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Phytochemical Screening and Antimicrobial effects of Methanolic Extract of *Phyllanthus amarus* on Selected Clinical and Environmental Bacteria

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Abstract
The methanol extract of Phyllanthus amarus whole plant was analyzed for the phytochemical, proximate, mineral composition and antimicrobial properties using standard procedures. Steroids, glycoside, carbohydrates, tannins, alkaloids, flavonoids, eugenols, phenolics and terpenoids were present while saponins were absent in the plant extract. Proximate analysis of the plant gave moisture content (14.20 %), crude protein (4.06 %), fats (8.42 %), crude fibre (15.10 %), ash content (5.95 %), carbohydrate (52.27 %) and the metals analysed for were Fe (97.56 mg/Kg), Cu (32.30 mg/Kg), Ni (69.08 mg/Kg), Pb (0.00 mg/Kg), Na (16.30 mg/Kg), K (62.02 mg/Kg). Bacillus subtilis, Micrococcus cerus, Corynebacterium spp, Staphylococcus albus, Listeria spp, Eshcerichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Staphylococcus aureus and Proteus vulgaris were used for the antimicrobial test and the Minimum Inhibitory Concentration (MIC) ranged from 8.70 mm to 12.80 mm for all concentrations (1000 mg/ml, 500 mg/ml, 250 mg/ml and 125 mg/ml) while the Minimum Bactericidal Concentration (MBC) ranged from 8.20 mm to 14.20 mm. The results suggest that Phyllanthus amarus has significant antimicrobial effects and nutritional components which can be further explored.

Keywords: Antimicrobial, Bactericidal, Extract, Mineral, Phytochemicals

Introduction
The use of drugs derived from plants has been in practice for a very long time because of the high rate of mortality caused by bacterial infections and other diseases in human population and its significance cannot be over emphasized with the recent trend of high percentage of bacteria resistance to the present day antibiotics (Adebayo and Adegoke, 2008; Komolafe and Adegoke 2008). Medicinal plants are considerably useful and economically important. They contain many active constituents which are used in the treatment of many diseases (Ahirrao and Patil , 2010).

Modern research has paved way for the discovery of plants of potential value in the treatment of a wider range of ailments. Among these plants is *Phyllanthus amarus,* which has

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been in use in ayurvedic medicine (or Ayurveda, that is traditional Hindu system of medicine, which is based on the idea of balance in body systems and uses diet, herbal treatment and yogic treatment), *P. amarus* has quite a number of tradomedical uses including internal use for jaundice, gonorrhea, skin ulcer, menstruation and diabetics (Abo *et al.,* 2008, Adeneye *et al.,* 2006-a).

Phyllanthus amarus is a plant of the family *Euphorbiaceae* and has about approximately 800 species which are found in tropical and subtropical countries of the world. The name '*Phyllanthus*' means 'leaf and flower' and named so because of its appearance where flower, fruit and leaf appear fused. *P. amarus* is a branching annual glabrous herb which is 30-60cm high and have slender, leaf-bearing branchlet, distichous leaves which are sub-sessile elliptic- oblong, obtuse, rounded base. Flowers are yellowish, whitish, or greenish. The auxillary male flowers are in groups of one to three whereas females are solitary. *P. amarus* is a small herb common to central and southern India. It can grow to 30 to 60cm in height and bloom with yellow flowers (Zubair *et al.,* 2017). Fruits are smooth capsules present underneath the branches and seeds are trigonous , pale brown with longitudinal parallel ribs on the back .The plant has been found in Philippine, Cuba, Nigeria and among others. In India *P. amarus* is widely distributed in cultivated and waste lands. In Nigeria, the plant is called '*Eyin Olobe*' in Yoruba, '*Geeron- Tsuntsaayee*' (Bird's millet) in Hausa, '*Enyikwonwa*' in Ibo and "*carry me go seed*" in pidgin English (Oluboyo *et al.,* 2016).

P. amarus as a herb has found traditional usefulness in the treatment of several health problems such as diarrhea, diabetes, dysentery, dropsy, jaundice, intermittent fevers, urinogenital disorders, scabies and for wound treatment (Schlage 2002). It is applied topically for the treatment of several skin ulcers, sores, swellings and itchiness, wounds, bruises, scabies, ulcer and sores edematous swellings, tubercular ulcer, ringworm, scabby and crusty lesions (Saranraj and Sivasakthivelan, 2012). It has also found use in the treatment of several disease conditions commonly associated with women such as leucorrhoea, menorrhagia and mammary abscess and can act as galactagogue. Fresh leaf paste has wound healing capacity and it is used to cure white spots on the skin. The stem juice is also used for wound healing. The root extract is used to cure stomach pain while the flower paste is applied externally as antidote for snake bite (Bahar *et al.,* 2011).

Materials and Methods

Collection of samples

Phyllanthus amarus plant was collected from the natural habitat within Owo metropolis in Ondo State, Nigeria. The plant was identified accordingly at the Department of Plant Biology and Biotechnology, University of Benin, Benin City, Nigeria. The roots were cut off to avoid soil contamination and the whole plant was dried in the laboratory for four weeks (one month). The dried plant sample was ground to powder with an all-steel electric blender, kept in an air-tight glass jar and stored in a refrigerator until required for analysis.
The environmental bacterial isolates used for the antimicrobial activity were obtained from the Department of Microbiology, University of Benin, while the clinical isolates were procured from the University of Benin Teaching Hospital (UBTH), Benin City, Nigeria.

Laboratory procedures

Freshly prepared reagents were used for the phytochemical screening. All other solvents used were of analytical grade and were used without further purification.

Extraction of the plant
The powered sample of *P. amarus* (178g) was extracted with methanol using a soxhlet extractor. The crude extract obtained was then concentrated using a rotary evaporator. Further concentration of extract was carried out in a water bath at a controlled temperature of 50^0C.

Phytochemical Screening
Phytochemical screening was carried out on the powdered plant using standard procedures as described by Sofowora (1993) as well as Trease and Evans (2003).

Proximate and heavy metal analysis
The proximate composition and light metal analysis were carried out using the methods described by Ikhuoria *et al*., (2008). The heavy metal content was determined by first digesting the crude plant sample using standard methods (A.O.A.C., 2005) and thereafter analysed using atomic absorption spectrophotomer, AAS (Buck Scientific VGP210, U.S.A., 2005).

Antimicrobial assay
Antimicrobial activity and minimum inhibitory concentration (MIC) Test
Cultures of five clinical bacterial isolates: *Escherichia coli, Pseudomona aeruginosa, Klebisiella pneumoniae, Staphylococcus aureus* and *Proteus vulgaris* and five environmental bacterial isolates: *Bacillus subtilis, Micrococcus cerus, Corynebacteria spp, Staphylococcus albus*,, and *Listeria spp* were used for the antibacterial assay. Pure erythromycin, chloramphenicol, streptomycin, ciprofloxacin, amoxicillin, and gentamycin from Oxoide™, were used as reference standard drugs for comparing results and were obtained from the University of Benin Teaching Hospital. The drugs were selected based on availability as they are the commonest antibiotics used for treating infections in Benin City.

The sensitivity of the test organism to the methanolic extract of *P. amarus* was carried out using the cork boring agar diffusion method described by Ebi and Afieroho (2011) while the minimum inhibitory concentration was determined by the agar dilution technique described by Baron and Finegold (1990). Both tests were carried out in the Laboratory of the Department of Microbiology, University of Benin. The experiment was carried out in triplicates.

Minimum Bactericidal Concentration (MBC)
This refers to the minimum concentration of an antimicrobial drug that causes a 3-logarithmic decrease (99.9% killing) in the size of the standard inoculum. In general, if the MBC is no more than four times the MIC, the antibacterial agents are regarded as bactericidal. Sometimes, the MBC of some drugs is very close to its MIC. And if the MBC of the tested drug against the tested microorganism is $\geqq$ 32 times the MIC, the microorganism is said to have developed resistance to the tested drug (Creative Bio-lab, 2021).

Results and Discussion
The phytochemical screening of *Phyllanthus amarus* plant extract revealed the presence of steroids, glycoside, carbohydrates, tannins, alkaloids, flavonoids, eugenols, phenolics and terpenoids (Table 1). Saponins was absent. The results obtained are comparable to those obtained by some researchers on *P. amarus* plant (Foo and Wang, 1992; Zubair *et al.,* 2017).

Tanins have been shown to have astringent properties (Okwu and Josiah, 2006). This may be responsible for the ability of *P. amarus* extract to hasten healing of wounds and inflamed mucous membranes. Alkaloids in this plant may be responsible for its anti-malaria and analgesic properties and its use in the treatment of stomach disorders. Patel *et al*,. (2011) and Okwu (2004) reported that alkaloids and their synthetic derivatives have antispasmodic and bactericidal effects and therefore can be used as basic medicinal agents. The presence of flavonoids (an anti-oxidant) and phenolic compounds in this plant seems to be responsible for its antimicrobial anti-inflammatory, nephroprotective and hepatoprotective effects (Kasuya *et al*,. 2003 and Adeneye *et al*,. 2006-b)

Table 1: Results of phytochemical screening of *Phyllanthus amarus* leaf extract.

Phytochemical	Results
Carbohydrate	++
Phenolic compounds	++
Saponins	--
Terpenoids	++
Alkaloid	++
Tannins	++
Steroids	++
Flavonoids	++
Eugenols	++
Glycosides	++

Key: ++ = present; -- = absent

Table 2: Proximate composition of *Phyllanthus amarus*

Compound	Value (%)
Moisture Content	14.20
Crude Protein	04.06
Fat and Oil	08.42
Crude Fibre	15.10
Ash Content	05.95
Carbohydrate	52.27

Table 3 Mineral composition of *Phyllanthus amarus*

Metals	Values (mg/Kg)
Fe	97.56
Cu	32.30
Pb	00.00
Ni	69.08
Na	16.30
K	62.02

Table 2 shows the proximate composition of *P. amarus*. The carbohydrate content (52.27 %) is quite remarkable implying that apart from being medicinal, the plant is also a source of energy for the consumer. Also, some carbohydrates isolated from medicinal plants have been shown to exhibit anti-diabetic action (Akhtar *et al*., 1981 and Osadebe *et al*., 2004). *P. amarus* may also owe its medicinal property to this feature. The value of the moisture content (14.20 %) shows that the dry plant is not highly susceptible to microbial attack. However, the total ash value of 5.95 % suggests that the amount of inorganic substances in the plant is not very high and therefore the plant may be classified as a poor source of dietary inorganic salts.

The values for the selected heavy metals in *P. amarus* are shown in Table 3. The results show that Pb was not detected in the plant hence it may not pose serious health challenge to its consumers. The level of Fe in plant was found to be relatively high compared to other elements analyzed. This makes the plant a suitable candidate for the formulation of effective remedies for iron deficiency.

The antibiotic sensitivity pattern of the microorganisms to the selected antibiotics is shown in Table 4. The result shows that some of the organisms like *E. coli*, *P. aeruginosa* and *P. vulgaris* were resistant to most of the antibiotics. *B. subtilis* and *K. pneumoniae* were sensitive to erythromycin, streptomycin, gentamycin but resistant to chloramphenicol, ciprofloxacin, and amoxicillin. *S. albus* was sensitive only to erythromycin. However, *Listeria spp. M. cerus*, *Corynebacteria spp S. aureus* were found to be resistant to all the antibiotics they were tested against.

Table 4. Sensitivity of selected antibiotics on the microorganisms

	GMC (10.0µg/ml)	ERC (15.0µg/ml)	CPL (30.0µg/ml)	STC (10.0µg/ml)	CFC (30.0µg/m)	AMC (30.0µg/ml)
	Zone of inhibition(mm)					
E. coli	19.0	13.0	11.0	15.0	16.0	10.0
P. aeruginosa	16.5	14.0	14.0	10.0	14.0	9.0
K. pneumoniae	11.6	16.0	17.0	18.0	12.0	12.0
S. aureus	12.6	21.2	12.4	11.8	12.0	11.8
P. vulgaris	12.0	17.0	15.0	12.0	13.0	10.0
B. subtilis	17.0	16.0	11.0	15.0	7.5	11.0
M. cerus	14.0	11.0	12.5	13.5	8.5	10.0
Cornebact. spp	12.0	12.5	1.8	11.0	9.0	12.0
S. albus	13.0	19.6	11.5	12.0	12.5	11.5
Listeria spp.	11.0	8.7	10.6	11.8	10.8	9.7

Strength of antibiotic < 14.5 = resistant; Strength of antibiotic > 14.5 = sensitive
KEY: GMC = gentamycin, ERC = erythromycin, CPL = chloramphenicol, STC = streptomycin, CFC = ciprofloxacin, AMC = amoxicillin

Table 5 shows the antimicrobial activity of methanol extract of *P. amarus* on bacterial isolates The antimicrobial assay of the plant extract on the clinical isolates shows that the zone of inhibition for *S. aureus* for all different concentrations ranged from 16.40mm - 25.00mm, *E. coli* ranged from 12.50mm - 16.30mm, *P. aureginosa* ranged from 15.80mm - 21.20mm, *K. pneumoniae* ranged from 11.20mm-13.50mm and *P. vulgaris* ranged from 12.10mm - 16.20mm.

The antimicrobial assay of the plant extract on the environmental isolates shows that the zone of inhibition ranges were from 18.50mm – 12.70mm for *B. subtilis,* 10.80mm – 10.10mm for *M. cerus,* 11.20mm – 10.60mm for *Corynebacterium spp.,* 16.50mm – 12.80mm for *S. albus,* and 10.20mm – 8.70mm for *Listreia spp*. This is an indication that the methanol extract of *P. amarus* contains substances that can inhibit the growth of some microorganisms. The observed inhibitory effect was more as the concentration of the extract was increased. This is an indication that the microbial effect of the extract is dose dependent. However, the extract was not able to significantly inhibit the growth of *E. coli, P. aueuginosa, K. pneumoniae, S. aureus* and *P. vulgaris*. This may be because these organisms possess a mechanism of inactivating the active ingredients in the extracts or other mechanisms which such as exclusion of the substance from the cell or modification of the target site of the substance (Dhandapani *et al*., 2007).

Table 5. Antimicrobial activity of methanol extract of *P. amarus* on bacterial isolates

	Concentration of *P. amarus* extract			
	1000mg/ml	500mg/ml	250mg/ml	125mg/ml
	Zone of inhibition (mm)			
E. coli	16.30	14.50	12.70	12.50
P. aeruginosa	21.20	19.40	17.60	15.80
K. pneumoniae	13.00	13.50	12.40	11.20
S. auerus	25.00	21.30	18.10	16.40
P. vulgaris	16.20	14.70	13.50	12.10
B. subtilis	18.50	13.60	13.90	12.70
M. cerus	10.80	10.70	10.90	10.10
Corynebact. spp	11.20	10.50	10.70	10.60
S. albus	16.50	14.10	13.60	12.80
Listeria spp	10.20	9.50	9.40	8.70

strength of antibiotic. < 14.5 = resistant; > 14.5 = sensitive

The minimum bactericidal concentration (MBC) exhibited by the methanol extract of *Phyllanthus amarus* is shown in Table 6. *E. coli, P. aeroginosa, S. aureus, P. vulgaris*, *K. pneumonia* and *B. subtilis*, which have earlier been shown to be multi-drug resistant organisms, had a bactericidal zone of inhibition greater than 10mm. Thus the extract may be used as an alternative to orthodox antibiotics as it will be relatively cheaper to procure. Also microorganisms are less likely to develop drug resistance to the plant extract and as a medicinal plant, it is expected to be relatively free of contraindications (Akinjogunla *et al*., 2010).

Table 6. Minimum bactericidal concentration of 125mg/mL *P. amarus* extract

Microorganism	Bactericidal zone (mm)
E. coli	11.7
P. aeruginosa	12.5
K. pneumoniae	10.8
S. auerus	14.2
P. vulgaris	10.6
B. subtilis	11.3
M. cerus	-
Corynebact. spp.	8.9
S. albus	11.5
Listeria spp.	8.2

The methanol extract of *P. amarus* could inhibit the growth of *B. subtilis, M. cerus, Corynebact. spp., S. albus* and *Listeria spp.,* but was not able to totally eliminate these micro-organisms as potently as the standard antibiotic drugs as seen in Table 3. The plant may therefore be used as a cheaper alternative to orthodox antibiotics.

Conclusion

The results obtained have shown that *Phyllanthus amarus* contained considerable amount of some important chemical compounds. Also the resourcefulness of this plant has allayed or lessened the fear in therapeutic failure of antibiotics to some life threatening infectious diseases in some countries. Moreover, the plant extract has proved to be a potential herbal drug which if explored by the appropriate bodies could be a useful product in medical science, open a possible wide door for profit and increase employment opportunities for the pharmaceutical industry.

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