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Phytochemical Composition and Anti-microbial Properties of *Petersianthus macrocarpus* (P.Beauv.) Liben (Lecythidaceae) Stem Bark Extract and Fractions

Joseph M. Nwosu[1], Fabian I. Eze[2]*, Peter C. Ayogu[3], Jacob U. Inuaeyen[2], Stephanie O. Emmanuel[2], Ezinne B. Ugwu[2], Onyinyechukwu Y. Ugonabo[2], Matthias O. Agbo[2], Chidimma G. Ezema[4], Benjamin E. Ezema[1*]

[1]*Department of Pure and Industrial Chemistry, University of Nigeria Nsukka, 410001, Nigeria*
[2]*Department of Pharmaceutical and Medicinal Chemistry, University of Nigeria Nsukka, 410001, Nigeria*
[3]*Department of Applied Microbiology and Brewing, Enugu State University of Science and Technology, Enugu, Nigeria*
[4]*National Centre for Energy Research and Development, University of Nigeria Nsukka, 410001, Nigeria*

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ABSTRACT

Petersianthus macrocarpus(Lecythidaceae) is commonly used in traditional medicine in southeastern part of Nigeria for treating infectious diseases. In the current study, qualitative and quantitative phytochemical composition, and antimicrobial properties of 95% aqueous methanolic stem bark extract, and various solvent fractions of *P. macrocarpus*, were evaluated. The total phenolics content (TPC) and total tannins content (TTC) were evaluated spectrophotometrically according to the Folin-Ciocalteu's protocol, while the total flavonoids content (TFC) was assessed according to the aluminum chloride colorimetric assay. The aqueous methanolic extract showed 706.61 ± 0.20 mg gallic acid equivalents GAE/g of extract, 131.42 ± 0.06 mg GAE/g of extract and 249.6 ± 0.14 mg quercetin equivalents QE/g of extract for TPC, TTC and TFC respectively. The ethyl acetate fraction showed higher TTC than the absolute methanol fraction but lower TPC and TFC. The antimicrobial properties of the extracts were evaluated against clinical strains of bacteria and fungi using the agar disc diffusion technique. All the evaluated samples showed inhibitory effect at 5.0 mg/mL against the tested bacteria and fungi, with the inhibition zone diameter (IZD) values ranging from 10 - 20 mm. The ethyl acetate fraction of the extract showed minimum inhibitory concentrations (MIC) of 0.81 mg/mL and 0.82 mg/mL against *Aspergillus niger* and *Candida albicans* respectively. *P. macrocarpus* stem bark is a rich source of phenolics, tannins and flavonoids which may be responsible for its ethno-medicinal use in treating microbial infections.

Keywords: *Petersianthus macrocarpus*, Quantitative phytochemical, Anti-microbial, Clinical.

Introduction

Petersianthus macrocarpus (P.Beauv.) Liben (Lecythidaceae) is an evergreen tree which occurs mainly in the West and Central African rain forests and is harvested from the wild for local use as a medicine and source of wood.[1] It is widely used in the folkloric medicine of the southeastern part of Nigeria, where it is locally known as *Ogbu Onya*, for the relief of pains, malarial fever, as an anti-cancer agent and for treating skin infections.[2-3] In Cameroon, the stem bark is commonly taken as a remedy the infections and disorders of the gastrointestinal tract, including dysentery.[4] Previous reports on the pharmacological properties of *P. macrocarpus* portray the plant as a potential source of new drug leads. The aqueous stem bark extract has been proved to be a rich source of some triterpenic saponins with both estrogenic and anti-estrogenic potency.[5] The antimicrobial activities of various extracts of the stem bark against laboratory strains of bacterialand yeasts implicated in gastrointestinal disorders in Cameroon has been reported.[4]

*Corresponding author. E mail: fabian.eze@unn.edu.ng; benjanin.ezema@unn.edu.ng
Tel: +2348064714208; +2348066072738

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The stem bark extracts have been shown to exhibit strong filaricidal effect against Loa loa.[6] High concentrations of the bark extract have been reported to act on smooth muscles, heart-muscles and interfere with the oestrus cycle, conception and pregnancy.[4] An ethanolic leaf extract of the plant showed antiproliferative activity on human colon cancer cells.[2] The stem bark extract has also been confirmed to demonstrate strong anti-oxidant and anti-nociceptive properties.[3,7]

The phytochemical investigation on the ethanol extract of dry powdered stem bark of *P. macrocarpus* showed the presence of flavonoids, alkaloids, saponins, terpenoids, tannins, steroids, acidic compounds and reducing sugars.[8] The n-butanol extract of the stem bark has previously yielded two new triterpenoid saponins, petersaponins III and IV.[9]

Though research has been carried out on the preliminary phytochemical composition of *P. macrocarpus* stem bark extracts, report on the quantitative determination of the major classes of the phytochemicals in the extracts and fractions is lacking. Moreover, edaphic and other environmental factors are known to influence the phytochemical composition of plants and the previous report was on the Cameroonian species of the plant. Whereas the anti-microbial data on the laboratory strains of micro-organisms are available,[4] we are not aware of the data against clinical isolates of *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus cereus*, *Aspergillus niger and Candida albicans*. Multidrug-resistant microorganisms are on the increase nowadays due to indiscriminate use of the conventional antimicrobial drugs and the mutation of pathogens,[10] hence, the need to develop new anti-microbial drugs from plants and test them on the clinical isolates of the organisms which may have resisted the usual antibiotics. The aim of the current study, therefore, was to investigate the qualitative and quantitative phytochemical composition and the antimicrobial properties of *P. macrocarpus* (P.Beauv.) Liben

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(Lecythidaceae) stem bark extract and fractions on the clinical isolates of *S. aureus, E. coli, P. aeruginosa, B. cereus, A. niger and C. albicans*

Materials and Methods

Chemicals and equipment
Analytical grade of methanol, n-hexane, dimethyl sulphoxide (DMSO) and ethyl acetate (Sigma-Aldrich, Germany), gallic acid (Qualikems, India), quercetin (BDH, England) and the Folin-Ciocalteu's Phenol Reagent (FCPR, Lob. Chem., India) were used. All other chemicals used were of AnalaR grade. Concentration of extracts was done in rotary evaporator (Büchi Rotavap. R-200, Germany), while all spectral data were recorded on UV-visible spectrophotometer (UV-1800, Shimadzu, Japan),

Test microorganisms
Clinical isolates of the following microorganisms, implicated in infections and disorders, were sourced from the Pharmaceutical Microbiology Department, University of Nigeria. These include four species of bacteria namely *S. aureus, E. coli, P. aeruginosa, B. cereus*, and two fungal species: *A. niger* and *C. albicans.*

Plant materials
The stem bark of *P. macrocarpus* Liben was obtained from Ariam-Usaka community in Abia State, Nigeria during October 2018. The plant was authenticated at the Int. Centre for Ethnomed. & Drug Dev. (InterCEDD), Nsukka, Nigeria. A voucher sample (with code no: P. Macrocarpus/InterCEDD/043) was kept in the herbarium.

Extraction of plant material
The stem bark of *P. macrocarpus* (239.0 g) was air-dried, crushed and macerated with 2.5 L of 95 % aqueous methanol[11] at 25 ºC for 24 h and filtered. The filtrate was concentrated at 40°C *in vacuo* at reduced pressure to afford the crude extract CE, 21.1 g (8.82 %). A portion (12.0 g) of the CE was purified using vacuum liquid chromatography (VLC) in which silica gel column was eluted sequentially with n-hexane, ethylacetate and absolute methanol to give the solvent fractions: n-hexane soluble fraction (PMHF), 0.3 g (2.5%), ethyl acetate soluble fraction (PMEF), 3.5g (29.17%), and methanol soluble fraction (PMMF), 7.9 g (63.83%) respectively.

Qualitative phytochemical analysis
The qualitative phytochemical tests were carried out on the crude extract and solvent fractions following the standard techniques,[12-14] while the quantitative analysis was carried out as previously reported. [14-20]

Evaluation of the total phenolics content (TPC)
The TPC was estimated by way of the modified Folin-Ciocalteu technique with gallic acid as a reference standard. [16] Briefly, distilled water (9 mL) was added to 1 g each of the test extract and fractions in 25 mL flasks followed by dilute (1:10) FCPR (25 mL). Each of the mixtures was allowed to stand for 5 min after which 10 mL of 7.5% Na_2CO_3 solution was added and made up to the mark with water. Incubation of each mixture lasted for 1h 30 min at 25 ºC. Various standard solutions of gallic acid were similarly incubated to generate the calibration graph. The absorption frequencies of the test samples and standard solutions were read against the reagent blank at a wavelength of 760 nm. The TPC was obtained from the calibration graph and given as milligrams of gallic acid equivalent (GAE) per gram of the extract and fractions. Triplicate measurement was done for each sample and the mean obtained.

Determination of total tannins content (TTC)
The TTC was also determined according to the modified Folin-Ciocalteu protocol with gallic acid as a reference standard.[18] In brief, 7.5 mL of distilled water was added to 1.0 g each of the extract and the solvent fractions in a 10 mL volumetric flask. Dilute (1:10) FCPR (0.5 mL) was added to each of the mixtures, followed by 35 % Na_2CO_3 solution (1 mL). The mixtures were made up to mark with distilled water before incubation in the dark for 30 min at 25 ºC. The absorption frequencies of the solutions were read against at a wavelength of 725 nm. The TTC was calculated from the calibration curve (generated using gallic acid) and given as milligram of gallic acid equivalent (GAE) per gram of the extract and fractions.[19] The determinations of the total tannins content in the extract and fractions were done in triplicates.

Estimation of the total flavonoids content (TFC)
The TFC measurement in the plant samples were carried out according to the slightly modified aluminum chloride colorimetric assay protocol, with quercetin as a reference standard.[16] Distilled water (4 mL) was added to each of the test extract and fractions (1.0 g each) in 10 mL volumetric flasks followed by a 5% sodium nitrite (0.30 mL). A solution of 10 % $AlCl_3.6H_2O$ (0.30 mL) was added to each of the mixtures after 5 min, followed by 2 mL of 1 M NaOH solution after additional 5 min and made up to the mark with water. Various standard solutions of quercetin were similarly treated with sodium nitrite and $AlCl_3.6H_2O$ to generate the calibration graph. The absorption frequencies of all the solutions extract, fractions and standard solutions were read at 510 nm. The TFC was calculated appropriately from the calibration graph and given as milligram of quercetin equivalent (QE) per gram of extract and fractions.[20] The TFC data were obtained in triplicates.

Evaluation of the antimicrobial activity
The agar disc diffusion protocol [21-22] was followed. Solutions of each of the extract and fractions (5 mg/mL) were prepared separately in 10 % solution of DMSO. The wells were filled with the extract and fractions (0.05 mL) with the aid of Pasteur pipette. Standard antibiotic (tetracycline) and antifungal (fluconazole) were used as positive controls while sterile distilled water and DMSO served as negative controls. IZDs were determined using a metre rule after incubating plates at 37ºC for 24 h (for bacteria) and 25ºC for 48 h (for fungi). The results were tabulated and graphs of logarithm of concentration against IZD square were plotted. The MICs of the test samples and controls were read from the regression equations of the plots of logarithm of the sample concentration against the square of IZD.

Statistical analysis
All experimental data were obtained in triplicate and given as mean of three vaues ± standard deviation of (3n) measurements. Graphical analyses were done using Microsoft Office Excel applications (Microsoft Inc. USA).

Results and Discussion

The preliminary qualitative phytochemical screening of the 95% aqueous methanolic extract and solvent fractions of the stem bark of *P. macrocarpus* revealed the presence of saponins, tannins, flavonoids and terpenoids as summarized in Table 1.
The TPC, TFC and TTC of the extract and solvent fractions were calculated from the regression equations: $y = 0.0011x + 0.0794$, $R^2 = 0.988$; $y = 0.001x + 0.0924$, $R^2 = 0.974$ and $y = 0.0033x + 0.0746$, $R^2 = 0.9926$ respectively. The results are presented in Table 2.

Table 1: Qualitative phytochemical composition
KEY: + = present; - = Not detected.

Secondary metabolites	Relative Abundance			
	CE	PMEF	PMMF	PMHF
Saponins	+	+	+	-
Tannins	+	+	+	-
Flavonoids	+	+	+	-
Alkaloids	-	-	-	-
Terpenoids	+	+	-	+
Steroids	-	-	-	+

Table 2: Quantitative phytochemical composition of the extract and solvent fractions

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Sample code	TPC(mg GAE/g)	TFC (mg QE/g)	(TPC/TFC)	(TTC) (mg GAE/g)
CE	706.61 ± 0.20	249.6 ± 0.14	2.83	131.42 ± 0.06
PMHF	ND	ND	ND	ND
PMEF	165.4 ± 0.10	59.3 ± 0.01	2.79	74.96 ± 0.02
PMMF	448.4 ± 0.15	141.3 ± 0.08	3.17	11.22 ± 0.01

ND = Not detected. Values are presented as mean ±SD (n = 3).

The inhibition zone diameter (at 5 mg/mL solution) and the MIC of the test extracts, as a function of antimicrobial activities, are given in Tables 3 and 4 respectively. The qualitative phytochemical test on various extracts of the stem bark of *P. macrocarpus* has confirmed that it contains flavonoids, tannins, saponins and terpenoids as the major components (Table 1). The quantitative phytochemical evaluation revealed that the crude methanol extract, CE, contained 706.61 ± 0.2 mg (GAE)/g of extract, 131.42 ± 0.06 mg GAE/g of extract and 249.6 ± 0.14 mg (QE)/g of extract for TPC, TTC and TFC respectively. The ethyl acetate fraction of the extract was found to have higher TTC (74.96 ± 0.02 mg GAE/g of fraction) than the methanol fraction of the extract (11.22 ± 0.01 mg GAE/g of fraction). However, the methanol fraction showed higher TPC and TFC (448.4 ± 0.15 mg GAE/g and 141.3 ± 0.08mg QE/g of fraction respectively) than the ethyl acetate fraction (165.4 ± 0.10 mg GAE/g and 59.3 ± 0.01 mg QE/g of fraction respectively). It is evident from the results that the plant is a rich source of phenolics, tannins and flavonoids.

The role of polyphenols as strong antioxidants has been reported.[23] Reactive oxygen rich species and associated free radicals have been proved to cause various human ailments such as inflammatory and metabolic disorders, diabetes mellitus, cancers, rheumatoid arthritis, AIDS and cellular aging.[24] The secondary metabolites of plants are known to be produced as a defense mechanism against predation by many microorganisms, insects and herbivores.[25] Thus, the abundance of phenolics, tannins and flavonoids in the stem bark of *P. macrocarpus* as revealed by this study may be responsible for its anti-microbial properties and other ethnopharmacological uses.

The results of the antimicrobial assay on the extract and solvent fractions against selected pathogenic microorganisms (Tables 3 and 4) show that the extract and all the fractions evaluated exhibit inhibitory action at 5.0 mg/mL against all the microorganisms tested with the IZD ranging from 10 - 20 mm. Ethyl acetate fraction of the extract showed the highest IZD of 20 mm against *A. niger*.

The most susceptible bacterium was *E. coli*, followed by *B. cereus*, while *P. aeruginosa* and *S. aureus* had equal average susceptibility to the extract and solvent fractions. On the other hand, *A. niger* was the most susceptible fungus followed by *C. albicans* which also showed the least average susceptibility of all the test microorganisms against 5.0 mg/mL of extract and solvent fractions. The MIC values obtained are given in Table 4. Test samples with MIC values less than 100 µg/mL (i.e. 0.1 mg/mL) were considered to have good antimicrobial activity; 100 to 500 µg/mL (i.e. 0.1 to 0.5 mg/mL) were considered moderate; from 500 to 1000 µg/mL (i.e. 0.5 to 1.0 mg/mL) the antimicrobial activity was weak; above 1000 µg/mL, the extract/fraction was considered inactive.[26] An MIC value less than, or equal to, 8000 µg/mL against any of the tested fungi, was also considered to indicate good activity.[27] The results obtained, therefore, showed that the extract and fractions presented mild activities against the pathogenic bacteria tested. The activity could, however, be improved by further purification. The extract and solvent fractions presented very good activities against the two pathogenic fungi tested. Ethyl acetate fraction was the most active against *A niger* and *C albicans* with MIC of 0.81 mg/mL and 0.82 mg/mL respectively, followed by methanol fraction with MIC of 0.86 mg/mL and 1.25 mg/mL respectively.

Table 3: Anti-microbial susceptibility profile of the crude extract and fractions

Sample	Inhibition zone diameter (IZD) (mm)					
	Ec	***Pa***	***Bc***	***Sa***	***Ca***	***An***
CE	17	13	11	17	13	18
PMMF	**14**	**13**	**17**	**14**	**13**	**18**
PMEF	**13**	**15**	**15**	**10**	**13**	**20**
Tetracycline	**19**	**25**	**16**	**15**	-	-
Fluconazole	-	-	-	-	**25**	**25**

Ec = E. coli; Pa = P. aeruginosa; Bc = B. cereus; Sa = S. aureus; Ca = C. albicans; An = A. niger; - = Not Tested

Table 4: Minimum inhibitory concentration (MIC) of the extract and fractions against the test microorganisms

Sample	MIC (mg/mL)					
	Ec	***Pa***	***Bc***	***Sa***	***Ca***	***An***
CE	1.25	2.50	2.50	1.25	2.50	0.86
PMMF	1.25	1.25	0.74	1.25	1.25	0.86
PMEF	1.25	0.76	1.25	2.50	0.82	0.81
Tetracycline	0.92	0.43	0.76	0.39	-	-
Fluconazole	-	-	-	-	-	-

Ec = E. coli; Pa = P. aeruginosa; Bc = B. cereus; Sa = S. aureus; Ca = C. albicans; An = A. niger; - = Not Tested

Conclusion

The results of the present work indicate that the stem bark of *P. macrocarpus* contains high quantities of phenolic compounds and exhibits fairly good anti-microbial properties against the tested bacteria and fungi. The presence of large quantities of flavonoids, saponins, tannins and polyphenols in methanol extract and solvent fractions may account for their antimicrobial activities. This lends credence to the use of the plant parts in folk medicine for the treatment of various diseases whose symptoms might involve fungal and/or bacterial infections. It also underscores the importance of the ethno botanical approach for selection of plants in the discovery of new bioactive compounds. Further purification and isolation of pure compounds from the extracts may result in the discovery of new, potent antimicrobial drug leads. The plant can also be used to formulate potent anti-microbial herbal medicines for treating diseases caused by bacterial infection.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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