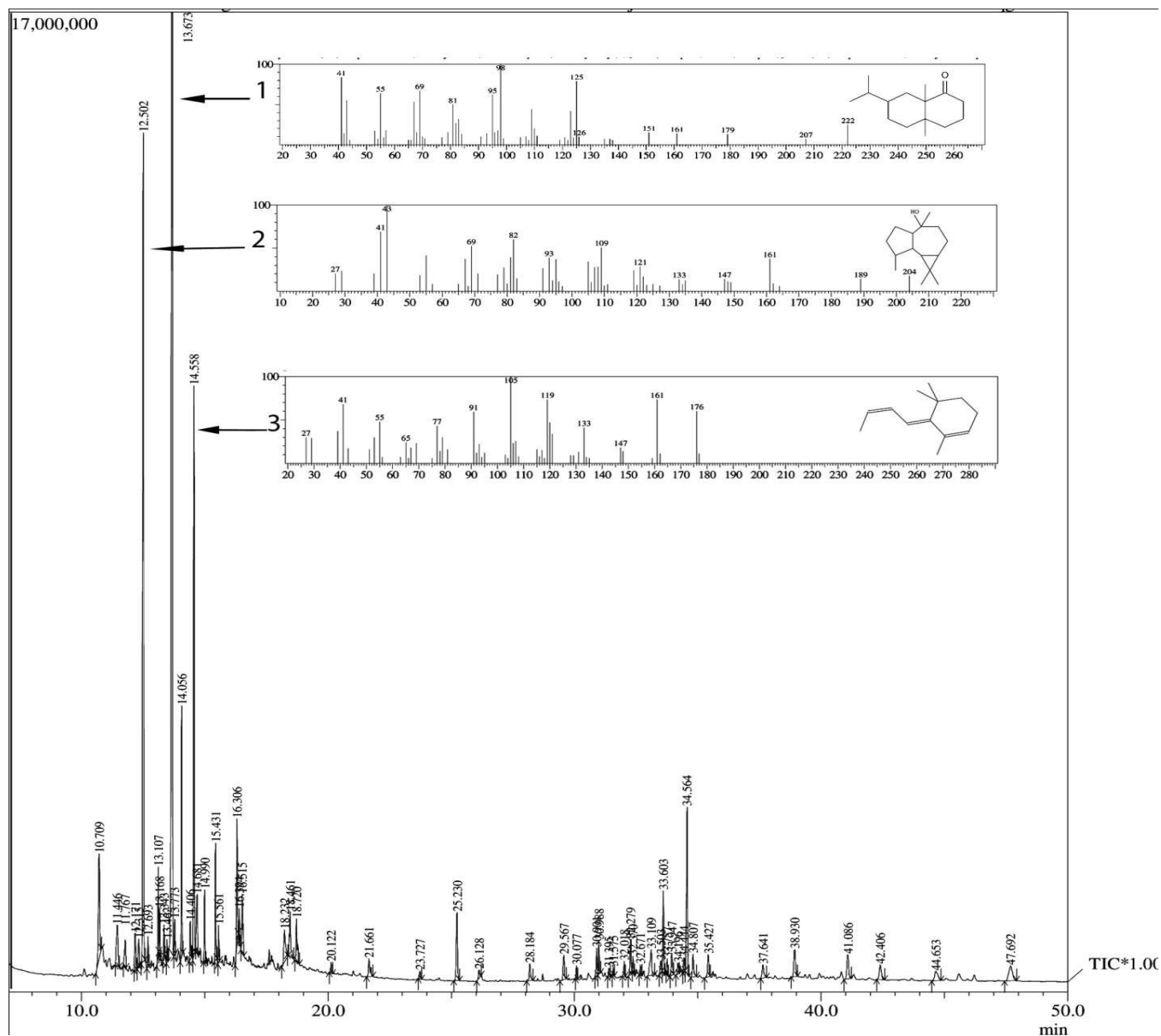
On GC-MS analysis, ethyl acetate extract structures revealed the presence of sesquiterpenes, steroids, oil and flavonoids (Table 3). 58 distinct peaks were identified by GC-MS while the compounds identified through the NIST05.LIB database. The major compound present in the extract was Valeranone with RT: 13.67 and total: 28.00 % availability. Mass spectrum of major compound was shown in Figure 1, molecular formula C₁₅H₂₆O, molecular weight 222 with tranquilizing and anticonvulsant activities. Compound also reduces aggressiveness. Other major components were Epiglobulol (RT: 12.50, area 16.86%), Megastigma-4,6e, 8e triene (RT: 14.55, area 9.12%), valerenal (area 3.30%), isosativene (RT: 10.70, area 2.77%) and hexadecanoic acid (area 2.39%). Further, the biological activities of major components were given in Table 4.

Table 4. GC-MS spectra of stem ethyl acetate extract of *P. pterocarpum*.

Peak	R. Time	Area %	Name	Molecular formula	Molecular weight	Similarity index	Properties known
1.	10.709	2.77	Isosativen	C ₁₅ H ₂₄	204	88	Antiseptic, bactericidal, antiinflammatory analgesic
2.	11.446	1.35	(+)-Cycloisosativene	C ₁₅ H ₂₄	204	90	"
3.	11.767	0.67	(-)- α -Panasinsen	C ₁₅ H ₂₄	204	85	-
4.	12.171	0.73	2,5,5-Trimethyl-3-hexyn-2-ol	C ₉ H ₁₆ O	140	83	-
5.	12.315	0.55	1-Ethyl-4,4-dimethyl-cyclohex-2-en-1-ol	C ₁₂ H ₂₂ O ₂	198	80	-
6.	12.502	16.86	Epiglobulol	C ₁₅ H ₂₆ O	222	85	Antiseptic, cytotoxic
7.	12.693	0.52	Viridiflorol	C ₁₅ H ₂₆ O	222	94	Antimicrobial, antifungal
8.	13.168	0.67	Bicyclogermacrene, Cordinol	C ₁₅ H ₂₄	204	82	Antifungal
9.	13.342	0.57	δ -Cedrol	C ₁₅ H ₂₆ O	222	81	Antioxidant
10.	13.673	28.00	Jatamansone, Valeranone	C ₁₅ H ₂₆ O	222	95	Tranquilizing, reducing aggressiveness, anticonvulsant
11.	14.056	3.30	Valerenal	C ₁₅ H ₂₂ O	218	96	CNS-depressant, antispasmodic effects
12.	14.558	9.12	Megastigma-4,6e, 8e triene	C ₁₃ H ₂₀	176	85	Antimicrobial, fragrance
13.	14.990	1.04	Valerenic acid	C ₁₅ H ₂₂ O ₂	234	89	CNS-depressant, sleep enhancing, antispasmodic, sedative
14.	15.431	1.54	1,2-benzenedicarboxylic acid	C ₁₆ H ₂₂ O ₄	278	95	Insecticidal, pesticide, antitumor
15.	16.306	2.39	Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	256	95	Antioxidant, nematocide, hypocholesterol-mic , pesticide
16.	18.232	1.20	9,12-Octadecadienoic acid	C ₁₈ H ₃₂ O ₂	280	91	Anticarcinogenic antiatherogenic, antioxidant, anti-inflammatory
17.	18.461	1.19	9-Octadecenoic acid ethyl ester	C ₂₀ H ₃₈ O ₂	310	92	-
18.	18.720	1.00	1-Docosene	C ₂₂ H ₄₄	308	93	Antimicrobial, anticarcinoma
19.	21.661	0.59	1-Tricosanol	C ₂₃ H ₄₈ O	340	94	Hepatoprotective
20.	25.230	1.79	Di-N-Octyl phthalate	C ₂₄ H ₃₈ O ₄	390	97	γ -ray absorbers, plasticizers, lubricants
21.	29.567	0.62	Ethyl docosanoate	C ₂₄ H ₄₈ O ₂	368	90	Antibody production
22.	30.901	0.53	Octacosane	C ₂₈ H ₅₈	394	93	Mosquitocidal, antifeedant
23.	30.988	0.62	n-Hentriacontanol-1	C ₃₁ H ₆₄ O	452	95	Herbicidal, anti-inflammatory, in rheumatism, gout, jaundice, anti-pyretic
24.	32.370	0.24	β -Sitosterol	C ₂₉ H ₅₀ O	414	83	Antimicrobial, antiinflammatoryanalgesic, antipyretic
25.	33.109	0.91	Cholesteryl propanoate	C ₃₀ H ₅₀ O ₂	442	82	-
26.	34.196	0.24	Cholesta-4,6-dien-3-ol, benzoate	C ₃₄ H ₄₈ O ₂	488	78	Antitumor, anti-inflammatory, antioxidant, antiulcerogenic,
27.	34.807	0.63	Vitamin E acetate	C ₃₁ H ₅₂ O ₃	472	91	Antioxidant
28.	41.086	0.89	Lupeol	C ₃₀ H ₅₀ O	426	89	Antitumor, cardioprotective, hepatoprotective, antimicrobial
29.	42.406	0.63	Stigmast-4-en-3-one	C ₂₉ H ₄₈ O	412	90	Antiplasmodial, antimicrobial, hypoglycemic, cardioactive
30.	44.653	0.60	17-[5-(1-Hydroxy-1-methyl-ethyl)-2-methyl-tetrahydro-furan-2-yl]	C ₃₀ H ₅₀ O ₅	490	78	-
31.	47.692	1.10	Cholest-4-ene-3,6-dione	C ₂₇ H ₄₂ O ₂	398	83	Antifungal
		100.0					

Components were tentatively identified based on library and literature searches and only those components showing matches exceeding 80% were selected.

Figure 1. Chromatogram of *P. pterocarpum* bark ethyl acetate extract containing (1) Jatamansone, (2) Epiglobulol and (3) Megastigma-4,6e, 8e triene.



On GC-MS reports, isolated secondary plant metabolites exert a wide range of biological activities (as documented in Table 3) on physiological systems [21, 22] also reported hexadecanoic acid, ethyl ester and n-hexadecanoic acid, unsaturated fatty acid, docosatetraenoic acid and octadecatrienoic acid as antimicrobial, anti-inflammatory, antioxidant, hypocholesterolemic, cancer preventive, hepatoprotective, antiarthritic, antihistimic, antieczemic and anticoronary properties. Presence of lipophilic compounds (mainly long chain fatty acids) can be closely related to its antibacterial activity, as these compounds easily pass through lipidic coating of bacteria [23] and coagulate cellular proteins.

Results of the present study indicate that stem extracts possessed appreciable antioxidant and antimicrobial activities i.e. higher/similar to standards ascorbic acid (Vitamin C), quercetin (for antioxidant activity) and gentamycin, ketonocozole (for antimicrobial activity). On GC-MS analysis 45 compounds were identified and quantified. In

several compounds higher biological activities were reported. The wood of the plant appeared to have the highest concentrations of the antioxidants and coincidentally, traditional healers also use the stem (wood) concoctions in dysentery, gargles and tooth powder. The investigated plant extracts may be useful to pharmaceutical and natural therapies for the treatment of infectious diseases in human and plants.

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