

## Full Length Research Paper

# Antibacterial and antifungal activities of leaf extracts of *Phyllanthus amarus* Schum and Thonn.

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The leaf extracts of *Phyllanthus amarus* Schum and Thonn. a common Western Nigerian weed, was investigated for phytochemical constituents and antimicrobial activities. Hexane extracts demonstrated the highest extraction of 59% followed by Acetone (57%) and water (48%). Saponins, tannins, alkaloid flavonoids, balsam, anthraquinones and phenols were extracted in an acidic medium. Twelve microorganisms (8 bacterial species and 4 fungal species) were investigated for antimicrobial activities. Generally, the fungal species showed lower minimum inhibitory concentration (MIC) than the bacterial species. The test organisms showed close susceptibility to the leaf extracts when compared with Bacitracin and Erythromycin. Generally, the combination of the leaf extract with either bacitracin or erythromycin alone demonstrated synergistic effect thus showing the potential benefit of combining these standard antibiotics with the leaf extracts of *P. amarus* for combating infections.

**Key words:** *Phyllanthus amarus*, leaf extracts, phytochemicals, antimicrobial property antibiotic.

## INTRODUCTION

*Phyllanthus amarus* belongs to the family Euphorbiaceae. It occurs as a weed throughout the Southern and Western part of Nigeria. It is called "Eyin-Olobe" or "yoloba" among the Yoruba speaking populations of Western Nigeria. According to folklore medical research, it is used widely by the local people for the treatment of gonorrhoea, genito-urinary diseases, asthma, diabetes, typhoid fever, jaundice, stomach ache, dysentery, hypertension and ringworm (Odugbemi, 2008). Other reports claim the use of the plant juice extracted from the stem for ophthalmic condition (Idika and Niemogha, 2008).

Foo and Wong (1992) have reported the presence of tannin, phyllanthusin D in the plant. Similar reports have reported the occurrence of hypophyllanthin (Mahidol et

al., 1994), lignans (Singh et al., 2009) and alkaloids (Houhgton et al., 1996).

Venkateswaran et al. (1987) have shown that the leaf extract of the plant possess the ability to inhibit hepatitis viruses A and B. The plant extract was equally shown to inhibit the replication of a variety of RT inhibitor resistant Human Immunodeficiency Virus-I (HIV-I) strains. It has been reported to inhibit chemically induced liver tumor in rats (Joy and Khuttan, 1988), increase the life span of rats with hepatocellular carcinoma (Rajeshkumar and Kuttan, 2000); exert protective effect on gentamicin and acetaminophen induced nephrotoxic rats (Adeneye and Berebo, 2008). It exhibits gastro-intestinal potentials in human subjects (Odetola and Akojenu, 2000) and possess some antimalarial activity (Tona et al., 1999).

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The essential oil and fractional samples have also been demonstrated by Ogunlesi et al. (2009) to possess antimicrobial activity.

In Nigeria, the Federal Government has established the National Agency for Food and Drug Administration Control (NAFDAC) with the purpose of screening and monitoring the production of orthodox drugs among others. Within the last five years, several traditional health practitioners have submitted many plant-based natural medications to the regulatory body for assessment and approval. Accordingly, some of them have been approved and have since been patented for the use by the general population. However, the agency does not have the essential equipments, required personnel, funds and political will to conduct a thorough evaluation of the various herbs submitted to it.

Thus they have no basis to accept or reject the claims of these traditional medical practitioners concerning the composition and efficacy of these herbs. As a result, NAFDAC usually writes on each of these approved drugs the inscription. "These claims have not been evaluated by NAFDAC". Since folklore medicine is already widely used by the people largely due to poverty and accessibility to these orthodox medicines by the rural populace, it does not seem wise to discourage this practice by NAFDAC which at least ensure that the herbal drugs are wholesome (Kuti, 1997). However, it will seem rather sensible to embark on an aggressive scientific investigation into the efficacy toxicity and contra indications of these herbs so as to give a meaningful scientific direction to the use of herbs. This is the basis of the present effort on *P. amarus*.

Similarly, many of the reports of the antimicrobial activities of the plants have either utilized the oil extract alone (Ogunlesi et al., 2009) or used plants collected from Asia (Joy and Khutta, 1988; Tona et al., 1999). There is the need to carry out these tests with plants obtainable from our locality. This is because ecotypes often have different concentrations of the active substances across regions of the world.

## MATERIALS AND METHODS

### Plant material and preparation

The leaves of *Phyllanthus amarus* were collected from Ijebu-Ode in Ogun State. This plant was authenticated at El-kaf Herbarium, Olabisi Onabanjo University. The leaves were dried in an oven at 45°C for fourteen days. The dried leaves were powdered and stored in a sterile bottle at room temperature. Water, acetone, and hexane extraction were carried out by using Soxhlet extractor (Quickefit U.K). Powdered dried leaves (50 g) were extracted with 250 ml of each solvent (Akinyemi et al., 2005).

### Test organisms

The bacteria used were *Neisseria gonorrhoea*, *Salmonella typhi*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Staphylococcus aureus*,

*Escherichia coli*, *Streptococcus pneumonia*, *Bacillus subtilis*, while the fungi were *Aspergillus niger*, *Aspergillus tamari*, *Candida albicans* and *Fusarium oxysporium*.

The standard strains were from stocks of culture collections maintained in the Microbiology Department of International Institute of Agriculture Research and Technology IAR&T, Jericho, Ibadan, Oyo State. Bacteria were maintained on nutrient agar slants and fungi on Sabouraud Dextrose agar at 4°C and sub cultured monthly.

### Preliminary photochemical studies

The extract was subjected to various phytochemical tests to determine the active constituents present in the crude aqueous, acetone, and hexane extracts. The slightly modified methods of Sofowora et al. (1982) and Trease and Evans (1989) were used.

### Determination of pH value

The pH was measured using a pH meter of a glass electrode. The glass electrode was immersed in water for several hours before use. The measurement started about 5 min after the equipment was switched on and the detecting unit was rinsed well with water and cleaned with a piece of filter paper. The pH meter was adjusted at one pH value and the temperature compensation dial was rotated to set the temperature of the pH standard solution. The detecting unit was then immersed in the pH standard solution and measurement taken about 2 min when the pH meter is set to the pH of the standard solution.

The detecting unit was removed from the standard solution, washed well with water and gently blotted with a piece of filter paper to remove water. It was then immersed in the sample solution, and the pH value measured. The temperature of the sample solution and that of the pH standard solution was the same.

### Antimicrobial activity leaf extract

#### Disc diffusion method

The powdered leaf was dissolved in water, acetone and hexane. Discs of about 6 mm diameter were made from Whatman's No.1 filter paper using a paper puncher. Batches of 100 discs were transferred into Bijou bottles and sterilized in the oven at 121°C for 15 min. The sterilized discs were soaked with 2 drops of the extract using a sterile Pasteur pipette and allowed to dry at room temperature.

#### Standardization of inoculum:

The inocula were prepared from the stock cultures which were maintained in nutrient agar slant at 4°C and subculture in nutrient broth using a sterilized wire loop. The density of suspension inoculated into the media for test was determined by comparison with 0.5 McFarland standard of Barium sulphate solution (Cheesbrough, 2002). Spore suspension for fungal bioassay was prepared according to the procedure of Murugan et al. (2007).

A sterile cotton swab was then used to spread the resulting suspension uniformly on the surface of the oven-dried Isosensitive Agar (oxid) and Sabouraud dextrose agar plates (Sterillin) for bacteria and fungi, respectively. Sterile forceps were used to place each of the discs on the agar plates aseptically and the inoculated plates were then incubated at 37°C for 24 h for bacteria strains and at 25°C for 72 h for the fungal strains. Antimicrobial activity was evaluated by noting the zone of inhibition against the test organisms.

**Table 1.** Phytochemical constituents of the leaf extract of *Phyllanthus amarus*.

Parameters determined	WE	ACE	HEX
pH	4.40	5.20	5.70
% Extraction	48	57	59
Saponins	-	+	+
Tannins	+	+	+
Alkaloids	-	-	TRACE
Flavonoids	-	+	+
Balsams	-	-	+
Anthraquinones	-	-	+
Phenol	+	+	+

+ = Present; - = absent.

**Table 2.** The minimum inhibitory concentration (MIC) of the different extracts on the test organisms.

Organisms	WE	ACE	ETH	HEX	METH	Bacitracin (30 µg)
<i>Neisseria gonorrhoea</i>	1300	1200	1000	1000	950	800
<i>Salmonella typhi</i>	1200	1100	1000	900	900	800
<i>Pseudomonas aureginosa</i>	1300	1200	1100	950	850	600
<i>Proteus vulgaricus</i>	1200	1100	950	900	800	1000
<i>Staphylococcus aureus</i>	1200	1100	1000	950	1000	800
<i>Eschericia coli</i>	1100	1000	900	850	900	800
<i>Streptococcus pneumonia</i>	1000	900	800	750	800	700
<i>Bacillus subtilis</i>	1000	900	800	800	900	600
<i>Aspergillus niger</i>	600	500	400	350	400	500
<i>Aspergillus tamari</i>	500	400	350	300	400	400
<i>Candida albicans</i>	600	500	450	400	500	300
<i>Fusarium oxysporum</i>	400	300	250	200	300	300

WE = Water extract; ACE = acetone extract; ETH = ethanol extract; HEX = hexane extract; METH = methanol extract.

### Minimum inhibitory concentration (MIC)

The estimation of MIC of the crude extracts was carried out using the method of Akinpelu and Kolawole (2004). Two-fold dilutions of the crude extract was prepared and 2 ml aliquots of different concentrations of the solution were added to 18 ml of pre-sterilized molten nutrient agar and SDA for bacteria and fungi respectively at 40°C to give final concentration regimes of 0.050 and 10 mg/ml.

The medium was then poured into sterile Petri dishes and allowed to set. The surface of the medium was allowed to dry under laminar flow before streaking with 18 h old bacterial and fungal cultures.

The plates were later incubated at 37°C for 24 h and at 25°C for up to 72 h for bacteria and fungi respectively, after which they were examined for the presence or absence of growth. The MIC was taken as the lowest concentration that prevented the growth of the test microorganisms.

### RESULTS

Table 1 shows the results of the phytochemical constituents of the leaf extracts of *P. amarus*. Hexane demonstrated the highest percentage (59%) extraction followed by Acetone (57%) and water (48%) respectively.

Thus saponins, tannins, alkaloids, flavonoids, balsam, anthraquinones and phenols were all extracted by hexane. Acetone extracts did not contain alkaloids, balsams and anthraquinones while the water extract only contained phenols and tannins. All the extracts were acidic.

Table 2 shows the minimum inhibitory concentration of the different extracts on the test organisms. Generally, the methanol extract had the highest activity against all the test organisms used. This was followed by hexane, ethanol, acetone and water extracts respectively. The fungal species used demonstrated lower MIC generally when compared to the bacterial species thus indicating their increased susceptibility to the extract. The hexane extract has a lower MIC (850) than the methanol extract (900). This trend is also observable for all the four fungal species. *F. oxysporum* emerged as the most susceptible micro-organisms to all the extracts as compared to all the other strains. *S. aureus* showed a higher MIC (1000) for methanol extract than hexane (950). This was also observed for *S. pneumoniae* (850 against 900), *E. coli* (900 versus 850) and all the fungal species. The gram

**Table 3.** Synergistic effect of the combination of the extracts with erythromycin and bacitracin.

Organisms	Zone of inhibition (mm)					
	E	B	EB	E	ER	EER
<i>N. gonorrhoea</i>	8	10	12	8	11	14
<i>S. typhi</i>	8	11	13	8	12	15
<i>P. aureginosa</i>	9	12	14	9	14	16
<i>P. vulgaricus</i>	10	13	15	10	14	16
<i>S. aureus</i>	11	10	12	12	11	13
<i>E. coli</i>	12	10	13	11	12	15
<i>S. pneumoniae</i>	10	11	12	11	13	15
<i>B. subtilis</i>	9	8	10	8	9	12
<i>A. niger</i>	6	5	7	7	6	9
<i>A. tamari</i>	6	7	8	6	8	9
<i>C. albicans</i>	6	7	8	6	8	10
<i>F. oxysporium</i>	4	6	5	4	5	9

E = Extracts only, B = bacitracin, EB = extracts/bacitracin, ER = erythromycin, EER = extracts/erythromycin.

positive species of *S. pneumoniae* and *S. aureus* both showed lower MIC for hexane than methanol extract. The gram negative species of *N. gonorrhoea*, *S. typhi*, *P. aureginosa* and *P. vulgaricus* had lower MIC for methanol extract as compared with the hexane extract. For all the test organisms, the water extract had the highest MIC when compared with all the other extracts.

Table 3 shows the effects of the standard antibiotics erythromycin and bacitracin on the test organisms separately as well as their effect when combined with the extract. It also shows the effect of the extract alone on the test organisms. From the table, it is observed that the zone of inhibition of the extract alone was less than either bacitracin or erythromycin alone in the case of *N. gonorrhoea*, *S. typhi*, *P. aureginosa*, *P. vulgaricus*, and *S. pneumoniae*. However, from the bacteria group both *B. subtilis* and *S. aureus* had slightly higher zone of inhibition in the extract alone than both bacitracin and erythromycin alone.

Among the fungi species used, *A. niger* showed a higher MIC when bacitracin or erythromycin is applied alone. However, the other three fungal species of *A. tamari*, *C. albicans* and *F. oxysporium* showed slightly lower MIC for the extract alone as compared with either bacitracin or erythromycin alone.

The situation, however, is consistent when the synergistic effect of the combination of the extract with either bacitracin or erythromycin is considered. In all cases, the combination showed a higher zone of inhibition than any of them when applied separately.

## DISCUSSION

All the extracts were acidic (pH 4.40 - 5.70) with the water extract showing the highest acidity. The acidity of

the extract alone could have contributed to the overall antibiotic properties of the plants. This is because bacteria have been known to be generally sensitive to acidic environment.

The fungi species demonstrated higher sensitivity to the extract than the bacterial species. This is a welcome development because most antibiotics are active against bacteria than fungi. The study indicates the potential of the leaf extracts of *P. amarus* as an antimicrobial agent.

Specifically, the most sensitive organism of all the test organisms is *F. oxysporium* which demonstrated the lowest MIC for all the extract types used as well as the lowest MIC for hexane extract as compared with all the other extracts. The result is consistent with that of Ogunlesi et al. (2009) who tested the oil extracts of the leaf and seeds of *P. amarus* for antimicrobial activity. However, the results obtained by them indicated that there was no activity against *P. aeruginosa*. The result obtained in this study indicated that *P. aeruginosa* is sensitive to the crude hexane extracts used. The difference may be explained by the fact that the active ingredients to which *P. aeruginosa* was sensitive might have been presented in the crude extract used but not in the oil extract used previously. This may suggest the preference of the crude extract for further analysis as compared to the oil extract alone.

The gram negative and gram positive species used did not show any significant difference in their susceptibility to the extract. This suggests the use of *P. amarus* extract as a broad-spectrum antibiotic herb for most common human infections.

It is suggested that for all purposes the hexane extract should be used when testing the efficacy of the plant because it extracted more of the classes of phytoconstituents than the water and acetone extracts. The phytoconstituents are bioactive and they are known

to be bactericidal, pesticidal or fungicidal in nature (El-astal et al., 2005; Senjobi et al., 2011).

Most of the test organisms used in this study are associated with various human and animal infections. *P. vulgaricus* causes wound infections and urinary tract infections (Parekh, 2007). Similarly, *E. coli* is a chief cause of septicemia (Black, 1996).

The extract when combined with either bacitracin or erythromycin showed synergistic effect than when any of them was used alone. This shows the potential of the leaf extract with other standard antibiotics for either increasing their potency or making them broader. Furthermore the fact that the plant is abundant and grows extensively as a weed makes it cheap to access and therefore less expensive than the classical antibiotics. Since there has been no reported case of side effect, it is believed that the plant can be used by the local populace pending the outcome of further studies on the toxicity of the plant extract.

## Conclusion

The leaf extract of *P. amarus* in this study showed a broad-spectrum activity against gram negative and gram positive bacteria as well as fungal species. This could be probably due to the phytoconstituents identified from the extract. The results also confirm the use of the plant in traditional medicine. It is suggested that the extract should be purified, concentrated and individually tested so as to identify the specific bioactive element(s) responsible for the antimicrobial activity.

## Conflict of interests

The authors declared that there is no conflict of interests regarding the publication of paper.

## REFERENCES

- Adeneye AA, Benebo AS (2008). Protective effect of the aqueous leaf and seed extract of *Phyllanthus amarus* on gentamicin and acetaminophen – induced nephrotoxic rats. *J. Ethnopharmacol.* 118:318-323.
- Black JG (1996). *Microbiology: Principles and Application*. Prentice Hall, New York. P 260.
- El-astal ZY, Aera A, Aam A (2005). Antimicrobial activity of some medicinal plant extracts in Palentine. *Pal. J. Med. Sci.* 21(2):187.
- Foo LY, Wong H (1992). *Phyllanthus* Ilin D, an unusual hydrolysable tannin from *P. amarus*. *Phytochemistry* 31:711-113.
- Houhgton PJ, Woldemariam TZ, O'shea S, Thyagarajam SP (1996). Two securinega-type alkaloids from *P. amarus*. *Phytochemistry* 43:715-717.
- Idika N, Niemogha MT (2008). The Diversity of Uses of Medicinal Plants in Nigeria: In A Textbook of Medicinal Plants from Nigeria. Odugbemi T (Ed.), University of Lagos Press: Lagos, ISBN 978-978-48712. pp. 71-79.
- Joy KL, Kuttan R (1998). Inhibition of *P. amarus* of hepatocarcinogenesis induced by N-Nitrosodiethylamine. *J. Clin. Biochem. Nutr.* 24:133-139.
- Mahidol C, Sahakitpichan P, Ruchurawat S (1994). Bioactive natural products from Thai plants. *Pure Appl. Chem.* 66:2353-2356.
- Odetola AA, Akojenu SM (2000). Anti-diarrhoeal and gastro-intestinal potentials of the aqueous extract of *P. amarus*. *Afr. J. Med. Sci.* 29:119-122.
- Odugbemi T (2008). A Textbook on Medicinal Plants from Nigeria. University of Lagos Press, Lagos, Nigeria. ISBN: 978-978-48712-9-7. pp. 542-612.
- Ogunlesi MW, Okiei EA, Osibote S, Muotoh C (2009). Antimicrobial activity of the essential oil and the fractional samples obtained from the leaves and seeds of *phyllanthus amarus* (Euphorbiaceae). *Res. J. Phytochem.* 3:77-84.
- Parekh J (2007). In Vitro screening of antibacterial activity of aqueous and alcoholic extracts of various Indian plant species against selected pathogens from Enterobacteriaceae. *Afr. J. Microbiol. Res.* 1(6):92-99.
- Rajeshkumar NV, Kuttan R (2000). *P. amarus* extract administration increases the life span of rats with hepatocellular carcinoma. *J. Ethnopharmacol.* 73(1):215-219.
- Kuti OR (1997). National Drug Policy in Nigeria. *J. Publ. Health Pol.* 13:367-373.
- Senjobi CT, Moody JO, Ettu AO (2011). Antimicrobial and cytotoxic effects of *cnidoscolus aconitifolius* (Miller) Johnson. *J. Agric. Biol. Sci.* 2(2):021-025.
- Singh MN, Tiwari K, Shanker R, Verma K, Gupta AK, Gupta MM (2009). Two new lignans from *phyllanthus amarus*. *J. Asian Nat. Prod. Res.* 11:562-568.
- Sofowora A (1982). *Medicinal Plants and Traditional Medicine in Africa*. 1<sup>st</sup> Edition. John Wiley and Sons Ltd. 131:168-171.
- Tona L, Ngimbi NP, Tsakala M, Mesia K, Cimanga K, Apers S, De Bruyne T, Pieters L, Totte J, Vlietinck AJ (1999). Antimalarial activity of 20 crude extracts from nine African Medicinal Plants used in Kinshassa, Congo. *J. Ethnopharmacol.* 68(1):193-203.
- Trease GE, Evans WC (1989). *Pharmacognosy* 13<sup>th</sup> Edition. Bailliere Tindall, London. pp. 176-180.
- Venkateswaran PS, Milliman I, Blumberg BS (1987). Effects of extract from *phyllanthus niruri* on hepatitis B and woodchuck hepatitis viruses: *In vitro* and *in vivo* studies. *Proc. Natl. Acad. Sci. USA* 84(1):274-278.