Research Article

Antimicrobial activities, antioxidant and phytochemical analysis of methanol and aqueous leaf extract of *Phyllathus muellerianus* (KUNTZ) Exell

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ABSTRACT
The development of resistance in many microorganisms to synthetic drugs, inability of these synthetic drugs to provide cure for some diseases and infections calls for serious attention. Due to this development, the antimicrobial activity, phytochemical analysis and antioxidant properties of aqueous and ethanol leaf extracts of *Phyllanthus muellerianus* was studied. Well in agar diffusion method was used for the antimicrobial test. Chemical methods was used for the preliminary phytochemical screening and the *in vitro* antioxidant tests. The aqueous leaf extract possessed higher antibacterial potency (14.0±0.16–37.3±0.03 mm) on the test bacteria species and less of antifungal activity (5.0±0.2–16.0±0.0 mm) than the antibacterial (6.2±0.5–20.8±0.3 mm) and antifungal antibiotics (6.5±0.07-17.7±0.4 mm) respectively. Methanol extract only inhibited *Candida krusei* with inhibition of 4.5±0.3 mm. In both the qualitative and quantitative phytochemicals screened, aqueous extract was of higher yield than methanol extract. The *in vitro* antioxidant assay of the leaf extract in free radical scavenging (DPPH), ferric reducing antioxidant and hydroxyl radical scavenging properties yielded values able to represent antimicrobial potentials which may make the plant serve as alternative medicine for cure of the many microbial disease origins that plaque around the world. As valuable antimicrobial potential, phytochemical constituents and *in vitro* antioxidant properties was exhibited by *P. muellerianus* leaf extract, it may enhance a large segment of the world population to rely upon the use of traditional system of medicine for disease healing with novel plants.

Keywords: Inhibition; Plant extract; *Phyllanthus muellerianus*; Antioxidant; Phytochemicals

INTRODUCTION
Among the depredations of war and famines that strikes the world, infectious diseases are other factors that negate the pleasure of man. Despite the advancement in the understanding of microbial diseases and control, incidence of epidemics resulting from drug resistant and the emergence of new diseases, the health care of individuals and communities are still being threatened by pathogens. The treatment options for some microbial origin diseases have become limited due to the emergence of multi-drug resistant strains (1). It is a popular phenomenon of antibiotics being used against microbial infections but over the years, reduction from the use of the existing antibiotics as a result of microorganisms forming resistance to their inhibitory activities, has increased greatly the employment of plant origin drugs. They have been found to yield higher active compounds for pharmacological prospects and of less side effects. Plants have a bright roll to play in healing of diseases because out of the thousands species of plants, very few have been studied where only 1 – 10% of them are yet employed by man for effective therapeutic measure. Plants are found all over the world and this however facilitated their effective pharmacological potency over the many available antibiotics that are now ineffective intreating some diseases. Plant chemicals known as phytochenmicals are the requirements from plants for effective therapy. These plant chemicals are not evenly distributed among plants as some are found to be more from one plant to the other and each has their respective pharmacological function. Saponin for example, exerts some antibacterial activity by combining with cell membrane to elicit changes in cell morphology, leading to cell lyses (2). It was reported that polyphenols such as Gallic acid act possibly by binding to bacterial dehydrofolatereductase (DHFR) enzymes, inhibition

of super coiling activity of *Escherichia coli* bacterial gyrase by binding to the ATP binding site of gyrase B and binds to bacterial DNA therapy inducing topoisomerase IV enzyme-mediated DNA cleavage and bacterial growth statics (3). Antioxidants protect cells against the damaging effects of reactive oxygen species such as singlet oxygen, superoxide, peroxyl radicals, hydroxyl radicals and peroxide which results in oxidative stress leading to cellular damage (4).*Phyllanthus mullerianus* is an evergreen shrub with numerous, more or less straggling stems from the base and sometimes with a climbing habit. This plant has arsenal as medicinal values as a multipurpose plant employed in many countries to attack various diseases. The leaves, stem and roots have been used to heal stomach upset, dysentery, infertility and wounds. Apart from its medicinal value, the leaves are cooked for soup and the fruits are edible as other beneficial fruits. Hence the many diseases that plaque around us needs urgent alternative medical strategies for control as many synthetic antibiotics are becoming ineffective, the aims of this research are therefore to investigate the *in vitro* antimicrobial, phytochemical constituents and antioxidant properties of *P. mullerianus*.

Materials and Methods

Plant material

The leaves of *P. muellerianus* was collected from forest at Federal Polytechnic Ado-Ekiti, Ekiti State of Nigeria and was authenticated by Mr. Olatunji a botanist in the Department of Biological Sciences, Afe Babalola University, Ado-Ekiti, Ekiti State. A voucher specimen was deposited at the Herbarium of the Department. The leaves were washed thoroughly under running tap water and were air dried for two weeks at room temperature. After drying, the plant leaves were milled (using mechanical blender) into fine powder and transferred in to air tight containers with proper labeling. 500 g each of finely ground plant material was soaked in 1000 ml of methanol and water solutions, respectively for 24 h. The extracts were filtered through muslin cloth and through Whatman No 1 filter paper. Aqueous extract was concentrated in water bath regulated at 55 °C and methanol extract was concentrated with rotary evaporator. Before use, the extracts were reconstituted with 5% dimethylsulfoxide (DMSO).

Test microorganisms

Seventeen pathogenic microorganisms were used and they include *Bacillus cereus*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Vibrio mimicus*, *Proteus mirabilis*, *Salmonella typhi*, *Propinobacterium acnes*, *Enterobacter aerogenes*, *Escherichia coli*, *Arthrobacter mysorens*, *Pedicoccus* sp, *Shigella dysentriae* and *Pseudomonas aeruginosa*. Fungi used include *Candida krusei*, *Candida glabrata*, *Candida albican*, and *Aspergillus flavus*. These microorganisms were obtained from the Microbiology research laboratory of Afe Babalola University, Ado-Ekiti. They were purified and maintained on double strength Nutrient Agar and potato dextrose media for bacterial and fungal isolates respectively. Twenty-four hour pure cultures were prepared for bacterial and 72 h cultures for fungal isolates for analyses.

Antimicrobial extracts test

The extracts were tested for antimicrobial activity by agar diffusion method. Freshly prepared Muller Hinton Agar medium were seeded with 24 h cultures of bacterial inoculums of 1-2 x 10^7cfu/ml 0.5 Mcfarland standards while fungal inoculums were seeded on Potato dextrose Agar. The seeded agar plates were punched with 7 mm diameter sterile cork borer to create wells. The wells were filled with 50 μL of plant extracts (aqueous and methanol). The bacterial plates were incubated at 37 °C for 24 h, while the fungal plates were incubated at 28±2 °C for 72 h. After incubation, zone of growth inhibition for each extract was measured.

Determination of antibiotic sensitivity

The susceptibility of the microbial strains to different antibiotics was tested using disc diffusion method (5). Antibacterial agents from different classes of antibiotics were used which included Pefloxacin, Gentamycin, Ampiclox, Zinnacef, Amoxacilln, Ciprofloxacin, Streptomycin, Septrin, Erythromycin, Chloramphenicol, Sparfloxacin, Augumentin, Pefloxacin, Tarivid, Ciprofloxacin, Tetracycline and Chloramphenicol. For fungal strains, nystatin was used.

Phytochemical Screening of extracts

The criterion of preliminary screening of secondary metabolites was carried out according to the common phytochemical methods as described by Trease and Evans (6); Harborne and Williams, (7).

Test for Alkaloids (Wagner's reagent)

A fraction of extract was treated with 3-5 drops of Wagner's reagent (1.27 g of iodine and 2 g of potassium iodide in 100 ml of water) and observed for the formation of reddish brown precipitate (or) coloration (7)

Test for Terpenoids (Salkowski Test)

One gram of each extract was added to 2 ml of chloroform. Concentrated sulphuric acid (3 ml) was carefully added to form a layer. A reddish brown

coloration of the interface indicates the presence of terpenoids (Trease and Evans (7)

Test for Tannins

One gram of the extract was boiled in 10 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black coloration (Trease and Evans (7)

Test for Saponins (Foam test)

Two milliliters of extract was added to 6 ml of water in a test tube. The mixture was shaken vigorously and observed for the formation of persistent foam that confirms the presence of Saponins (Trease and Evans (7)

Test for Cardiac glycosides (Keller Kelliani's test)

Five milliliters of each extract was treated with 2 ml of glacial acetic acid in a test tube and a drop of ferric chloride solution was added to it. This was carefully underlayed with 1ml concentrated sulphuric acid. A brown ring at the interface indicated the presence of deoxy sugar characteristic of cardiac glycosides. A violet ring may appear below the ring while in the acetic acid layer, a greenish ring may form (Trease and Evans (7)

Test for Phenols (Ferric chloride test)

A fraction of the extracts was treated with 5% ferric chloride and observed for formation of deep blue or black colour (8)

Test for reducing sugars (Fehling's test)

To 2 ml of extracts, 3-5 drops of Fehling solution A and B was added, and boiled for 30 seconds. Brick- red colour indicates the presence of reducing sugar.

Test for Flavonoids

To a portion of the dissolved extract, few drops of 10 % ferric chloride solution were added. A green or blue colour indicates the presence of phenolic nucleus (7).

Sterols and Steroids (Salkowski's Test)

One milliliter of extract was treated with 2 ml of chloroform and equal amount of concentrated sulphuric acid was added, upper layer is turns to red indicates the presence of the sterols and steroids (7)

In vitro antioxidant screening

Determination of the level of ferric reducing antioxidant property

The method as described by Buricova and Reblova (8) was adopted but with little modifications. Each (0.1 gram) of methanol and aqueous plant extract were dissolved in 20 ml of distilled water and filtered through Whatman's No 1 filter paper. The filtrate (2.5 ml) was taken and 2.5 ml of 100% (w/v) phosphate buffer (pH 6.6) and 2.5 ml of 100% (w/v) potassium ferrocyanide were added. Standard a prepared by calibration of freshly prepared solution of ascorbic acid in deionized water was used for this purpose (concentration from 0 to 4 mg/100 ml). All preparation was carried in triplicates. The mixture was incubated at 50 °C for 15 minutes. Trichloroacetic acid (10% v/v) was added, followed by the addition of 5 ml of distilled water and 1 ml of 0.1% ferric chloride. The absorbance of the ferric standard and the test samples were read at 700 nm against reagent blank. The contents of the oxidizable substances were expressed as mg of ascorbic acid/g of dry plant weight material.

Quantification of free radical scavenging

The method used was almost the same as used by Ibanez et al. (9) but with modifications. An aliquot of 0.5 ml of 0.1 ml 1, 1- diphenyl 1-2 picrylhdrazyl (DPPH) radical (Sigma Aldrich, St Louis, USA) in the concentration of 0.05 mg/mL in methanol was added to a test tube with 1 ml of the aqueous and methanol plant extract each at a concentration of 20 mg/ml. The reactants were mixed at room temperature (28 ± 2 ^{0}C) and kept at this room temperature for 20 minutes. The absorbance was read at 520 nm with a spectrophotometer. The absorbance of the DPPH radical solution containing the plant extract was expressed as mg of L-ascorbic (Sigma Chemical Co, St Louis, USA) per 1 gram of the plant extract. Calibration was used in such a case, where the plant extract was replaced with a freshly prepared solution of ascorbic acid in deionized water (concentration from 0 to 1.6 mg/100 ml). The experiment was performed in triplicate.

The percentage of DPPH free radical was calculated using the following formula:

$$\text{DPPH scavenging effect (\%)} = \frac{A_0 - A_1}{A_0} \times 100$$

Where A_0 is the absorbance of the control, and A_1 is the absorbance of extract or positive control.

Assay for Hydroxyl radical scavenging

The capacity to scavenge hydroxyl radical by the extract was measured according to the modified method proposed by Halliwell et al. (10)(1987) with slight modification. The hydroxyl radicals are generated by iron-ascorbate-EDTA-H_2O_2, which then react with deoxyribose to form thiobarbituric acid reactive substance (TBARS). This substance yields pink chromogen at low pH while heating with trichloroacetic acid (TBA). The reaction mixture contained 4 mMdeoxyribose, 0.3 mM ferric

chloride, 0.2 mM EDTA, 0.2 mM ascorbic acid, 2 mM H_2O_2 and various concentrations of the plant extract in different tubes. The tubes were capped tightly and incubated for 30 minutes at 37 °C. Then 0.4 ml of 5% (v/v) TBA and 0.4 mL of 1% (v/v) TBA were added to the reaction mixture which was kept in a boiling water bath for 20 minutes. The intensity of pink chromogen developed was measured spectrophotometrically at 532 nm against the blank sample. Ascorbic acid was used as a positive control. Every preparation was carried in triplicates.

The hydroxyl radical scavenging activity of the leaf extract was reported as % inhibition of deoxyribose degradation and calculated using the following formula:

$$\% \text{ Inhibition} = \frac{A_0 - A_1}{A_0} \times 100$$

Where A_0 was the absorbance of the control sample and A_1 was the absorbance of the extract or positive control.

Table 2. Qualitative phytochemicals analysis of *Phyllanthus muellerianus* leaf extracts

Parameters	Aqueous	Methanol
Saponin	++	+
Tannin	+++	+++
Phenols	+++	+++
Flavonoids	++	++
Alkaloids	+++	+
Terpenoids	+	-
Steroids	+	-
Cardiac Glycosides	+	+
Reducing sugars	++	++

Key: + = present, - = absent, ++ = moderately present, +++ = strongly present

Table 3.Quantitative phytochemical analysis of *Phyllanthus muellerianus* leaf extracts

Parameters	Aqueous	Methanol
Phenols	0.89±0.00	0.75±0.00
Flavonoids	3.75±0.06	2.04±0.04
Tannin	0.75±0.12	0.60±0.00
Alkaloids	207.29±0.09	43.00±0.00

Values are expressed as mean ± SEM of triplicat

Table 4 .*In vitro* antioxidant screening of *Phyllanthus muellerianus* leaf extracts Antioxidant tests

	AE	ME
FRAS DPPH (mg of ascorbic acid assay dry plant material)	86.74±0.21	56.26±0.37
FRAP (mg of ascorbic acid dry plant material)	0.74±0.13	1.04±0.03
HRS (%)	0.51±0.14	0.44±0.35

Values are expressed as mean ± SEM of triplicates

Key: AE – Aqueous extract; ME – Methanol extract; FRAS – Free radical antioxidant property; FRAP – Ferric reducing antioxidant property; HRS - Hydroxyl radical scavenging .

Evaluation of extracts durability

To evaluate the shelf life of the antimicrobial extracts, durability study of the extracts were tested at regular intervals of five days. This was evaluated by checking the activities of the extracts against test microorganisms by agar well diffusion techniques. Onto 5 mm diameter well created on pre-seeded Mueller Hinton agar plates, 0.55 ml extract was added and the plates were incubated at 37 °C for 24 h. Inhibition created on each test microbe with the extracts were measured and compared with the initial susceptibility result on day one.

Evaluation of Time-Killing Kinetics

The extracts rate of killing was evaluated by using their least MIC values on each test microbe by the criteria of Miyasaki et al. (11). Overnight bacterial broth culture was serially diluted to 5×10^5 CFU/ml with nutrient broth supplemented with 1 ml of extract. The culture was incubated at 37 °C in a shaker water bath regulated at 160 rpm. Aliquot of culture was obtained at time intervals of 0, 1, 6, 12, 24 h, serially diluted with nutrient broth and 1 ml was plated onto nutrient agar plate. The plates were incubated at 37 °C for 18 h, after which viable colonies were counted and results were expressed in $\log_{10}$ CFU and plotted against time for each test microbe. The experiments were performed in triplicate.

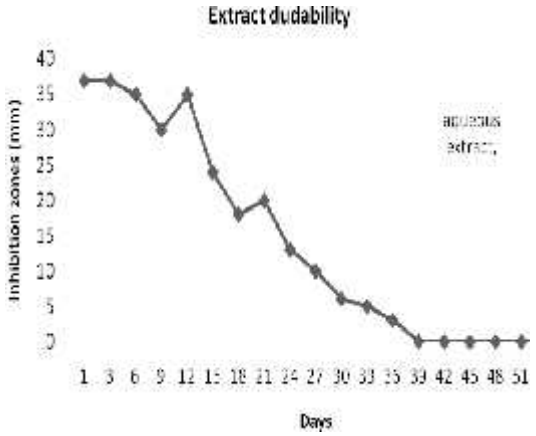

Figure1. Duration of extract potency

Statistical analysis

The obtained data were subjected to statistical analysis and expressed as mean ± standard deviation (SD) by one way analysis of variance (ANOVA). Least significant difference was performed and values were resolved to be significantly different at ($P < 0.5$)

Results

The highest zone of inhibition with the crude aqueous leaf extract of *P. muellerianus* was 37 mm on *S. aureus*. This was followed by *P. aeruginosa* with inhibition of 30 mm. Meanwhile, other test bacteria species were inhibited with zones from between 14 – 24 mm. Though methanol extract of the leaf was ineffective on the test bacteria species, it inhibited *C. krusei* with a zone of 4.5±0.3 mm. The aqueous leaf extract also showed antifungal property by most inhibiting *C. glabrata* with zone of 16.0±0.00 mm. This was followed by *C. albicans* which was inhibited with a zone of 14 mm. Lowest inhibition of 5 mm was exerted on *C. krusei*. Highest inhibition of 20.8 mm was recorded for reference antibiotics on *B. cereus*. Meanwhile, *S. aureus*, *E. coli* and *E. aerogenes* were inhibited with zone of 20 mm with some of the antibiotics. Other test bacteria species were inhibited with zones of between 6 to 19 mm. Table 1). Nystatin the reference antifungal, most inhibited *A. flavus* with zone of 17 mm, followed by *C. albicans* with zone of 14 mm, *C. krusei* with 13 mm and least inhibited *C. glabrata* with zone expression of 6 mm making it a better antifungal agent on the test fungi species (Table 1).Table 2 expresses the preliminary phytochemicals screened from the aqueous and methanol leaf extracts of *P. muellerianus*, where tannins and phenols yielded much results, followed by flavonoids, saponins, alkaloids and steroids with less yield from the methanol extract. Higher yield of tannins, phenols and alkaloids was screened from the aqueous extract and were followed by saponnins, flavonoids, reducing sugars and cardiac glycoside with lesser yields. Quantitative amounts of 0.75%, 2.04%, 0.60% and 0.43% of phenols, flavonoids tannins and alkaloids were respectively recorded from methanol leaf extract, while from the aqueous leaf extract, it was 0.89%, 3.75%, 0.75% and 207 for phenol, flavonoids, tannins and alkaloids respectively (Table 3).Table 4 expresses the *in vitro* antioxidant properties of the aqueous and methanol extracts with differences in values. The leaf aqueous extract Free radical scavenging activity was 86.72 μmol (TE), ferric reducing power of 0.74 μmol (AAE) and hydroxyl radical scavenging activity of 0.51%. From the methanol leaf extract, values of free radical scavenging activity of 56.26 μmol (TE), ferric reducing power of 1.04 μmol (AAE) and hydroxyl radical scavenging activity of 0.44% were recorded.The aqueous leaf extract was actively potent for 21 days after preparation, but however dropped until 39 days when inhibition of the test microbe was no longer effective (Fig. 1).*S. aureus*, *C. albicans* and *V. mimicus* were suppressed fewer than 24 h of extract treatment. *C. krusei* and *E. aerogenes* were totally eliminated fewer than 36 h of extract treatment while other test microbes were totally killed fewer than 48 h of extract treatment (Fig. 2).

Discussion

The Aqueous leaf extract of *P. muellerianus* showed higher degree of antimicrobial activity against all the susceptible tested microorganisms than the methanol extract which had partial or no inhibition zone on the tested microorganisms. The better activity with water extracts could be attributed to the

extracts being prepared according to the traditional methods which involved extracting with hot water or boiling in water for several hours. Though the traditional medical practitioners uses water often because it is a solvent mostly at their disposal to achieve successful healing, their success may be due to administration of the concoctions/or decoctions in large quantities, and the treatment in most cases involves the use of the extracts for a long period of time (12). These methods however, check against resistance of microorganisms when infections or diseases are treated with plant extracts. Though Clarkson et al. (13) has reported less inhibition of microorganisms with aqueous leaf extract of *P. mullerianus*, we observed higher antimicrobial effect with the aqueous extract against *P. aeruginosa, S. aureus,* and *Proteus mirabilis*.

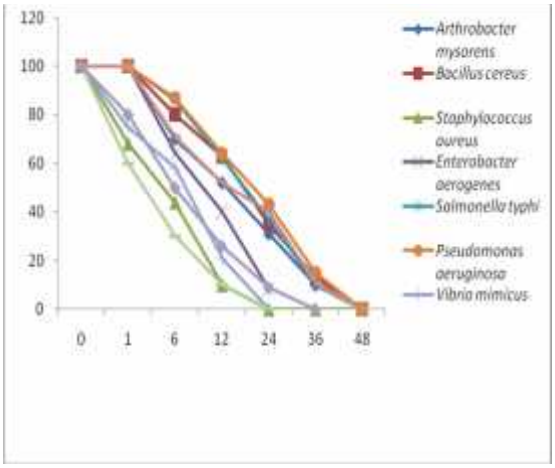

Figure 2. Killing rates of the extracts

The differences in results might be due to the method of reconstitution of the crude extract, plant age, climatic condition and method of extract preparations.Earlier reports on antimicrobial activity of *P. muellerianus,* revealed inhibition of *E. coli*, *S. aureus*, *Paeruginosa*, *S. pyogenes* and *C. albicans* (4), *B. cereus*, *S. typhi*, *K. pneumoniae*, *P.* mirabilis, *S. flexneri* (14) with varying degree of inhibition. However, the antimicrobial result obtained in this study, presents higher inhibition of these microorganisms that has been reported. *P. muellerianus* become one of the plants valuable medical importances employed by traditional healers in Nigeria because of its representative role in disease causing pathogens that affect the populace. Based on report by Ofokansi et al. (15), the leaf extract of *P. muellerianus* posed antibacterial activity against *P. aeruginosa*, *P. mirabilis* and *E. faecalis* at 50 mg/ml and could possibly be used in combination with ciprofloxacin to treat infections caused by *P. aeruginosa*. *P. muellerianus* can assist in controlling infection caused by *P. aeruginosa* and *S. aureus* which are an opportunistic microorganism and can cause food poisoning, skin infection and urinary tract infection (Ofokansi et al. (15). This leaf extracts had antifungal property as it inhibited growth of *Candida* spp, which is in line with the report of Ofokansi *et al*. (15). Earlier researches conducted on *Phyllanthus* spp extracts established that the *Phyllanthus* family has high antimicrobial effect on *Candida* spp most especially *Candida albicans* which is a dimorphic fungus and causes lots of diseases ranging from oral and genital infections in human (16).This study shows that various phytochemicals, including phenols, alkaloids and flavonoids are present in the leaf of *P. muellerianus*. Ofokansi et al. (15), has reported similar phytochemical constituents in earlier their study. However Awomukwu et al. (17) has reported lesser values in quality of phytochemicals from the leaves of *P. muellerianus* than what was observed in our study. These chemical compounds screened from the leaves could be responsible for the inhibition of microorganisms as a proof of its medical importance in traditional medicine. The antimicrobial test with the leaf extract of *P. muellerianus* in this study performed better activity than the commercial antibiotics. This might be due to the various active phytochemical constituents present the leaves than the synthetic drug where chemical constituents are separated and embedded singly on discs. However, this separated therapeutic agent are made to address a particular disease hence are not paired to work in combination as plant extracts do. *S. pyogenes* and *S. aureus* are known to play vital pathogenicity in wounds and were inhibited with the leave aqueous extract of *P. muellerianus*. This finding is in correlation with Agyare et al. (18) who have reported that the aqueous leaf extract of *P. muellerianus* and its major isolate, geraniin stimulate cellular activity, differentiation and collagen synthesis of human skin keratinocytes and dermal fibroblasts. The inhibition of *E. coli*, *P. aeruginosa*, *S.aureus* and *S. pyogenes* in this study further strengthened the aqueous leaf extract of *P. muellerianus* may exhibit wound healing activity hence these bacteria species are known for wound infections.In a study on the phytochemical constituents of aqueous extract of *P. amarus*, the following constituents were detected: alkaloids, saponins, tannis, anthraquinones,

flavones, carotenoids, reducing compounds, cardiac glycoside, steroids and triterpenes, coumarins, and volatile oils, as reported by Odetola and Akojenu, (19), of which were also screened in this study.. Most importantly with the phytochemicals for their value in medicine, flavonoids are known to prevent gastric ulcer due to the astringent and antimicrobial properties, which appear to be responsible for gastro-protective activity (20). Phenols and phenolic compounds have been extensively used in disinfections and remain the standard with which other bactericidal agent is compared (21). Phenol was present in leaf extracts as reported by Wahle et al. (22) which show the presence of phenols in several plants and its ability to inhibit cancer cells *in vitro* and *in vivo*. Generally, phytochemicals are known to confer certain health benefits such as anti-inflammatory, antimicrobial, antihypertensive, and anti-diabetic effects reported by Oikeh et al. (23). More importantly, there have been no side-effects or toxicity reports for many years on this plant (24).Antioxidant activity of *P. muellerianus* leaf extracts was investigated using DPPH radical scavenging method. Antioxidant efficacy of methanol and aqueous extracts were found to be higher and comparative with standard ascorbic acid at concentration of 0.1 mg/ml. Reports have shown that reducing the risk of chronic diseases and prevent progression of diseases linked to ROS is possible either by enhancing the body's natural antioxidant defenses or by supplementing with proven dietary antioxidants (25). This has mandatorily necessitated the search for potent and non-toxic antioxidants agents to prevent the progression of oxidant implicated chronic diseases (26).The presence of phenolic compounds like flavonoids and tannins may present the aqueous leaf extract of *P. muellerianus* for the free radical scavenging effects observed; hence they act as primary antioxidants (27). Polyphenolic compounds are known to have antioxidant activity and therefore the activity of *P. muellerianus* known to contain high amount of geraniin and other polyphenols may be responsible for its notable *in vitro* antioxidant observed in this study. However, Belguidoum et al. (27), have reported similar findings.The results also presented high reducing FRAP of the aqueous leaf extract of *P. muellerianus* The result obtained correlates with the findings of Boakye et al. (4). The High FRAP activity of the aqueous leaf extract of *P. muellerianus* may be due to the high tannin content since the antioxidant activity of tannins is mediated through reducing power and scavenging activity as reported by Minussi *et al*. (28). The reducing power ability of this extract may suggest that ROS such as H_2O_2, O_2 and OH may be neutralized via hydrogen atom transfer (14). The quality *in vitro* antioxidant activity as observed in this study may suggest the vital role it could play in healing some diseases and in support of its use by traditional healers. Synthetic drugs have been known to be accompanied by several contradictions and guidelines such as nausea, anaemia, method of use, time frame, rashes etc. while most medicinal or herbal plants are asymptomatic and have little or no adverse effect (29). Based on this finding, *P. muellerianus* plant may serve as alternative to synthetic drugs and further research needs to be done especially towards the mechanism of biological activity of phytochemicals from this plant.

Conclusion

From this study, *P. muellerianus* leaf extract shows high concentration of phytochemical constituents, antimicrobial values and *in vitro* antioxidant properties. This may enhance a large segment of the world population to rely upon traditional system of medicine in healing disease. Moreover, natural antioxidants have potential advantages over various diseases with oxidative stress as *P. muellerianus* aqueous leaf extract can be an alternative out of all the numerous medicinal plants.

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Table1.Crude extract of *P. muellerianus* and antibiotics inhibition on test microorganisms

Microorganisms	Aqueous extract	Methanol extract	Synthetic drug PEF	CN	APX	Z	AM	E
	SXT	S	NY	CPX	OFX	SP	AU	CH
Arthrobacter mysorens	15.8±0.3	-	14.0±1.00	6.8±0.3	-	6.2±0.4	-	
	16.6±0.4	12.7±0.3	11.1±02	-	13.9±0.2	-	-	-
-								
Bacillus cereus	14.0±0.2	-	15.8±0.8	12.3±0.3	-	18.2±0.4	-	
	13.5±0.5	20.8±0.3	18.5±0.5	-	19.5±0.5	-	-	-
-								
Staphylococcus aureus	37.3±0.0	-	20.5±0.5	-	-	16.5±0.5	17.6±0.4	
13.5±0.5	16.9±0.2	14.5±0.5	-	8.8±0.3	-	-	-	-
Enterobacter aerogenes	23.3±0.2	-	17.9±1.0	-	-	-	-	
-	-	-	-	20.5±0 5	20.3±0.3	17.5±0.5	14.8±0.8	
-								
Salmonella typhi	24.6±0.4	-	-	14.8±0.8	-	-	-	
-	-	15.3±0.3	-	-	-	-	-	-
Pseudomonas aeruginosa	30.0±0.2	-	-	-	-	-	-	
-	-	-	-	-	-	-	-	-
Vibrio mimicus	22.0±0.1	-	-	12.3±0.3	-	-	-	
-	-	-	-	-	13.9±0.1	-	-	-
Proteus mirabilis	21.0±0.3	-	13.6±0.6	-	-	-	-	
-	-	-	-	19.5±0.5	12.6±0.6	-	-	
13.9±0.1								
Klebiella pneumoniae	-	-	-	-	-	-	-	
-	-	-	-	11.5±0.5	-	-	-	-
Escherichia coli	-	-	-	20.5±0.5	-	18.5±2.0	-	
14.8±0.3	14.0±0.0	19.5±1.5	-	14.0±1.0	-	-	-	-
Shigelladysentriae	24.5±0.3	-	-	-	-	-	-	
-	-	12.3±0.3	-	-	-	-	-	-
					Nystatine			

Candida glabrata	16.0±0.00	-	6.5±1.0
Aspergillus flavus	10.0±0.00	-	17.7±0.4
Candida albcans	14.5±0.8	-	14.5±0.1
Candida krusei	5.0±0.2	4.5±0.3	13.5±1.0

Values are expressed as mean ± SEM of triplicates
Key: PEF – Pefloxacin; CN – Gentamycin; APX – Ampiclox; Z – Zinnacef; AM – Amoxacilln; CPX – Ciprofloxacin; NY – Nystatin; S – Streptomycin; SXT – Septrin; E – Erythromycin; CH – Chloranphenicol; SP – Sparfloxacin; AU – Augumentin; PEF – Pefloxacin; Nil – No zone of inhibition; - not applicable; OFX – Tarivid.