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Original article

Phytochemical composition and antibacterial efficacy of *phyllantus amarus linn* against bacteria isolated from ready-to-eat foods vended in Akure, Nigeria

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ABSTRACT

Background: *Phyllantus amarus* has been exploited in different parts of the world because of its pharmacological value. This value is known to be as a result of the phytochemical constituents found in different parts of the plants. In this paper, we examined the phytochemicals inherent in the leaves of *P. amarus*, both qualitatively and quantitatively, while also evaluating its antimicrobial efficacy against selected pathogenic bacteria. **Methods**: Test bacteria were isolated from ready-to-eat food samples. Extracts of *P. amarus* were prepared using water, ethanol and n-hexane as extracts and then screened for their bioactive components and antibacterial activities. The chemical components and their percentage abundance were evaluated using Varian GC – MS equipment alongside Mass Spectrometer (MS) 3000 equipped with Agilient MS capillary column. **Results:** Phytochemicals such as Flavonoids, Tannins, Phenolics Amino Acids, Saponins, among others were detected in various extracts of the plant. Ethanol extract at 300 mg/ml showed the highest potency against *Salmonella typhi* with a zone of inhibition 24.67±0.88 mm but had no effect on *Citrobacter freundii*. Water extract at 300 mg/ml induced zone of inhibition of 15.00±1.73 mm and 15.00±1.16 mm against *Staphylococcus aureus* and *Salmonella typhi* respectively, while *Shigella dysenteriae* was the least susceptible with an inhibition zone of 12.67±0.33 mm. **Conclusions**: The study demonstrated that extracts of *Phyllanthus amarus* have antimicrobial potency on bacterial isolates. These antibacterial properties are due to the presence of some phytochemicals and bioactive compounds.

Introduction

The wide spread of multiple antibiotic-resistant (MAR) bacteria has grossly limited the effectiveness of antimicrobial therapy, and by this, antimicrobial resistance became one of the most important areas of research in biomedical sciences [1]. This widespread resistance to antibiotics is partly connected to the extensive administration of antibiotics for the treatment of diseases [2]. In view of this public health challenge experienced globally, the present study therefore, suggests an alternative method for treating bacterial infections by evaluating the phytochemical and antimicrobial properties components of *Phyllanthus amarus* extracts.

Phyllanthus amarus is a medicinal plant naturally found growing in parts of Africa, America and South-East Asia [3]. The plant usually grows to a

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height of 30 – 60 cm, in soil of high moisture with light shade and reaches maturity in 2 – 3 months [4,5].

Various ethnic groups, particularly amazonian tribes, have utilized *P. amarus* in folkloric medicine to cure gallstones and kidney stones; in ayurvedic medicine for the treatment of bronchitis, anemia, and diabetes; and in Malay traditional medicine for diarrhea, kidney ailments, and gonorrhea [6]. In recent times, there have been preclinical and clinical studies looking into the plant's supposed liver-protective capabilities and its effect on hepatitis B viral infection [7].

In general, medicines derived from natural sources are believed to be more suitable for the human body when compared to drugs of synthetic origin [8]. Some medicinal plants have been used customarily for a long time while recent scientific studies have revealed the existence of a good link between the traditional use of some of these plants which further strengthens the search for pharmacologically active components from plants [9]. According to the World Health Organization (WHO), about 60 - 80% of the world's populace still rely on traditional medicine for the treatment of common diseases [10].

The medicinal value of *P. amarus* is reportedly linked to the presence of some phytochemicals in it, such as flavonoids (quercetin-3-0-glucoside and ruin), tannins (geraniin, amariin and gallocatechin) and alkaloids (phyllantine, quinolizidine type, securinine, norsecurinine, isobubbialine and epibubbialine) [11,12]. In this study, we examined the qualitative and quantitative phytochemical composition of P. amarus as well as its antimicrobial efficacy on some selected intestinal pathogenic bacteria.

Material and methods

Collection and authentication of plant materials

Fresh leaves of *P. amarus* were obtained from Ilere community around the Federal University of Technology, Akure (FUTA), Nigeria, and were identified by experts at the department of Crop, Soil and Pest Management, FUTA, Nigeria. The plant materials collected were thoroughly washed with running water, dried in the shade for 2 weeks, and then ground into a coarse powder using an electric blender (Electroline model IS 4780, CM/L 7902804). The powder was stored in a sterile container for further use as described by **Mishra et al.** [13].

Preparation of plant extracts

The powdered plant materials were immersed in 250 ml of different solvents (n-hexane, ethanol and distilled water) contained in 500 ml sterile conical flasks and placed in a shaker incubator for 4 days. The solutions were then filtered through Whatman No. 1 filter paper and the solvents were left to evaporate. The extracts were stored at 4 ℃ until further use [14].

The stock solution of the leaves extract was prepared by dissolving 1g of the resultant residue of the extracts after evaporation into 30% of dimethyl sulfoxide (DMSO) prepared with saline water to give a concentration of 100mg/ml as the stock solution. Furthermore, the concentrations of 200mg/ml and 300mg/ml were also prepared using the same stock solution. The tubes containing the different concentrates were stored in the refrigerator at 4 ℃ for further use.

Phytochemical analysis

The leaves extract of *P. amarus* was analyzed for the presence or absence of the following phytochemicals: alkaloids, terpenoids, tannins, saponins, flavonoids, and quinines using standard methods described by **AOAC** [15].

Identification of biologically active compounds in plant extracts

The method of **Olusola-Makinde et al**. [16] was used for the evaluation of the chemical components using a Varian GC – MS equipment (Varian 4000 mass spectrometer, USA) alongside a mass spectrometer (MS) 3000 equipped with Agilient MS capillary column (30 m × 0.25 mm, i.e., film thickness).

Isolation and identification of bacteria

All the media used in this study were prepared according to the manufacturer's instructions. The method described by **Onifade and Omololu** [17] was used for the isolation of bacteria from ready-to-eat food samples. Inoculum was standardized using 0.5 McFarland's standard as described by **Isunu et al.** [18].

Bacteria isolates were identified using cultural and molecular techniques described by **Olutiola et al.** [19] and **Adeboboye et al.** [20] respectively.

Antibiotic sensitivity test

This test was carried out in accordance with Kirby-Bauer antibiotic protocol (KB testing or disc diffusion antibiotic sensitive testing). The test was carried out using antibiotic-impregnated wafers to determine the antibiotic susceptibility pattern of the bacterial isolates [21].

Antibacterial Assay of *P. amarus* extracts

The assay for the antibacterial activity of extracts of *P. amarus* was carried out following the method described by **Isunu et al.** [18]. Positive control was maintained with ciprofloxacin, which is a standard antimicrobial drug, while wells containing the solvent alone were maintained as the negative control. The plates were then incubated for 18 hours at 37℃ and the diameter of zones of inhibition was measured in mm.

Determination of minimum inhibitory concentrations (MIC) of extracts of *P. amarus*

The extracts that showed antimicrobial activity were reconstituted by dissolving 0.5 g of each in 10 ml of 30% of DMSO and then sterilized by passing through a sterile millipore membrane filter (0.45U). However, different concentrations of the extracts (50, 25, 12.5, 6.25, 3.125 mg/ml) were used. The tube with the least concentration of extract without growth after incubation was taken and recorded as the minimum inhibitory concentration.

Determination of minimum bactericidal concentrations (MBC) of extracts of *P. amarus*

Streaks were taken from the two lowest concentrations of the extract tubes exhibiting invisible growth (from inhibition zone of MIC tubes) and sub-cultured onto sterile Tryptone soya agar (TSA) plates. The plates were incubated at 35℃ for 24hrs, then examined for bacterial growth in corresponding to plant extract concentration. MBC was taken as the concentration of plant extract that did not exhibit any bacterial growth on the freshly inoculated agar plates as described by **CLSI** [22].

Statistical analysis

Data obtained were subjected to one-way analysis of variance while the means were compared by Duncan's New Multiple Range Test at 95 % confidence level using Statistical Package for Social Sciences version 26.0. Differences were considered significant at $p \leq 0.05$.

Results

Phytochemical composition of *P. amarus*

The phytochemical constituents of *P. amarus* as shown in **tables (1)** and **(2)** include Flavonoids, Tannins, Phenolic Amino Acids, Saponins, Glycosides, Steroids, Alkaloids, Terpenoids, and Phlobatannins. N-hexane extract appears to contain most of these phytochemicals in relatively high quantity, though alkaloids and terpenoids were absent. A lot of phytochemicals were absent from the water extracts like Saponin, Steroids, Alkaloids, and Phlobatannins. Table 2 shows the quantity of phytochemicals present in *Phyllanthus amarus* leaves. The N-hexane extracts had the highest abundance of glycosides (981.35±2.57) ditto the water extract (302.70±18.78), followed by Phenol (61.59±3.62 n-hexane), (48.46±0.55 ethanol), (48.27±1.05 water), saponins and steroids were absent in the water extracts. Tannins are poorly extracted in all three extracts (0.31±0.03 (water), 0.39±0.04 (ethanol) and 0.28±0.03 (N-hexane).

Compounds detected in the ethanol extract of *P. amarus*

Table (3) Shows the compounds detected in the Gas Chromatography - Mass Spectrometry of *Phyllanthus amarus* ethanol extract. 16 organic compounds were detected in the sample with their equivalent peak areas and structures.

Identity of bacterial isolates

All the six bacterial isolates in **table (4)** showed different biochemical reactions and were characterized accordingly. The isolates include *Pseudomonas aeruginosa, Shigella dysenteriae, Salmonella typhi, Escherichia coli, Citrobacter freundii, Staphylococcus aureus*. **Table (5)** reveals the molecular identity of the bacterial isolates. The length of amplified products was 1500 base pair. The sequences obtained were blasted in National Centre for Biotechnology Information (NCBI) database. Based on the 16s rRNA sequences, the following bacteria were confirmed using the 16s rRNA sequence identification from the NCBI database with their respective accession number: *Pseudomonass aeruginosa* DSM 50071, *Shigella dysenteriae* ATCC 13313, *Citrobacter freundii* ATCC8090, *Staphylococcus aureus* S33R, *Escherichia coli* strain NBRC 102203.

Antibiotic sensitivity profile of isolates

Table (6) shows the antibiotic sensitivity profile of the bacterial isolates. The results showed that Ciprofloxacin had the highest potency effect on all the bacteria with zone range of 23.33±0.67 against *S. dysenteriae* and 29.00±0.58 mm against *P. aeruginosa*. This is because it is a broad spectrum antibacterial drug to which most Gram-negative bacteria are highly susceptible *in vitro* and many Gram-positive bacteria are susceptible or moderately susceptible. This antibacterial agent exerted its highest effect on *P. aeruginosa* while the least effect was on *S. dysenteriae, E. coli* and *C. freundii* respectively. While there was no significant difference in the effect of Augmentin and Gentamycin

For *shigella dysentriae* with zone range of 13.00±0.00 and 13.67±0.33 mm respectively, both exhibited the highest potency against *shigella dysentriae*, Septrin had no effect on the bacteria. Septrin appeared to have the least effect with zero zones on three bacteria (*S. dysenteriae, E. coli* and *C. freundii*). *Staphylococcus aureus* showed the least susceptibility against Amoxicillin and Ampiclox at 0.00 mm zone while Ciprofloxacin exerted the highest effect on the bacteria at zone 25.33±0.67 mm.

Antibacterial activity of extracts of *P. amarus*

Tables 7, 8 and 9 show the effect of *P. amarus* water, N-hexane and ethanol extracts on isolates respectively. For the water extract at all the concentrations, there was no effect of the extract between the 100 mg/ml and 200 mg/ml concentration, except at the 300 mg/ml concentration, the extract had the highest potency at the said 300mg/ml on *Staphylococcus aureus* and *Salmonella typhi* at 15.00±1.73 mm and 15.00±1.16 mm respectively, *Shigella dysenteriae* was the least susceptible of all the bacterial isolates at the 300 mg/ml (12.67±0.33).

For the n-hexane extract, *Salmonella typhi and Staphylococcus aureus* had the highest zones of inhibition at 100 mg/ml with zone range of 17.00±0.58 mm and 18.00±1.53 mm respectively, with significant difference between the 200 mg/ml and 300 mg/ml concentrations. *C. freundii* showed the least susceptibility with zone range of 4.00±4.00 and 4.33±4.33 respectively. For the ethanol extract, there was no effect on *C. freundii* across the three concentrations. The extract showed the highest potency on *Salmonella typhi* with a zone of inhibition 24.67±0.88 mm at 300mg/ml.

Minimum inhibitory concentrations and minimum bactericidal concentrations

As shown in **table (10)**, the Minimum Inhibitory Concentration (MIC) of *Phyllanthus amarus* extracts revealed that the MIC values for ethanol extract against *Citrobacter freundii* and *Shigella dysenteriae* are both 25 mg/ml. The Minimum Bactericidal Concentration (MBC) of the extracts on the bacterial isolates stood at 25mg/ml and 50mg/ml and this could be traced to *Salmonella typhi* isolate at both ethanol and N-hexane extracts of *P. amarus* respectively as shown in **table (11)**

Table 1. Qualitative phytochemical properties of *Phyllanthus amarus* leaves

Phytochemicals	Water extracts	Ethanol Extracts	N-Hexane Extracts
Flavonoids	+	+++	++
Tannins	++	++	++
Phenolic Amino Acids	++	++	++
Saponins	-	+	++
Glycosides	+	-	+
Steroids	-	+	+
Alkaloids	-	-	-
Terpenoids	+	+	-
Phlobatannins	-	-	+

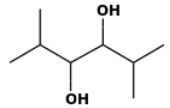
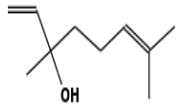
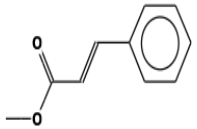
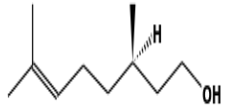
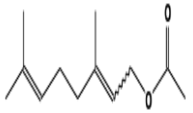
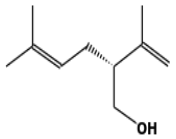
Key: + = Present, - = Not Present

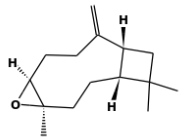
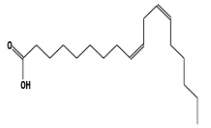
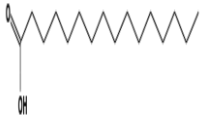
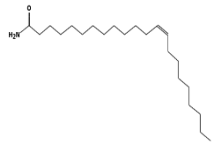
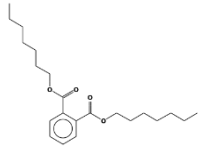
Table 2. Quantitative phytochemical properties of *Phyllanthus amarus* leaves

Phytochemicals	Water Extract	Ethanol Extracts	N-Hexane extracts
Saponins	NP	0.42 ± 0.01^{a}	0.89 ± 0.05^{b}
Flavonoids	29.00 ± 1.00^{a}	35.00 ± 1.00^{b}	42.67 ± 0.84^{c}
Tannins	0.31 ± 0.03^{a}	0.39 ± 0.04^{a}	0.28 ± 0.03^{a}
Terpenoids	23.67 ± 2.05^{a}	59.92 ± 5.09^{b}	NP
Glycosides	302.70 ± 18.78^{a}	NP	981.35 ± 2.57^{b}
Phenol	48.27 ± 1.05^{a}	48.46 ± 0.55^{a}	61.59 ± 3.62^{b}
Steroids	NP	2.77 ± 0.66^{a}	3.60 ± 0.08^{a}

Values on the row with the same superscript letter are not significantly different from each other at 0.05 level of significance
Key: NP – Not Present

Table 3. Compounds detected in the Gas Chromatography - Mass Spectrometry of *Phyllanthus amarus* ethanol extract.

Peak #	RT	Compound Detected	Mol. Formula	MW	Peak Area %	Comp %w	m/z	Structures
1	5.52	3,4-Hexanediol, 2,5-dimethyl-	$C_8H_{18}O_2$	146	6.02	5.01	41, 73, 146	
2	9.38	Linalool	$C_{10}H_{18}O$	154	4.87	5.08	43, 71, 154	
3	11.47	2-Propenoic acid, 3-phenyl-, methyl ester, (E)-	$C_{10}H_{10}O_2$	162	4.44	5.46	51, 103, 162	
4	14.90	6-Octen-1-ol, 3,7-dimethyl-	$C_{10}H_{20}O$	156	10.32	9.50	41, 69, 156	
5	15.48	2,6-Octadien-1-ol, 3,7-dimethyl-, acetate	$C_{12}H_{20}O_2$	196	5.30	3.92	43, 69, 196	
6	16.50	4-Hexen-1-ol, 5-methyl-2-(1-methylethenyl)-, (R)-	$C_{10}H_{18}O$	154	6.11	6.90	41, 69, 154	

7	17.25	Caryophyllene oxide	$C_{15}H_{24}O$	220	3.61	2.19	43, 79, 220	
8	19.17	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	2.58	3.16	43, 74, 270	
9	26.99	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	270	9.46	10.26	41, 67, 270	
10	27.75	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	10.06	7.94	43, 73, 256	
11	30.99	Isopropyl stearate	$C_{21}H_{42}O_2$	326	13.76	14.10	43, 102, 326	
12	32.51	11-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	296	5.59	4.73	41, 56, 296	
13	35.50	13-Docosenamide, (Z)-	$C_{22}H_{43}NO$	337	8.17	9.41	41, 72, 337	
14	38.76	1,2-Benzenedicarboxylic acid, diheptyl ester	$C_{22}H_{34}O_4$	362	4.51	3.98	41, 149, 362	

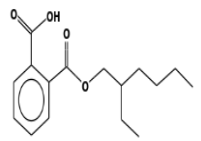
15	40.50	Mono(2-ethylhexyl) phthalate	$C_{16}H_{22}O_4$	278	3.42	4.12	41, 70, 278	
16	42.00	Squalene	$C_{30}H_{50}$	410	1.50	3.97	41, 69, 410	

Table 4. Biochemical characteristics of bacterial isolates

S/N	Suspected Organism	Gram Stain	Cell Shape	Glucose	Sucrose	Lactose	H_2S	Gas	Manitol	Fructose	Rhamnose	Raffinose	Xylose	Catalase	Citrate	Motility	Indole	Urease	MR	VP
1	*Pseudomonas aeruginosa*	-	Rod	-	-	-	-	-	+	+	-	-	-	+	+	+	-	-	-	-
2	*Shigella dysenteriae*	-	Rod	+	-	-	-	-	+	+	-	+	-	+	-	-	-	-	+	-
3	*Salmonella typhi*	-	Rod	+	-	-	+	-	+	+	-	-	+	+	-	+	-	-	+	-
4	*Escherichi coli*	-	Rod	+	+	+	-	+	+	+	-	-	+	+	-	+	+	-	+	-
5	*Citrobacter freundii*	-	Rod	+	+	+	+	+	+	N	N	N	N	+	+	+	-	-	+	-
6	*Staphylococcus aureus*	+	Cocci	+	+	+	-	-	+	+	-	-	-	+	+	-	-	+	+	+

Key: N – Not determined

Table 5. Molecular identity of bacterial isolates

S/N	Cultural and biochemical identities	16s rRNA sequence identification	Max Identity	Accession number
1	*Pseudomonass aeruginosa*	*Pseudomonass aeruginosa* DSM 50071	92%	NC010554.1
2	*Shigella dysenteriae*	*Shigella dysenteriae* ATCC 13313	95%	NR 026332.1
3	*Citrobacter freundii*	*Citrobacter freundii* ATCC8090	100%	NR 028894.1
4	*Staphylococcus aureus*	*Staphylococcus aureus* S33R	99%	NR 037007.2
5	*Escherichia coli*	*Escherichia coli strain NBRC* 102203	100%	NC010473.1

Table 6. Antibiotic sensitivity test results of isolates

S/N	Isolates	SXT	E	CH	CPX	AM	AU	APX	CN
1	*S. dysenteriae*	0.00±0.00^{a}	ND	10.33±0.33 ^{b}	23.33±0.67^{c}	12.67±0.33^{d}	13.00±0.00^{d}	ND	13.67±0.33^{d}
2	*E. coli*	0.00±0.00^{a}	ND	11.33±0.33^{b}	27.67±0.33^{c}	13.00±0.58^{b}	0.00±0.00^{a}	ND	11.67±0.67^{b}
3	*C. freundii*	0.00±0.00^{a}	ND	11.33±0.33^{b}	24.00±0.57^{c}	12.33±0.33^{b}	11.33±0.33^{b}	ND	10.67±0.33^{b}
4	*S. typhi*	10.33±0.33^{a}	ND	12.00±0.58^{a}	25.00±0.58^{b}	10.67±0.33^{a}	11.67±0.33^{a}	ND	18.33±0.33^{c}
5	*P. aeruginosa*	20.33±0.33^{a}	ND	23.00±0.58^{b}	29.00±0.58^{c}	18.67±0.33^{a}	20.00±0.58^{a}	ND	20.67±0.67^{a}
6	*S. aureus*	11.00±0.00^{a}	10.67± 0.33^{a}	ND	25.33±0.67^{b}	0.00±0.00^{c}	ND	0.00±0. 00^{c}	16.33±1.86^{d}

Values on the row with the same superscript letter are not significantly different from each other.
KEYS: SXT= Septrin (5mg/ml), E= Erythromycin, CH = Chloramphenicol (5mg/ml), CPX= Ciprofloxacin (5mg/ml), AM=Amoxicillin (5mg/ml), AU= Augmentin (5mg/ml), APX= Ampiclox, CN= Gentamycin (5mg/ml), ND= Not Determined

Table 7. Effects of different concentrations of *P. amarus* water extract on bacterial isolates

S/N	Isolates	100mg/ml	200mg/ml	300mg/ml	Positive control	Negative control
1	*S. aureus*	0.00±0.00^{a}	0.00±0.00^{a}	15.00±1.73^{b}	29.00±0.58^{c}	0.00±0.00^{a}
2	*E. coli*	0.00±0.00^{a}	0.00±0.00^{a}	13.33±0.88^{b}	28.00±0.53^{c}	0.00±0.00^{a}
3	*S. dysenteriae*	0.00±0.00^{a}	0.00±0.00^{a}	12.67±0.33^{b}	29.00±0.88^{c}	0.00±0.00^{a}
4	*C. freundii*	0.00±0.00^{a}	0.00±0.00^{a}	12.33±0.88^{b}	29.67±1.45^{c}	0.00±0.00^{a}
5	*S. typhi*	0.00±0.00^{a}	0.00±0.00^{a}	15.00±1.16^{b}	27.33±1.00^{c}	0.00±0.00^{a}

Values on the row with the same superscript letter are not significantly different from each other at $p \leq 0.05$

Table 8. Effects of different concentrations of *P. amarus* N-hexane extract on the bacterial isolates

S/N	Isolates	100mg/ml	200mg/ml	300mg/ml	Positive control	Negative control
1	*S. aureus*	18.00±1.53^{b}	23.00±1.16^{c}	23.33±0.88^{c}	30.00±0.58^{d}	0.00±0.00^{a}
2	*E. coli*	0.00±0.00^{a}	7.33±3.71^{b}	13.33±0.33^{c}	28.00±0.88^{d}	0.00±0.00^{a}
3	*S. dysenteriae*	0.00±0.00^{a}	12.33±0.88^{b}	16.00±0.58^{c}	23.33±0.88^{d}	0.00±0.00^{a}
4	*C. freundii*	0.00±0.00^{a}	4.00±4.00^{b}	4.33±4.33^{b}	29.00±0.91^{c}	0.00±0.00^{a}
5	*S. typhi*	17.00±0.58^{b}	21.00±0.58^{c}	24.33±0.67^{d}	28.00±0.88^{e}	0.00±0.00^{a}

Values on the row with the same superscript letter are not significantly different from each other at $p \leq 0.05$

Table 9. Effects of different concentrations of *P. amarus* ethanol extracts on the bacterial isolates

S/N	Isolates	100mg/ml	200mg/ml	300mg/ml	Positive control	Negative control
1	*S. aureus*	18.00±0.58^{b}	20.00±0.58^{b}	20.00±0.58^{b}	30.00±0.58^{d}	0.00±0.00^{a}
2	*E. coli*	0.00±0.00^{a}	11.00±0.58^{b}	15.00±0.00^{c}	28.00±0.88^{c}	0.00±0.00^{a}
3	*S. dysenteriae*	15.00±0.58^{b}	19.00±0.58^{c}	20.00±0.58^{c}	23.33±0.88^{d}	0.00±0.00^{a}
4	*C. freundii*	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	29.00±0.91^{b}	0.00±0.00^{a}
5	*S. typhi*	17.00±1.16^{b}	20.00±0.58^{b}	24.67±0.88^{c}	28.00±0.88^{d}	0.00±0.00^{a}

Values on the row with the same superscript letter are not significantly different from each other at $p \leq 0.05$

Table 10. Minimum Inhibitory Concentration (MIC) of *Phyllanthus amarus* extracts

S/N	Bacterial Isolates	Water (mg/ml)	Ethanol (mg/ml)	N-Hexane (mg/ml)
1	*E. coli*	200.00	100.00	200.00
2	*Citrobacter freundii*	200.00	25.00	200.00
3	*Salmonella typhi*	200.00	50.00	200.00
4	*Shigella dysenteriae*	200.00	25.00	100.00
5	*Staphylococcus aureus*	200.00	50.00	100.00

Table 11. Minimum Bactericidal Concentration (MBC) of *Phyllanthus amarus* Extracts

S/N	Bacterial Isolates	Water (mg/ml)	Ethanol (mg/ml)	N-Hexane (mg/ml)
1	*E. coli*	200.00	100.00	100.00
2	*Citrobacter freundii*	200.00	50.00	200.00
3	*Salmonella typhi*	200.00	25.00	50.00
4	*Shigella dysenteriae*	200.00	100.00	100.00
5	*Staphylocoocus aureus*	200.00	100.00	200.00

Discussion

Medicinal plants are the basis of therapeutic treatments in developing countries. Recent years have also seen an increase in the use of herbal medicines in developed countries. The plants are used medicinally in different countries of the world and are a good source of many potent and powerful drugs [23]. The resistance of pathogens to antibiotics commonly used, the increase in opportunistic infections and the effect of toxicity due to the continued use of several drugs have led to increased attention paid to the search for new therapeutic agents from various sources, including plants, which are good starting materials for the discovery of new antimicrobial agents [24]. Secondary metabolites produced by plants constitute a source of bioactive substances. All over the world, plant scientists (Botanist) interest has increased due to the search for new drugs from plant origin. Secondary metabolites of plant have been reported to serve as defence mechanisms against predation by many microorganisms, insects and herbivores [25].

The qualitative phytochemical compositions of *Phyllanthus amarus* leaves extracts for the three solvents used in the extraction process (Water extracts, Ethanol Extracts and N-Hexane Extracts). The solvents were able to extracts metabolites like Flavonoids, Tannins, Phenolic amino acids, Saponins, Glycosides, Steroids, Alkaloids, Terpenoids and Phlobatannins. Phytochemical analysis revealed that the N-hexane extract appears to contain most of these phytochemicals in relatively high quantity, though alkaloids and terpenoids were absent. A lot of phytochemicals were absent from the water extracts like Saponin, Steroids, Alkaloids, Phlobatannins, making it the poorest extracted. In the Quantitative phytochemical properties of *Phyllanthus amarus* leaves, the N-hexane extracts had the highest abundance of glycosides (981.35±2.57) ditto the water extract (302.70±18.78), followed by Phenol (48.27±1.05 water), (48.46±0.55 ethanol), (61.59±3.62 N-hexane) Saponins and Steroids were absent in the water extracts. Tannins were poorly extracted in all the three extracts (0.31±0.03 (water), 0.39±0.04 (ethanol) and 0.28±0.03 (N-hexane).

In the Gas Chromatography - Mass Spectrometry analysis of PAE (*Phyllanthus amarus* ethanol extract), 16 organic compounds were detected with their equivalent peak areas and structures, the compounds include 3,4-Hexanediol, 2,5-dimethyl-, Linalool, 2-Propenoic acid, 6-Octen-1-ol, 3,7-dimethyl-, 2,6-Octadien-1-ol, 4-Hexen-1-ol, 5-methyl-2-(1-methylethenyl), Gluconic acid, 9,12-Octadecadienoic acid, Hexadecenoic acid, Mono(2-ethylhexyl) phthalate, 1,2-Benzenedicarboxylic acid, diheptyl ester, Squalene.

Based on the molecular identity of bacteria isolated from cooked food (rice, beans, eba and meat pie) and stools in Akure, the length of amplified products was 1500 base pair. Based on the 16s rRNA sequences, the following bacteria were confirmed using the 16s rRNA sequence identification from the NCBI database with their respective accession number: *Pseudomonass aeruginosa* DSM 50071, *Shigella dysenteriae* ATCC 13313, *Citrobacter freundii* ATCC8090, *Staphylococcus aureus* S33R, *Escherichia coli* str. NBRC 102203 and *Salmonella typhi* SKST. 16S rRNA gene was used in the molecular identification because it is conserved in bacteria, and contain hypervariable regions that can provide species-specific signature sequences, 16S rRNA sequencing is widely used in identification of bacteria and phylogenetic studies [26].

The antibiotic sensitivity test Results of isolates on Gram negative antibiotics disc showed that ciprofloxacin had the highest potency effect on all the bacteria with zone range of 23.33±0.67 and 29.00±0.58, this is because it is a broad spectrum antibacterial drug to which most Gram-negative bacteria are highly susceptible *in vitro* and many Gram-positive bacteria are susceptible or moderately susceptible. Unlike most broad spectrum antibacterial drugs, ciprofloxacin is effective after oral or intravenous administration [27]. This antibacterial agent exerted its highest effect on *P. aeruginosa* while the least effect was on *S. dysenteriae, E. coli* and *C. freundii* respectively. While there was no significant difference in the effect of augmentin and gentamycin for *Shigella dysentriae* with zone range of 13.00±0.00 and 13.67±0.33 respectively, both exhibited the highest potency against *Shigella dysentriae*, Septrin had no effect on the bacteria. Augmentin had the highest effect on *E. coli* (20.00±0.58), amoxicillin and chloramphenicol had the highest effect on *P. aeruginosa* (18.67±0.33) and (23.00±0.58) respectively. Septrin appeared to have the least effect with zero zones on three bacteria (*S. dysenteriae, E. coli* and *C. freundii*). *Staphylococcus aureus* was the only tested Gram positive bacteria, which showed the least susceptibility against amoxicillin and ampiclox at zero zone while Ciprofloxacin exerted the highest effect on the bacteria at zone 25.33±0.67.

The use of *Phyllanthus amarus* as herbal medicine had been well reported [28] because of its health enhancing properties that include antibacterial, antioxidants and anti-inflammatory, the plant had been referred to as the wonder plant [29]. The effect of *P. amarus* water extracts on isolates, for all the concentrations indicated that there was no significant effect of the extract between the 100 mg/ml and 200 mg/ml concentrations, except at the 300 mg/ml concentration where it had the highest potency of 15.00±1.73 and 15.00±1.16 on *Staphylococcus aureus* and *Salmonella typhi* respectively. *Shigella dysenteriae* was the least susceptible of all the isolates at the 300 mg/ml (12.67±0.33). The positive control showed potency at all the five isolates between 23.33±0.88 and 29.00±0.58 respectively. The positive control used was Ciprofloxacin.

The *P. amarus* N-hexane extracts exerted the highest zones of inhibition against *Salmonella typhi* and *Staphylococcus aureus* with values of 17.00±0.58 mm and 24.33±0.67 mm respectively, with great significant difference between the 200 mg/ml and 300 mg/ml concentration. *C. freundii* showed the least susceptibility with zone range of 0.00±0.00 and 4.33±4.33 respectively. Furthermore, the positive control showed high potency at range 30.00±0.58 and 23.33±0.88 respectively, while *C. freundii* demonstrated no effect across the three concentrations. These finding are similar to the report of **Saranraj and Sirasakthivelan** that reported susceptibility of eight organisms to *Phyllanthus amarus* including all the ones used in this study [29]. This result was also in line with the findings of **Olufemi and Debiri** [30] that reported antibacterial activity of *Phyllanthus amarus* against four multiple resistant bacteria, which thus implies that the extracts of *Phyllanthus amarus* is active against resistant bacteria responsible for food poisoning and food borne infections.

The Minimum Inhibitory Concentration (MIC) of *Phyllanthus amarus* extracts revealed that *C. freundii* and *S. dysenteriae* have their MIC at 25 mg/ml for ethanol extract. This may be due to the capacity of the composition in the extract compounds which have antibacterial properties. The variation in the effectiveness of the extracts against different microorganisms may be attributed to the phytochemical composition of the extracts and/or membrane permeability of the microbes for the chemicals and their metabolism.

Conclusion

The present study showed that fresh and dry extracts of *Phyllanthus amarus* showed antimicrobial activity on *S. typhi*, hence this

provides a scientific basis that reflects the idea of traditional healers for using this plant for curing of typhoid fever as well as other ailments. In conclusion, the results of the present study support the folkloric usage of the plant and suggest that both the water and ethanoic plant extracts possess compounds with antimicrobial properties that can be further explored for antimicrobial activity. This antibacterial study of the plant extracts demonstrated that folk medicine can be as effective as modern medicine to combat pathogenic microorganisms. The use of the plants in folk medicine suggests that they represent an economic and safe alternative to treat infectious disease.

Conflicts of interest

None.

Financial disclosure

None.

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Author's contributions

Author Onifade, A. K. designed the study. Author Adesina, W. developed the methodology, acquired the data, analysed and interpreted the data. Both authors read and approved the final draft of the manuscript.

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