



Antimicrobial activities, phytochemical and antioxidant analyses of *Phyllanthus muellerianus* (KUNTZ) Exell methanol and aqueous leaf extracts

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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, preliminary phytochemical analysis and antioxidant properties of aqueous and methanol leaf extracts of *Phyllanthus muellerianus* was studied. Well in agar diffusion method was used for the antimicrobial test. Chemical methods was used to preliminarily screen for phytochemical constituents and *in vitro* antioxidant quality. The aqueous leaf extract possessed higher antibacterial potency (14.0 ± 0.16 – 37.3 ± 0.03 mm) on the test bacteria species than the commercial antibiotics (6.2 ± 0.5 – 20.8 ± 0.3 mm) and antifungal (6.5 ± 0.07 – 17.7 ± 0.4 mm). Methanol extract only inhibited *Candida krusei* with zone of 4.5 ± 0.3 mm. In both the qualitative and quantitative phytochemicals screened, aqueous extract was of higher yield than methanol extract. The antioxidant of the leaf extract in free radical scavenging (DPPH) and hydroxyl radical scavenging activity were higher in aqueous extract than methanol extract while ferric reducing antioxidant was only more in methanol extract. As valuable antimicrobial potential, phytochemical constituents and antioxidant properties were exhibited by *P. muellerianus* leaf extract, it may enhance a large segment of the world population to rely upon the use of the plant as alternative medicine for healing of the many microbial disease origins that plaque around the world.

Keywords: Antimicrobial, Inhibition, Plant extract, *Phyllanthus muellerianus*; Phytochemicals

1. 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 occurrence of recently discovered diseases, the health care of very many people are still being



threatened by pathogens. The treatment options for some microbial origin diseases have become limited as result of the increasing trend of multi-drug resistant strains (Magiorakos et al., 2012). It is a popular phenomenon in using antibiotics to treat 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 imperatively yielded 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 distributed in all part of the world which however facilitated their effective pharmacological potency over the many available antibiotics that are now ineffective in treating some diseases. Plant chemicals known as phytochemicals are the requirements from plants for effective therapy. These plant chemicals are not of equal amounts among plants as some are found to be more from one plant to the other and each has their respective pharmacological function. Saponins for example, exerts some antibacterial activity by combining with cell membrane to elicit changes in cell morphology, leading to cell lyses (Moyo, 2011). 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 where it binds to ATP site of gyrase B and binds to bacterial DNA therapy inducing topoisomerase IV enzyme-mediated DNA cleavage and bacterial growth statics (Enwa et al., 2013). Terpenoids is also referred to as terpenes. Many terpenes have served good purposes in medicine. The artemisinin and paclitaxel as antimalarial and anticancer respectively are among the terpenes with immerse medical values (Guangyi et al., 2005). Flavonoids are source of antioxidant, it has been reported to increase the endothelial nitric oxide release and result in relaxation of blood vessels in conditions like hypertension and stroke (Andriantsitohaina et al., 2012), prevent hepatic steatosis and dyslipidemia in experimental models by either decreasing fatty acid synthesis or by increasing fatty acid oxidation (Assini et al., 2013) and also have antidiabetic action by acting on the biological targets that involved in diabetes mellitus type –2 such as aldose reductase and α - glucosidase (Tang et al., 2011; Jacques et al., 2013). Antioxidants keeps human cells from being damaged by reactive oxygen species (ROS) (Boakye et al., 2016).

Phyllanthus muellerianus is an evergreen shrub with turns all over the body of the plant, it also has the characteristics of climbing because of the flexibility of the branches. This plant has arsenal in medicine as a multipurpose plant employed in many countries to attack various diseases. The leaves, stem and even the roots are used to treat stomach upset, dysentery, infertility and wounds. Apart from its medicinal value, the leaves can be cooked as soup and the fruits are edible as other beneficial fruits.

Hence the many diseases that plague around us need 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. muellerianus*.

2. Materials and Methods

2.1 Plant material used

The leaves of *P. muellerianus* were collected from forest at Federal Polytechnic Ado-Ekiti, Ekiti State of Nigeria and were authenticated in Biological Sciences Department of Afe Babalola University, Ado Ekiti, Ekiti State by Mr. Adetunji. A voucher specimen was deposited at the Department's herbarium. Emphasis was focused on the plant leaves for its easy process than stem bark and roots; and mainly to give the plant a trial of its antimicrobial potency as geographical locations do have influence in plants' natural products. The leaves were rinsed 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 into air-tight containers with proper labeling. For crude extraction of the leaves, 500 g each of the ground leaf samples was weighed into two beakers. One was overlaid with 1000 ml of methanol and the other with also 1000 ml water solution. They were labeled appropriately and left for 24 h to obtain solutions containing the plant components. The extracts were passed through sterile muslin and finally through Whatman No 1 filter paper to obtain particles and sediment-free extract. Aqueous extract was concentrated in water bath regulated at 55 °C and methanol extract was concentrated with rotary evaporator. Before use, the extracts were mixed to solution with diluted dimethylsulfoxide (DMSO). DMSO was used to reconstitute the concentrated extracts to solution that can be obtained with pipette for distribution into wells created on microorganisms seeded agar plates.

2.2 Test microorganisms

Fifteen pathogenic microorganisms comprising of 11 bacteria species and 4 fungi species were used. The bacteria species include *Bacillus cereus*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Vibrio mimicus*, *Proteus mirabilis*, *Salmonella typhi*, *Escherichia coli*, *Enterobacter aerogenes*, *Arthrobacter mysorens*, *Shigella dysenteriae* and *Pseudomonas aeruginosa*; and the fungi species used include *Candida albicans*, *Candida glabrata*, *Candida krusei* and *Aspergillus flavus*. The pathogenic nature of these microorganisms necessitated their choice of use in this study. These microorganisms were sourced 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.

2.3 Antimicrobial extracts test

Using the agar well diffusion method, antimicrobial potency of the extracts were tested. Freshly prepared antimicrobial test medium (Mueller Hinton agar) was inoculated with 24 h test bacteria species of $1-2 \times 10^7$ Cfu/ml, a concentration matching with McFarland standards while fungal inoculums were plated with potato dextrose agar to test for antifungal activity of the extracts. The seeded plates for antimicrobial activity test were bored with 7 mm diameter sterile cork borer to create wells. The bored holes on each plate were filled with 50 μ l of plant extracts (aqueous and methanol). The cultured plates to determine antibacterial activity were incubated for 24 h at 37 °C incubated and the cultured plates for antifungal activity were incubated for 72 h at 28 \pm 2 °C. Inhibition of the microorganisms with the extracts was measured and recorded.

2.4 Determination of antibiotic sensitivity

The susceptibility of the microbial strains to different antibiotics was tested using disc diffusion method (NCCLS, 2000). Antibacterial agents from different classes of antibiotics were used which included Ciprofloxacin, Sparfloxacin, Zinnacef, Ampiclox, Streptomycin, Seprin,



Augmentin, Erythromycin, Gentamycin, Ampiclox, Amoxicillin, Pefloxacin, Tarivid, Tetracycline and Chloramphenicol. For fungal strains, nystatin was used.

2.5 Phytochemical Screening of extracts

The criterion of preliminary screening of secondary metabolites was carried out according to the methods described by Trease and Evan (1976); Harborne and Williams, (2000) to screen for alkaloids, terpenoids, tannins, saponins, cardiac glycosides, flavonoids, steroids, phenol and reducing sugars.

2.6 In vitro antioxidant screening

2.6.1 Determination of the level of ferric reducing antioxidant property

The method as described by Buricova and Reblova (2008) was adopted but with little modifications. Each (0.1 gram) of methanol and aqueous plant extract were dissolved into twenty milliliters of distilled water and filtered through Whatman's No 1 filter paper. 2.5 ml of 100% phosphate buffer and 2.5 ml of potassium ferrocyanide were added to 2.5 ml of filtered extract. Freshly prepared standard solution of 4 mg/100 ml ascorbic acid was used for the test. All preparation was carried out in triplicates. Mixture was incubated at 50 °C for 15 minute. 10% of trichloroacetic acid was added. This was followed by adding 1 ml of 0.1% ferric chloride and 5 ml of sterile distilled water. Using a spectrophotometer regulated at 700 nm, absorbance of the extracts and standard were read against blank and recorded. Oxidizable content in samples was expressed as mg of ascorbic acid/g of dry extract weight material.

2.6.2 Quantification of free radical scavenging

The method used was almost the same as used by Ibanez et al. (2003) but with modifications. An aliquot of 0.5 ml of 0.1 ml 1, 1- diphenyl 1-2 picrylhrazyl (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 mleach of the aqueous and methanol extract at a concentration of 20 mg/ml. The reactions were mixed in a temperature of 28±2 °C and stand 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 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.

2.6.3 Assay for Hydroxyl radical scavenging

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

2.7 Evaluation of extracts durability

To evaluate the shelf life of the antimicrobial extracts, durability study at regular intervals of five days was carried out. This was evaluated by checking the activities of the extracts against test microorganisms using the well-in-agar procedure. Onto 5 mm diameter well created on pre-seeded Mueller Hinton agar plates, 0.55 ml extract was filled 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.

2.8 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 Miyasakiet al.(2013). Overnight bacterial broth culture was 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 pour plated with nutrient agar. The plates were incubated at 37 °C for 18 h of which resultant colonies were counted and results expressed in \log_{10} CFU and plotted against time for each test microbe. Triplicate plating was done for the experiment.

2.9 Statistical analysis

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

3. Results

3.1. Antimicrobial activity

Zone inhibition of 37 mm was recorded on *S. aureus* with the leaf aqueous extract of *P. muellerianus*. This was followed by *P. aeruginosa* with inhibition of 30 mm. However inhibition zone of between 14 to 24 mm was recorded on other test bacteria species. 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. *P. muellerianus* leaf aqueous extract also exhibited 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 susceptible with zone of 20 mm to some of the antibiotics. Inhibition zones ranging from 6 – 19 mm was observed on other bacteria species (Table 1). Nystatin the reference antifungal, most inhibited *A. flavus* with zone of 17 mm and with 14 mm on *C. albicans* as the next inhibited among the fungi isolates. *C. krusei* was inhibited with 13 mm and it least inhibited *C. glabrata* with zone expression of 6 mm (Table 1).

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

| Bacteria species | Aqueous extract | Methanol extract | PEF | CN | APX | Z | AM | E | SXT | S | NY | CPX | OFX | SP | AU | CH |
|-------------------------------|-----------------|------------------|-----------|----------|-----|----------|----------|----------|----------|----------|----|----------|----------|----------|----------|----------|
| <i>Arthrobacter mysores</i> | 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±0.2 | - | 13.9±0.2 | - | - | - | - |
| <i>Bacillus cereus</i> | 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 | - | - | - | - |
| <i>Staphylococcus aureus</i> | 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 | - | - | - | - |
| <i>Enterobacter aerogenes</i> | 23.3±0.2 | - | 17.9±1.0 | - | - | - | - | - | - | - | - | 20.5±0.5 | 20.3±0.3 | 17.5±0.5 | 14.8±0.8 | - |
| <i>Salmonella typhi</i> | 24.6±0.4 | - | - | 14.8±0.8 | - | - | - | - | - | 15.3±0.3 | - | - | - | - | - | - |
| <i>Pseudomonas aeruginosa</i> | 30.0±0.2 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| <i>Vibrio mimicus</i> | 22.0±0.1 | - | - | 12.3±0.3 | - | - | - | - | - | - | - | - | 13.9±0.1 | - | - | - |
| <i>Proteus mirabilis</i> | 21.0±0.3 | - | 13.6±0.6 | - | - | - | - | - | - | - | - | 19.5±0.5 | 12.6±0.6 | - | - | 13.9±0.1 |
| <i>Klebsiella pneumoniae</i> | - | - | - | - | - | - | - | - | - | - | - | 11.5±0.5 | - | - | - | - |
| <i>Escherichia coli</i> | - | - | - | 20.5±0.5 | - | 18.5±2.0 | - | 14.8±0.3 | 14.0±0.0 | 19.5±1.5 | - | 14.0±1.0 | - | - | - | - |
| <i>Shigella dysenteriae</i> | 24.5±0.3 | - | - | - | - | - | - | - | - | 12.3±0.3 | - | - | - | - | - | - |
| Antifungal activities | | | | | | | | | | | | | | | | |
| Fungi species | Aqueous extract | Methanol extract | Nystatine | | | | | | | | | | | | | |
| <i>Candida glabrata</i> | 16.0±0.00 | - | 6.5±1.0 | | | | | | | | | | | | | |
| <i>Aspergillus flavus</i> | 10.0±0.00 | - | 17.7±0.4 | | | | | | | | | | | | | |
| <i>Candida albicans</i> | 14.5±0.8 | - | 14.5±0.1 | | | | | | | | | | | | | |
| <i>Candida krusei</i> | 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 – Amoxicillin; CPX – Ciprofloxacin; S – Streptomycin; SXT – Septrin; E – Erythromycin; CH – Chloranphenicol; SP – Sparfloxacin; AU – Agumentin; PEF – Pefloxacin; NY – Nystatin; Nil – No zone of inhibition; - not applicable; OFX – Tarivid.

3.2. Phytochemicals

Table 2 expresses the preliminary phytochemicals determined in the 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 were determined in the aqueous extract and were followed by saponins, 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 2. Qualitative phytochemicals of *P. muellerianus* leaf extracts

| Phytochemicals | Aqueous extract | Methanol extract |
|--------------------|-----------------|------------------|
| Saponins | ++ | + |
| Tannins | +++ | +++ |
| Phenols | +++ | +++ |
| Flavonoids | ++ | ++ |
| Alkaloids | +++ | + |
| Terpenoids | + | - |
| Steroids | + | - |
| Cardiac glycosides | + | + |
| Reducing sugars | ++ | ++ |

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

Table 3. Quantitative phytochemicals of *P. muellerianus* leaf extracts

| Phytochemicals | Aqueous extract | Methanol extract |
|----------------|-----------------|------------------|
| Phenols | 0.89±0.00 | 0.75±0.00 |
| Flavonoids | 3.75±0.06 | 2.04±0.04 |
| Tannins | 0.75±0.12 | 0.60±0.00 |
| Alkaloids | 207.29±0.09 | 43.00±0.00 |

Values are expressed as mean ± SEM of triplicates

3.3. Antioxidants

The *in vitro* antioxidant components of the extracts is expressed in table 4 with differences in values. The free radical scavenging activity of the leaf extract was 86.72 μmol (TE), ferric reducing power of 0.74 μmol (AAE) and hydroxyl radical scavenging potential of 0.51%. Observed and recorded values from the leaf methanol extract for free radical scavenging potential, ferric reducing power and hydroxyl scavenging ability are 56.26 μmol (TE), 1.04 μmol (AAE) and 0.44% respectively.

Table 4. *In vitro* antioxidants of *P. muellerianus* leaf extract

| Antioxidants | Aqueous extract | Methanol extract |
|---|------------------|------------------|
| FRAS DPPH(mg of ascorbic acid assay dry plant material) | 86.74 \pm 0.21 | 56.26 \pm 0.37 |
| FRAP(mg of ascorbic acid dry plant material) | 0.74 \pm 0.13 | 1.04 \pm 0.03 |
| HRS(%) | 0.51 \pm 0.14 | 0.44 \pm 0.35 |

Values are expressed as mean \pm SEM of triplicates

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

3.4. Shelf-life of extracts

The studied aqueous leaf extract was actively potent on test microbial species for 21 days after preparation, however, total ineffectiveness of the extracts was observed at day 39 (Fig. 1).

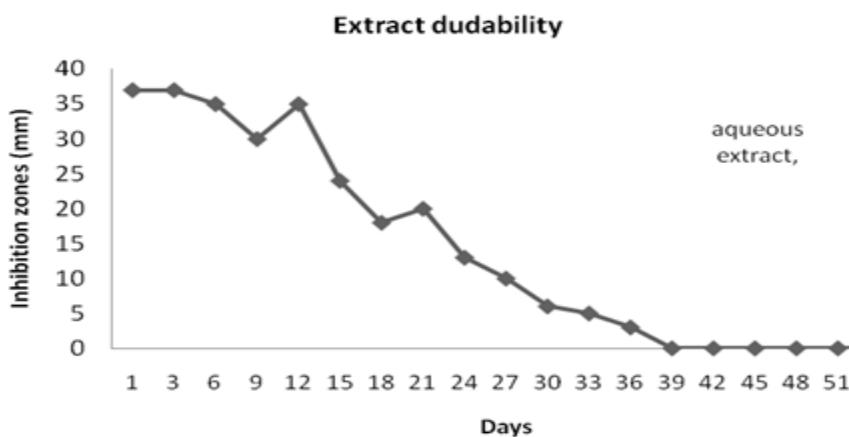


Figure1. Duration of extract potency

3.5. Kinetics of extract

Test isolates of *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).

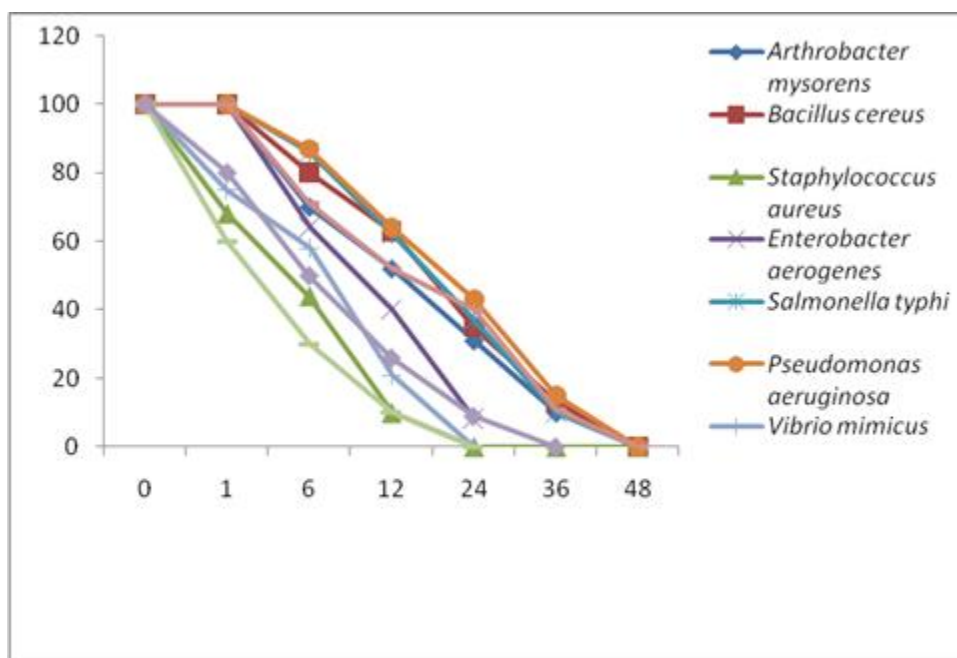


Figure2. Killing rates of the extracts

4. Discussion

4.1. Inhibitory effect of extracts

The leaf aqueous extract of the studied plants showed higher degree of antimicrobial potency on all the susceptible tested microorganisms than the methanol extract which had partial or no inhibition on the tested microorganisms. The better activity with water extracts might be referred to the improved scientific extraction methods to the traditional methods which involved extracting with hot water or boiling in water for several hours. Though the traditional medical practitioners use water often because it is a solvent mostly at their disposal to achieve successful healing, their success could be administration of the concoctions/or decoctions in large



quantities, and the treatment in most cases involves the use of the extracts for length of time (Yineger et al., 2008). These methods however, check against resistance of microorganisms when infections or diseases are treated with plant extracts. Though Clarkson et al.(2004) has reported less inhibition of microorganisms with the use of *P. muellerianus* aqueous leaf extract,we observed higher antimicrobial effect with the aqueous extract against *P. aeruginosa*, *S. aureus*, and *Proteus mirabilis*. The differences in results could be in 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 *P. aeruginosa*, *E. coli*, *S. pyogenes*, *S. aureus* and *C. albicans*(Boakye et al., 2004), *B. cereus*, *S. typhi*, *K. pneumoniae*, *P. mirabilis*, *S. flexneri*(Boakye et al., 2004) with varying degree of inhibition. *P. muellerianus* is one of the valuable plants of medical importance employed by traditional healers in Nigeria because of its representative role on disease causing pathogens that affect the populace. Based on report by Ofokansiet al. (2013), the leaf extract of *P. muellerianus* possessed antibacterial activity against *P. aeruginosa*, *P. mirabilis* and *E. faecalis* at 50 mg/ml and might be used to treat infections caused by the microbes. *P. muellerianus* has successfully assist in controlling infections of *P. aeruginosa* and *S. aureus* which are opportunistic bacteria species that can cause food poisoning, skin and urinary tract infections (Ofokansiet al. (2013). Antifungal activity of the leaf extract was manifest on *Candida* spp and it is in line with the report of Ofokansi *et al.* (2013). 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 mouth and urinary tract infections in man (Kotzekidou et al., 2008).

This study shows that various phytochemicals, including phenols, alkaloids and flavonoids are present in the leaves of *P. muellerianus*. Ofokansiet al. (2013), has reported similar phytochemical constituents in earlier study. However Awomukwuet al. (2014) 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 might result to the inhibition of microorganisms as a proof of its medical importance in traditional medicine. The antimicrobial test with the leaf extract in this study performed better activity than the commercial antibiotics. This could be traced to the various active phytochemical constituents

present in 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 aqueous extract. This finding is in correlation with Agyare et al. (2013) who in their earlier study stated that *P. muellerianus* leaf extract has antimicrobial activity and also its good effect in human physiology. The inhibition of *E. coli*, *P. aeruginosa*, *S. aureus* and *S. pyogenes* in this study further strengthened that extract from *P. muellerianus* may serve as wound healing substance hence these bacteria species are known for wound infections.

4.2. Phytochemical and antioxidant potentials of extracts

In a study on phytochemical constituents of the aqueous extract of *P. amarus*, the following phytochemicals were screened: alkaloids, saponins, tannins, anthraquinones, flavones, carotenoids, reducing compounds, cardiac glycoside, steroids and triterpenes, coumarins, and volatile oils, as reported by Odetola and Akojenu, (2000), which also we 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 (Rajeshwar et al., 2008). Phenols and phenolic compounds are used as disinfectants and have been a standard which other bactericidal agents are compared (Manikandan et al., 2016). Phenol was present in the leaf extracts as observed and Wahle et al. [2010], has reported that the presence of phenols in several plants have the ability to inhibit cancer cells *in vitro* and *in vivo*. In general, plant chemicals are known to have remedy for some health benefits like antihypertensive, antimicrobial, anti-diabetic and anti-inflammatory as reported by Oikeh et al. (2013). Interestingly, no side effect or toxicity have been reported for many years on this plant (Raphael et al., 2000)

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 0.1 mg/ml concentration of ascorbic acid standard. It has been reported that the risks of chronic diseases and diseases prevention linked to ROS is possible by supplementing with proven dietary antioxidants [Boakye et al., 2004]. This has resulted in

searching for quality natural antioxidant substances to eradicate the severe diseases implicated by oxidant (Sen and Chakraborty, 2011). The availability of phenol compounds like flavonoids and tannins manifested the quality scavenging activity of the aqueous extract hence they are active primary antioxidants (Belguidoum et al., 2015). Polyphenolic compounds are known for antioxidant effects and therefore the activity of *P. muellerianus* known to contain high amount of geraniin and other polyphenols may be responsible for its notable *invitro* antioxidant quality in this study. However, Belguidoum et al. (2015), has reported similar findings. The results also presented high reducing FRAP exhibited by the leaf aqueous extract of the employed plant. The result obtained correlates with the findings of Boakye et al. (2004). The quality FRAP observed could also be of the reason that the plant is rich in tannins as long as they are mediated through reducing power and scavenging activity as reported by Minussiet al. (2003). The reducing power ability of this extract may suggest that ROS such as H₂O₂, O₂ and OH may be neutralized by transferring through hydrogen atom (Boakye et al., 2004). The quality *in vitro* antioxidant activity as observed may suggest the vital role it could play in healing of 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 (Verma et al., 2004). 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 showed high concentration of phytochemical constituents, antimicrobial values and quality *in vitro* antioxidant. This may enhance a large segment of the world population to rely on the plant in the traditional system of medicine to heal diseases. 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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Competing interest

We, authors of this manuscript have no competing interest among us.

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