applied sciences

MDPI

Article

Evaluation of Saponin Extract from *Vitex doniana* and *Pentaclethra macrophylla* for Antibacterial Activity

Nneoma E. Akaniro-Ejim [1], Chibuike S. Ubani [2], Nkoyo I. Nubila [3], Alexander A. Nzei [4], Uchechukwu U. Nwodo [5,6,*] and Anthony I. Okoh [5,6]

1 Department of Biological Sciences, Godfrey Okoye University, Enugu 400211, Nigeria; nakaniro@yahoo.com
2 Department of Biochemistry, University of Nigeria, Nsukka 410001, Nigeria; chike.ubani@unn.edu.ng
3 Department of Pharmacology and Therapeutics, University of Nigeria, Nsukka410001, Nigeria; meldabassy@yahoo.com
4 Department of Sociology and Anthropology, University of Nigeria, Nsukka 410001, Nigeria; alexnzei@gmail.com
5 SAMRC Microbial Water Quality Monitoring Centre, University of Fort Hare, Private Bag X1314, Alice 5700, South Africa; AOkoh@ufh.ac.za
6 Applied and Environmental Microbiology Research Group (AEMREG), Department of Biochemistry and Microbiology, University of Fort Hare, Private Bag X1314, Alice 5700, South Africa
* Correspondence: UNwodo@ufh.ac.za; Tel.: +27-0-406022693; Fax: +27-0-862707453

Academic Editor: Helmut Martin Hügel
Received: 21 March 2016; Accepted: 31 May 2016; Published: 17 June 2016

Abstract: Saponins are pharmacologically active compounds that have been shown to ameliorate abnormal physiological processes and be aptly applied in folklore for the treatment of maladies occasioned by infectious agents. Consequently, saponins from *Vitex doniana* and *Pentaclethra macrophylla* were evaluated for antibacterial properties, as these herbs are used in folk medicine. Dried pulverized plant materials were defatted, and solvents with varying polarity were applied at varying ratios for the extraction of saponins. Phyto-chemistry was in accordance with standard methods, while an antibacterial assay was made through the agar well diffusion and micro broth dilution techniques. Phytochemical quantitation showed high concentrations of tannins, 231 $\pm$ 0.6 CE/g, and saponins, 58% from *V. doniana*. Similarly, *P. macrophylla* stem bark extract also showed high concentrations of tannins, 309 $\pm$ 2.42 CE/g, alkaloids, 71% $\pm$ 0.5%, and saponins, 87% $\pm$ 3.4%. The ethanol extracts of *V. doniana* inhibited the growth of *Staphylococcus aureus* (ATCC 11775) and a clinical strain with inhibition zone ranges of 15.5 $\pm$ 2.12 to 7.0 $\pm$ 0.0 (mm) against leaf extracts and 20.0 $\pm$ 1.41 to 7.0 $\pm$ 0.0 (mm) against stem bark extracts. Conversely, saponin extract from *V. doniana* showed a broad spectrum of activity, as it inhibited both Gram-negative and -positive test strains, *E. coli* clinical strain (20.0 $\pm$ 1.41 mm), *P. aeruginosa* clinical strain (18.5 $\pm$ 0.71 mm), *E. coli* ATCC 11775 (17.0 $\pm$ 0 mm), and *S. aureus* clinical strain (13.0 $\pm$ 1.41 mm). However, a broad spectrum was similarly achieved with *P. macrophylla* extracts, as all test bacteria genus was susceptible. Saponin fractions showed a high potency and broad spectrum antibacterial activity and thus a validation of the folklore applications and the potential for use as a drug or drug scaffold.

Keywords: phyto-constituents; folk medicine; cold maceration; aglycone; steroidal saponins

1. Introduction

The therapeutic properties of ethnobotanicals are adduced to the functional roles of their pharmacologically active phyto-constituents [1,2]. Consequently, ethnobotanicals have been reported to possess ameliorative potentials towards some physiological anomalies including hyper/hypo-tension, atherosclerosis, hypo/hyper-glycaemia, erectile dysfunction, antiphlogistic, immunomodulatory effects, anti-allergic activities, anti-neoplastic properties, and the corrective effects/management

of several metabolic syndromes in humans and livestock [3–5]. In the same vein, functional properties of ethnobotanicals unconnected with the amelioration of abnormal physiological processes in humans and livestock include fungicidal, antibacterial, and antiviral activities [2,6]. The plethora of desirable functions displayed by ethnobotanicals, as elicited by the myriad phyto-constituents, has been chronicled in several studies.

Vitex doniana and *Pentaclethra macrophylla* are folklore herbs common with herbal healers of Southeastern Nigeria; tribal communities in this geographical region use these herbs in folk medicine for the treatment of various ailments [7–10]. *V. doniana* and *P. macrophylla* are respectively known as black plum and African oil bean commonly. Further, *V. doniana* and *P. macrophylla* belong to the Verbenaceae and Leguminosae families, respectively [11,12]. These plants are widely distributed in the Eastern and Western parts of Nigeria [13–15]. Decoctions of *V. doniana* are administered orally in the treatment of gastrenteritis, diarrhea, and dysentry [8,16,17]. Similarly, the roots and leaves decoctions of *P. macrophylla* are used as a laxative and in the treatment of dysentery, while the ripe fruits and stem bark powder are applied externally to heal wounds and burns [10,18]. Additionally, *P. macrophylla* have been used as analgesics, anthelmintics, and for the treatment of gonorrhea, diarrhea, convulsion, and inflammatory diseases [7,12,18,19].

Saponins constitute a major phytochemical grouping, and over 100 vascular plant species have been documented to possess various kinds of saponin [20]. Saponins are structurally diverse and consist of polycyclic aglycones attached to one or more sugar side chains [21]. Traditionally, they are subdivided into triterpenoid and steroid glycosides [21–23]. Saponins exert a wide range of pharmacological activities including antibacterial, antifungal, antiviral, anti-parasitic, anti-inflammatory, hypocholesterolemic, and hypoglycemic [20,22,24]. In cognizance of the folk practices of cold maceration in water or admixtures of locally brewed alcohol of *V. doniana* and *P. macrophylla* before administration, the reported work aimed at extracting saponins from *V. doniana* and *P. macrophylla* and respectively evaluating their antibacterial activity.

2. Materials and Methods

2.1. Plant Materials

The leaves and stem bark of *V. doniana* and *P. macrophylla* were collected from the woodlands of Obollo-afor (6.92° N, 7.52° E) and Orba (6.51° N, 7.27° E), respectively, in Enugu North Senatorial Zone, Enugu State, Nigeria. The plants were identified taxonomically by Mr JO Ozioko as *Vitex doniana* and *Pentaclethra macrophylla*, and voucher specimens (ViD/a03 and PeM/05) were deposited at the herbarium of the Department of Botany, University of Nigeria, Nsukka. The plant materials were collected in line with the ethical guide of the University of Nigeria, Nsukka, on herbal research.

2.2. Maceration and Extraction of Plant Materials

Fresh leaves and fleshy stem bark of *V. doniana* and *P. macrophylla* were harvested, thoroughly rinsed with distilled water, and air-dried at room temperature (28 $\pm$ 2 °C). Dried plant portions were mechanically pulverized using a hammer mill (Henan Always Machinery, Zhengzhou, China). Ethanol extraction was carried out in accordance with the method described by [3]. About 50 g of the pulverized leaves and stem bark was macerated in 200 mL of absolute ethanol (BDH Chemicals, Birmingham, England) for 4 h. Each portion was filtered using Whatman No. 1 filter paper (Sigma-Aldrich, Johannesburg, South Africa) and filtrates concentrated to dryness at steady air current. Extracts were stored in sterile containers until further use.

2.3. Phytochemical Assay

Ethanol extracts of *V. doniana* and *P. macrophylla* leaves and stem bark were screened for the presence of saponins, alkaloids, tannins, anthraquinones, glycosides, flavonoids, and sterols following

the methods of Trease and Evans [25] and Harbone [26]. However, quantitation of these phytochemicals was in accordance with standard protocols [27,28].

2.4. Saponin Extraction

Extraction of saponin from the stem bark of *V. doniana* and *P. macrophylla* was carried out in accordance with the method Ajibade and Famurewa [29] with some modifications. Briefly, 100 g of the pulverized stem bark was defatted with 300 mL of petroleum ether (BDH Chemicals, Birmingham, England). The resulting marc was air-dried and depigmented in 300 mL of chloroform (BDH Chemicals, Birmingham, England). Afterwards, 300 mL of methanol (BDH Chemicals, Birmingham, England) was subsequently used to extract saponin form the defatted samples. The mixture was allowed to stand overnight, and the resulting aqueous residue was filtered and the filtrate evaporated to dryness. The methanol extract was further fractionated with 200 mL of distilled water-butanol (1:1 v/v) to get butanol extracts, which were precipitated with 50 mL of acetone (BDH Chemicals, Birmingham, England) to obtain crude saponins.

2.5. Test Bacteria

Clinical isolates of *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* were obtained from the clinical diagnostic laboratory of Microbiology Department, University of Nigeria Nsukka, while type cultures (*Escherichia coli* ATCC 11775, *Pseudomonas aeruginosa* ATCC 10145, and *Staphylococcus aureus* ATCC 12600) were obtained from Bioresource Development and Conservation Project (BDCP), Nsukka. The inoculum size of each test bacterial strain was standardized as described by the Clinical and Laboratory Standards Institute [30].

2.6. Antibacterial Assay

Ethanol extracts (leaves and stem bark) and crude saponin extracts of *V. doniana* and *P. macrophylla* were assayed for antibacterial activity using the agar well diffusion technique. A stock solution of 100 mg/mL concentration of each extract was prepared, from which varying concentrations (50, 25, 12.5, and 6.25 (mg/Ml)) were achieved. About 100 µL of standardized test bacterial suspension (1.5×108 CFU/mL) was seeded onto sterile Mueller–Hinton agar plates (Conda, Madrid, Spain) and spread evenly to achieve a confluent lawn of microbial growth [3]. The plates were left to dry, and a sterile cork borer 6 mm in diameter was used to bore wells. Afterwards, about 100 µL of each plant extract was introduced into the well. Plates were incubated at 37 °C for 24 h, and the bacterial inhibition zone diameter was measured on the plates to the nearest millimeter (mm). Ciprofloxacin (Oxoid, Hampshire, UK) served as a positive control, and experimentation was performed in triplicate assays.

2.7. Minimum Inhibitory Concentration Determination

The minimum inhibitory concentrations (MICs) of ethanol extracts, of the leaves and stem bark, and of the crude saponin fractions of *V. doniana* and *P. macrophylla* were respectively determined using the microbroth dilution technique [31]. The MICs were recorded as the lowest concentrations of the extract, which showed inhibition of bacterial growth.

2.8. Statistical Analysis

Assays were conducted in triplicate, and all data was subjected to analysis of variance (ANOVA) using the IBM Statistical Package for Social Scientists (IBM-SPSS) version 22 (IBM Coorp, Armonk, NY, USA, 2013). The results were presented as mean values with standard deviation.

3. Results

Quantitative assessment of *V. doniana* and *P. macrophylla* showed the presence of saponins, tannins, flavonoids, anthroquinones, and alkaloids, respectively. However, these phytochemicals were present at varying concentrations, and saponins were observed in higher measures than other constituents (Table 1). The quantities of secondary metabolites detected/quantified might have been predicated on the solvent of extraction, a position advanced on the premise that solvent polarity plays an integral role on the nature of phytochemical extracted. Nonetheless, ethanol served as the extraction solvent for the plant materials used for these studies due to the fact that locally brewed alcohol (ethanol) is used in folk practices for the preparations of the decoctions involving *V. doniana* and *P. macrophylla* administered in herbal remedies for disease conditions.

Table 1. Quantitation of the phytochemical composition of ethanol extracts of *Vitex doniana* and *Pentaclethra macrophylla*.

Phyto-constituent	*Vitex doniana*		*Pentaclethra macrophylla*	
	Leaves	**Stem Bark**	**Leaves**	**Stem Bark**
Tannins (CE/g)	231 ± 0.6	207 ± 0.4	152 ± 0.3	309 ± 2.42
Flavonoids (QE/g)	0.21 ± 0.01	0.39 ± 0.05	0.41 ± 0.06	69 ± 1.33
Anthroquinones (CE/g)	23 ± 0.21	19 ± 0.11	27 ± 0.21	55 ± 1.71
Alkaloids (%)	39 ± 0.7	33 ± 0.4	59 ± 0.7	71 ± 0.5
Saponins (%)	54.2 ± 0.58	81.2 ± 0.29	61.2 ± 0.17	87 ± 3.4

Antibacterial susceptibility assay showed that *V. doniana* inhibited the growth of the Gram-positive bacteria but not the Gram-negatives tested; inhibition zone diameters recorded against the type culture of *Staphylococcus aureus* (ATCC 11775) and the clinical strain ranged from 15.5 ± 2.12 to 7.0 ± 0.0 (mm) against the leaf extracts and from 20.0 ± 1.41 to 7.0 ± 0.0 (mm) against the stem bark extracts (Table 2). Conversely, *P. macrophylla* extracts showed activity against both Gram-positive and -negative test bacteria strains; leaf extract concentration of 50 mg/mL showed inhibition zone diameters (mm) of 20.0 ± 1.41 (*S. aureus* clinical strain), 18.5 ± 0.71 (*P. aeruginosa* clinical strain), 17.0 ± 0 (*P. aeruginosa* ATCC 10145), and 14.5 ± 2.12 (*E. coli* ATCC 11775), respectively. A similar pattern of activity was achieved with the stem bark extract, and the inhibition zone diameters ranged from 23.0 ± 2.83 to 17.0 ± 0 (mm) at 50 mg/mL (Table 2).

Saponin extracts of both *V. doniana* and *P. macrophylla*, respectively, exhibited activity across the divide of Gram status (Table 3). At 50 mg/mL, saponin extracted from *V. doniana* yielded inhibition zone diameters (mm) of 20.0 ± 1.41 (*E. coli* clinical strain), 18.5 ± 0.71 (*P. aeruginosa* clinical strain), 17.0 ± 0 (*E. coli* ATCC 11775), and 13.0 ± 1.41 (*S. aureus* clinical strain), respectively, against the listed test bacteria. Similarly, the saponin extracts of *P. macrophylla* showed activity against over 80% (5/6) of the test isolates, with inhibition zone diameters ranging from 18.5 ± 0.71 against *P. aeruginosa* to 10.5 ± 0.71 against *S. aureus* (both test bacteria are clinical strains). Consequently, saponin extracts from both *V. doniana* and *P. macrophylla*, respectively, inhibited the growth of all bacterial strains tested, with the exception of *S. aureus* ATCC 12600 (Table 3). The minimum inhibitory concentrations obtained from the results of the micro-broth dilution assay showed varying threshold concentrations for the growth inhibition of test bacteria; however, the MICs ranged from 0.12 to 0.78 (mg/mL), as is shown in Table 4.

Table 2. Bacteria growth inhibition zones of ethanol extract of *V. doniana* and *P. macrophylla*.

Plant Extract	Bacterial Strain	Mean Zone of Inhibition (mm) ± SD				
		50.0 mg/mL	**25.0 mg/mL**	**12.50 mg/mL**	**6.25 mg/mL**	**3.125 mg/mL**
V. doniana ethanolic leaf extract	*E. coli*	-	-	-	-	-
	E. coli ATCC 11775	-	-	-	-	-
	S. aureus	15.5 ± 2.12	13.0 ± 0	10.0 ± 1.41	7.0 ± 0	-
	S. aureus ATCC 12600	14.5 ± 0.71	12.5 ± 0.71	10.5 ± 2.12	-	-
	P. aeruginosa	-	-	-	-	-
	P. aeruginosa ATCC 10145	-	-	-	-	-
P. macrophylla ethanolic leaf extract	*E. coli*	15.0 ± 1.41	13.0 ± 0	10.5 ± 2.12	7.0 ± 0	-
	E. coli ATCC 11775	14.5 ± 2.12	11.5 ± 0.71	10.5 ± 0.71	8.0 ± 1.41	-
	S. aureus	20.0 ± 1.41	15.5 ± 0.71	11.5 ± 0.71	10.0 ± 0	8.5 ± 0.71
	S. aureus ATCC 12600	12.5 ± 0.71	9.5 ± 0.71	8.5 ± 2.12	-	-
	P. aeruginosa	18.5 ± 0.71	15.0 ± 1.41	11.5 ± 2.12	9.5 ± 0.71	-
	P. aeruginosa ATCC 10145	17.0 ± 0	14.0 ± 1.41	11.0 ± 1.41	-	-
V. doniana ethanolic stem bark extract	*E. coli*	-	-	-	-	-
	E. coli ATCC 11775	-	-	-	-	-
	S. aureus	17.5 ± 0.71	14.0 ± 0	10.0 ± 1.41	7.0 ± 0	-
	S. aureus ATCC 12600	20.0 ± 1.41	16.5 ± 0.71	11.0 ± 1.41	8.5 ± 0.71	7.0 ± 0
	P. aeruginosa	-	-	-	-	-
	P. aeruginosa ATCC 10145	-	-	-	-	-
P. macrophylla ethanolic stem bark extract	*E. coli*	17.0 ± 0	12.0 ± 1.41	11.5 ± 0.71	8.5 ± 0.71	-
	E. coli ATCC 11775	19.5 ± 0.71	17.0 ± 0	13.0 ± 1.41	9.0 ± 0	-
	S. aureus	18.5 ± 0.71	11.5 ± 0.71	9.5 ± 0.71	7.5 ± 0.71	-
	S. aureus ATCC 12600	23.0 ± 2.83	15.5 ± 0.71	12.5 ± 0.71	10.0 ± 0	8.5 ± 0.71
	P. aeruginosa	20.0 ± 1.41	18.0 ± 0.71	16.0 ± 0.71	13.0 ± 1.41	10.5 ± 0.71
	P. aeruginosa ATCC 10145	19.5 ± 3.54	14.5 ± 0.71	12.5 ± 0.71	10.5 ± 0.71	8.0 ± 0

-: no activity; ATCC: American Type Culture Collection; SD: Standard deviation.

Table 3. Bacteria growth inhibition zones of saponin extract of *V. doniana* and *P. macrophylla* stem bark.

Plant Extract	Bacterial Strain	Mean Zone of Inhibition (mm) ± SD					
		50.0 mg/mL	**25.0 mg/mL**	**12.50 mg/mL**	**6.25 mg/mL**	**3.125 mg/mL**	**CPF (100 mg/mL)**
V. doniana crude saponin extract	*E. coli*	20.0 ± 1.41	15.0 ± 1.41	10.5 ± 0.71	8.0 ± 0	7.0 ± 0	29.25
	E. coli ATCC 11775	17.0 ± 0	12.5 ± 0.71	10.5 ± 0.71	8.0 ± 0	-	28.85
	S. aureus	13.0 ± 1.41	10.0 ± 0	8.5 ± 0.71	-	-	24.85
	S. aureus ATCC 12600	-	-	-	-	-	22.25
	P. aeruginosa	18.5 ± 0.71	16.0 ± 0	13.5 ± 0.71	10.0 ± 1.41	7.5 ± 0.71	26.85
	P. aeruginosa ATCC 10145	15.0 ± 0	12.0 ± 1.41	9.5 ± 0.71	-	-	25.25
P. macrophylla crude saponin extract	*E. coli*	18.0 ± 1.41	14.5 ± 0.71	12.0 ± 1.41	9.5 ± 0.71	7.5 ± 0.71	29.25
	E. coli ATCC 11775	13.0 ± 1.41	10.0 ± 0	8.0 ± 1.41	-	-	28.85
	S. aureus	10.5 ± 0.71	8.0 ± 0	7.0 ± 0	-	-	24.85
	S. aureus ATCC 12600	-	-	-	-	-	22.25
	P. aeruginosa	18.5 ± 0.71	13.5 ± 0.71	12.0 ± 0	10.0 ± 1.41	7.5 ± 0.71	26.85
	P. aeruginosa ATCC 10145	13.5 ± 0.71	10.5 ± 0.71	9.0 ± 0	7.0 ± 0	-	25.25

CPF: ciprofloxacin; values are mean zone of inhibition (mm) ± standard deviation of three replicates; -: no activity.

Table 4. Minimum inhibitory concentration (MIC) of the plant extracts against test organisms.

Bacterial Strain	Minimum Inhibitory Concentration (mg/mL)					
	VDetl	**PMetl**	**VDets**	**PMets**	**VDs**	**PMs**
E. coli	-	0.195	-	0.195	0.12	0.12
E. coli ATCC 11775	-	0.195	-	0.195	0.195	0.78
S. aureus	0.195	0.12	0.195	0.195	0.78	0.78
S. aureus ATCC 12600	0.78	0.78	0.12	0.12	-	-
P. aeruginosa	-	0.195	-	0.12	0.12	0.12
P. aeruginosa ATCC 10145	-	0.78	-	0.12	0.78	0.195

VDetl: *V. doniana* ethanol leaf extract; PMetl: *P. macrophylla* ethanol leaf extract; VDets: *V. doniana* ethanol stem bark extract; PMets: *P. macrophylla* ethanol stem bark extract; VDs: *V. doniana* saponin extract; PMs: *P. macrophylla* saponin extract; -: no activity.

4. Discussion

The applications of saponins as pharmacologically active agents are described in various literary documentations on applications and potentials. Anticipated pharma activity includes anti-lipid peroxidation of plasma lipoprotein with the concomitant effect as a reduction in the risk of atherosclerosis [5], anti-diabetic activities from steroidal saponins of the *Polygonatum kingianum* in origin [32], and in the treatment of other ailments of human metabolic disorder and infectious agents [33]. The phyto-constituent analyses of the extracts of *V. doniana* and *P. macrophylla* indicated varying concentrations of tannins, flavonoids, anthroquinones, and alkaloids. The qualitative assay showed trace to non-detectable levels of falvonoids, anthroquinones, and alkaloids; however, upon quantitation, using a more sensitive technique, lower concentrations of these phytochemicals were detected. Nonetheless, saponins were detected in high concentrations without recourse to the techniques applied, thus an indication that the herbs are rich sources of saponins.

Antibacterial activity assessment of the ethanol extracts of *V. doniana* and *P. macrophylla* against Gram-positive and -negative bacteria showed a broad spectrum of activity for *P. macrophylla,* as all the genus bacteria tested were susceptible following zones of inhibition. Conversely, only Gram-positives were susceptible to the ethanol extract of *V. doniana*. The precise component(s) that may have potentiated activity alone, or in combination, against test bacteria from the respective extracts of *V. doniana* and *P. macrophylla* is (are) unknown and are not within the purview of this study. However, a phyto-constituent guided bio-assay, with combinatorial of respective phyto-constituent in a factorial, may be ideal to give an insight into the role of respective constituents on the herbal extracts.

In the same vein, various folkloric herbs have been validated for activity against several bacterial etiologic agents associated with human and livestock disease [34]. Similar activity has been shown to exist in Vitex species collected from the Southern African region [1]. Although, geographical boundaries impacts the variance of the pharmacologically active constituent of plants following prevailing conditions, which includes the interaction with other plants, pathogens, and weather conditions, as well as other biotic and abiotic factors.

Furthermore, in the assessment of saponin extracts from both *V. doniana* and *P. macrophylla* for activity against the same test bacteria used with the ethanol extracts, activity was recorded against the entire test bacteria genus. Saponin extracts from *V. doniana* showed more activity against both Gram-positive and -negative bacteria, indicating a broader spectrum of activity as compared to the ethanol extract of same plant. The ethanol extraction of *V. doniana* stem bark and leaves might only be able to extract constituents with activity against the Gram-positives. On the other hand, the saponin extracted may have been void of any adjuncts with activity inhibitory properties, as it is peculiar with plant extracts. It is worth mentioning that, the antibacterial activity recorded against the saponin extracted from *V. doniana* showed larger inhibition zone diameters as compared to the saponin extract from *P. macrophylla*. In effect, this phenomenon may be understood as structural variance in the aglycone backbone of the saponins from both plants. Whether or not the saponin's aglycones are

triternoid, steroidal, and/or a combination of both, the objectives of this study did not address whether the variation in activity is largely attributed to these differences.

Besides the obvious, namely, that saponins represent an important class of plant's secondary metabolites and has ameliorated physiological anomalies in humans and livestock, it has also contributed significantly to the bio-economy. The World Health Organisation's estimation that about 60% of the world's rural dwellers depend on herbal remedies [35] connotes the imperativeness of the exploration of phytochemicals for anti-infective properties. Hence, the efficacies of these herbs in folk medicine may be adduced to the antimicrobial activities of respective phyto-constituents, or a combination thereof, vaguely understood due to a paucity of information on the advanced concept.

5. Conclusions

The applications of *V. doniana* and *P. macrophylla* in folk medicine for the treatment of various ailments may be validated by the antibacterial activity recorded in this study. Additionally, the saponin extract from both plants showed high potency and a broad spectrum of activity against both Gram-positive and -negative bacteria tested. The saponins from *V. doniana* were active against bacterial species that were not susceptible to the ethanol extracts of the same plant. Saponins constituting important phytochemical grouping suggest the imperativeness of further studies on the saponins from *V. doniana* and *P. macrophylla* to elucidate the nature of the aglycone structures in these plants for novelty and the possibility for use as a scaffold in drug design.

Acknowledgments: With gratitude, we acknowledge the financial support from the Govern Mbeki Research and Development Centre (GMRDC) of the University of Fort Hare and South Africa Medical Research Council (SAMRC).

Author Contributions: Nneoma E. Akaniro-Ejim executed the benchwork and prepared the manuscript draft. Nkoyo I. Nubila co-participated in the research execution. Chibuike S. Ubani participated in data analysis and the revision of manuscript. Alexander A. Nzei collected the plant materials and contributed to the preparation of the manuscript at all times during the editorial process. Uchechukwu U. Nwodo designed and supervised the study and help revise the manuscript. Anthony I. Okoh prepared and proof-read the final version of the manuscript.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Nyiligira, E.; Viljoen, A.M.; van Heerden, F.R.; van Zyl, R.L.; Van Vuuren, S.F.; Steenkamp, P.A. Phytochemistry and *in vitro* pharmacological activities of South African *Vitex* (Verbenaceae) species. *J. Ethnopharmacol.* **2008**, *119*, 680–685. [CrossRef] [PubMed]
2. Nwodo, U.U.; Ngene, A.A.; Iroegbu, C.U.; Onyedikachi, O.A.L.; Chigor, V.N.; Okoh, A.I. *In vivo* evaluation of the antiviral activity of *Cajanus cajan* on measles virus. *Arch. Virol.* **2011**, *156*, 1551–1557. [CrossRef] [PubMed]
3. Nwodo, U.U.; Ngene, A.A.; Iroegbu, C.U.; Obiiyeke, G.E. Effects of fractionation on antibacterial activity of crude extracts of *Tamarindus indica*. *Afr. J. Biotechnol.* **2010**, *9*, 7108–7113.
4. Chan, K.W.; Iqbal, S.; Khong, N.M.H.; Ooi, D.J.; Ismail, M. Antioxidant activity of phenolics-saponins rich fraction prepared from defatted kenaf seed meal. *LWT Food Sci. Technol.* **2014**, *56*, 181–186. [CrossRef]
5. Liu, Z.; Nie, R.; Liu, Y.; Li, Z.; Yang, C.; Xiong, Z. The effects of total soy saponins on free radicals in the quadriceps femoris, serum testosterone, LDH, and BUN of exhausted rats. *J. Sport Health Sci.* **2016**. [CrossRef]
6. Fouedjou, R.T.; Teponno, R.B.; Quassinti, L.; Bramucci, M.; Petrelli, D.; Vitali, L.A.; Fiorini, D.; Tapondjou, L.A.; Barboni, L. Steroidal saponins from the leaves of *Cordyline fruticosa* (L.) A. Chev. and their cytotoxic and antimicrobial activity. *Phytochem. Lett.* **2014**, *7*, 62–68. [CrossRef]
7. Cousins, D.; Huffman, M.A. Medicinal properties in the diet of gorillas: An ethno-pharmacological evaluation. *Afr. Study Monogr.* **2002**, *23*, 65–89.
8. Ekeanyanwu, C.R. Traditional medicine in Nigeria: Current status and the future. *Res. J. Pharmacol.* **2011**, *5*, 90–94.
9. Ouattara, A.; Coulibaly, A.; Adima, A.A.; Ouattara, K.; Ouattara, K. Exploration of the antistaphylococcic activity of *Vitex doniana* (Verbenaceae) stem bark extracts. *Sch. Acad. J. Pharm.* **2013**, *2*, 94–100.

10. Cimanga, K.R.; Kikweta, M.C.; Tshodi, E.M.; Nsaka, L.S.; Mbamu, M.B.; Manienga, K.; Bumoyi, M.; Kambu, K.O. Antibacterial and antifungal screening of extracts from six medicinal plants collected in Kinshasa-Democratic Republic of Congo against clinical isolate pathogens. *J. Pharmacogn. Phytother.* **2014**, *6*, 24–32. [CrossRef]
11. Enujiugha, V.N. Nutrient changes during the fermentation of African oil bean (*Pentaclethra macrophylla* benth) seeds. *Pak. J. Nutr.* **2003**, *2*, 320–323.
12. Agbogidi, O. Response of African oil bean (*Pentaclethra Macrophylla* benth) seeds to soils contaminated with spent lubricating oil. *Afr. J. Environ. Sci. Technol.* **2010**, *4*, 492–494.
13. Agbede, J.; Ibitoye, A. Chemical composition of black plum (*Vitex doniana*): An under-utilized fruit. *J. Food Agric. Environ.* **2007**, *5*, 95–96.
14. Muhammad, M.B.; Binta, B.; Hamidu, M.R.; Paul, N. Antimicrobial activity of the leaves and stem bark of *Vitex doniana*. *Int. J. Biol. Sci.* **2013**, *3*, 1–5.
15. Osum, F.I.; Okonkwo, T.M.; Okafor, G.I. Effect of processing methods on the chemical composition of *Vitex doniana* leaf and leaf products. *Food Sci. Nutr.* **2013**, *1*, 241–245. [CrossRef]
16. Kilani, A. Antibacterial assessment of whole stem bark of *Vitex doniana* against some enterobactriaceae. *Afr. J. Biotechnol.* **2006**, *5*, 958–959.
17. Lagnika, L.; Amoussa, M.; Adjovi, Y.; Sanni, A. Antifungal, antibacterial and antioxidant properties of *Adansonia digitata* and *Vitex doniana* from Bénin pharmacopeia. *J. Pharmacogn. Phytother.* **2012**, *4*, 44–52. [CrossRef]
18. Okunrobo, L.; Ching, F.; Ifijeh, F. Antinociceptive activity of methanol extract and aqueous fraction of the stem bark of *Pentaclethra macrophylla* benth (mimosaceae). *J. Med. Plants Res.* **2009**, *3*, 101–104.
19. Okorie, C.; Oparaocha, E.; Adewunmi, C.O.; Iwalewa, E.; Omodara, S. Antinociceptive, anti-inflammatory and cytotoxic activities of *Pentaclethra macrophylla* aqueous extracts in mice. *Afr. J. Trad. CAM* **2006**, *2*, 44–53.
20. Mir, M.A.; Sawhney, S.S.; Jassal, M.M.S. Qualitative and quantitative analysis of phytochemicals of *Taraxacum officinale*. *Wudpecker J. Pharm. Pharmacol.* **2013**, *2*, 1–5.
21. Majinda, R.R. Extraction and isolation of saponins. *Methods Mol. Biol.* **2012**, *864*, 415–426. [PubMed]
22. Soetan, K.; Oyekunle, M.; Aiyelaagbe, O.; Fafunso, M. Evaluation of the antimicrobial activity of saponins extract of *Sorghum Bicolor* L. Moench. *Afr. J. Biotechnol.* **2006**, *5*, 2405–2407.
23. Vincken, J.P.; Heng, L.; de Groot, A.; Gruppen, H. Saponins, classification and occurrence in the plant kingdom. *Phytochemistry* **2007**, *68*, 275–297. [CrossRef] [PubMed]
24. Sparg, S.; Light, M.; van Staden, J. Biological activities and distribution of plant saponins. *J. Ethnopharmacol.* **2004**, *94*, 219–243. [CrossRef] [PubMed]
25. Trease, G.E.; Evans, W.C. Phytochemistry: Introduction and general methods. In *Pharmacognosy*, 11th ed.; Bailliere Tindall: London, UK, 1978; pp. 227–247.
26. Harborne, J.B. *Phytochemical Methods: A Guide in Modern Techniques of Plant Analysis*; Chapman and Hall Ltd.: London, UK, 1998; pp. 221–232.
27. Onyilagha, J.C.; Islam, S. Flavonoids and other polyphenols of the cultivated species of the genus Phaseolus. *Int. J. Agric. Biol.* **2009**, *11*, 231–234.
28. Zovko, K.M.; Kremer, D.; Gruz, J.; Strnad, M.; Bisevac, G.; Kosalec, I. Antioxidant and antimicrobial properties of *Moltkia petraea* (tratt.) Griseb. Flower, leaf and stem infusions. *Food Chem. Toxicol.* **2010**, *48*, 1537–1542. [CrossRef] [PubMed]
29. Ajibade, V.A.; Famurewa, O. Histopathological and toxicological effects of crude saponin extract from *Phyllanthus niruri*, L (syn. P. franternus. webster) on organs in animal studies. *Glob. J. Med. Res.* **2012**, *12*, 31–37.
30. Wikler, M.A. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically; Approved Standard—8th ed.*; NCCLS: Vilanova, PA, USA, 2003.
31. National Committee for Clinical Laboratory Standards (NCCLS). *Methods for Dilution in Antimicrobial Susceptibility Tests: Approved Standard*; NCCLS M2-A5; NCCLS: Vilanova, PA, USA, 1993.
32. Lu, J.M.; Wang, Y.F.; Yan, H.L.; Lin, P.; Gu, W.; Yu, J. Antidiabetic effect of total saponins from *Polygonatum kingianum* in streptozotocin-induced daibetic rats. *J. Ethnopharmacol.* **2016**, *179*, 291–300. [CrossRef] [PubMed]
33. Xiang, L.; Wang, Y.; Yi, X.; Feng, J.; He, X. Furospirostanol and spirostanol saponins from the rhizome of *Tupistra chinensis* and their cytotoxic and anti-inflammatory activities. *Tetrahedron* **2016**, *72*, 134–141. [CrossRef]

34. Hernandez, M.M.; Heraso, C.; Villarreal, M.L.; Vargas-Arispuro, I.; Aranda, E. Biological activities of crude plant extracts from *Vitex trifolia* L. (Verbenaceae). *J. Ethnopharmacol.* **1999**, *67*, 37–44. [CrossRef]
35. World Health Organization. *Traditional Medicine-Growing Needs and Potential*; WHO Policy Perspectives on Medicine, No. 2; WHO: Genève, Switzerland, 2002.