PHYTOCHEMICAL SCREENING AND ANTIBACTERIAL ACTIVITY OF AERIAL PARTS OF *Phyllanthus niruri* EXTRACTS AGAINST *Escherichia coli* ISOLATED FROM STOOL SAMPLES

* [1]Egharevba, P. A., [2]Idemudia, I. B., [1]Amengialue, O. O., [2]Saidu, J. Z. and [2]Ekhaise, F. O.

[1]Department of Biological Sciences, College of Science and Computing, Wellspring University, Benin City, Edo State, Nigeria
[2]Department of Microbiology, Faculty of Life Sciences, University of Benin, Benin City, Edo State, Nigeria
***Corresponding Author Email**: egharevba.precious@wellspringuniversity.edu.ng
Phone contact: +2348164550752;

ORCID: *https://orcid.org/0009-0008-5458-8408; https://orcid.org/0000-0003-4304-5738; https://orcid.org/0000-0002-0937-3036; https://orcid.org/0000-0002-3090-8672

Co-author Email: iyore.idemudia@uniben.edu; amengialue.orhue@wellspringuniversity.edu.ng; joy.saidu@uniben.edu; frederick.ekhaise@uniben.edu

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ABSTRACT

The increasing antibiotic resistance of bacterial pathogens necessitates the search for alternative antibacterial agents. Plants have a great deposit of antibacterial compounds that have huge therapeutic potential to treat/mitigate several diseases, without any detrimental outcome. *Phyllanthus niruri* has been widely used across continents as folk medicine for different ailments. This work sought to analyze the phytochemical profile and antibacterial effects of ethanol and aqueous extracts prepared from *Phyllanthus niruri* aerial parts against eleven (11) *Escherichia coli* strains obtained from stool samples. The extracts underwent qualitative and quantitative phytochemical evaluation, which identified the presence and concentration of several phytochemicals, including alkaloids, flavonoids, phenolics, saponins, and tannins. Ten (10) standard antibiotics were used to evaluate the test isolates' sensitivity to antibiotics, and the MAR index varied between 0.7 and 1.0, revealing a high level of antibiotic resistance in the test bacteria. Despite this, the ethanol extract showed zones of inhibition which varied between 8 ± 3.3 mm and 18 ± 2.7 mm, while the aqueous extract demonstrated inhibition zones that ranged from 5 ± 4.1 mm to 18 ± 4.2 mm against the test bacteria. The extracts exhibited significant antibacterial action against the evaluated bacterial strains, with minimum inhibitory concentrations (MICs) varying between 500 and 1000 mg/ml. However, no minimum bacteriocidal concentration (MBC) value was documented. These results suggests that the extracts of *Phyllanthus niruri* aerial parts have great potential as alternative antibacterial agents.

Keywords: Antibacterial, *Phyllanthus niruri*, aerial part, *Escherichia coli*, phytochemicals

INTRODUCTION

Escherichia coli is a key/notable member of the Enterobacteriaceae family (Rajesh and Rattan, 2008). It is a Gram-negative, facultative-anaerobic, rod-shaped bacterium, known to reside naturally in the gastrointestinal tract of humans and warm-blooded animals; it is among the most prevalent species within the intestinal flora (Lim *et al*., 2010). Additionally, the species *Escherichia coli* comprises numerous pathotypes that are responsible for a range of diseases. A minimum of six (6) distinct pathotypes are known as causative agents of enteric diseases, including diarrhoea and dysentery, while additional pathotypes are linked to infections that occur away from the intestine and examples of such extra-

intestinal infections include urinary tract infections and meningitis (Kaper *et al*., 2004).

Escherichia coli strains that obtain invasion factors develop virulence, thereby improving their capacity to adapt to new environments and allowing them to induce either mild, watery diarrhoea or inflammatory dysentery (Akinjogunla *et al*., 2010). Different strains of *Escherichia coli* have been identified as the etiologic agents responsible for outbreaks of diarrhoeal illness, with means of transmission identified as drinking water polluted with sewage. The strains consist of Enteropathogenic *E. coli* (EPEC), Enteroinvasive *E. coli* (EIEC), and Enterotoxigenic *E. coli* (ETEC), all of which are capable of causing diarrhoea in children and adults alike (Sule and Agbabiaka, 2008).

The development of multidrug resistance in different pathotypes of *Escherichia coli* isolated from particular cases of diarrhoea presents a notable issue (Alikhani, 2013). A significant escalation in *E. coli* resistance to frequently prescribed antibiotics for enteric illnesses (such as ampicillin, tetracycline, and co-trimoxazole) have been observed in the past few years. In developing nations, this resistance is primarily linked to the widespread, unguided use of antibiotics and inadequate prescription practices (Alikhani, 2013).

This increasing antibiotic resistance of *Escherichia coli* species, necessitates the search for alternative antibacterial agents.

The utilization of medicinal plants has historically been known to be a main basis of nature-based therapeutic medications for various infectious diseases worldwide (Egharevba *et al*., 2025). These plants contain antimicrobial compounds that have great therapeutic potential to treat numerous infectious diseases and are largely free of side effects (Amengialue *et al*., 2016). Despite their established traditional applications and encouraging pharmacological properties, there exists a substantial reservoir of unexploited potential within these plant materials, and this necessitates thorough investigations into their phytochemical profiles, bioactivity, and possible therapeutic uses to fully realize their medicinal benefits (Origbemisoye *et al*., 2024). Recently, there has been increased interest in plants that show biological efficacy, due to the affordable cost of producing them and reduced unwanted effects, in comparison to synthetic drugs (Amin *et al*., 2012).

Phyllanthus niruri is a small herb that is widely renowned for its extensive therapeutic characteristics, and it is utilized globally (Gazel *et al*., 2023). Research has indicated that *Phyllanthus niruri* extracts exhibit antibacterial, analgesic, anti-urolithiatic, antiviral, anti-inflammatory, antihyperuricaemic, hepatoprotective, hypoglycaemic, cardioprotective, and hypolipidaemic effects, as a result of its unique, biologically active constituents (Lee *et al*., 2016). The aim of the work done was to explore the phytochemical constitution of the extracts obtained from the aerial parts of *Phyllanthus niruri* by extraction with ethanol and water, to evaluate their antibacterial efficacy on *Escherichia coli* and compare it with that of antibiotics that are commercially available for the treatment of intestinal infections caused by *E. coli*.

Since there are very limited studies on the antibacterial activity of the aerial parts of *P. niruri* on *E.coli* isolated from stool samples, this study will provide baseline data and valuable insight into the efficacy of *P. niruri* against *Escherichia coli*, which World Health Organization (WHO) has tagged as an organism of public health concern. Furthermore, the study will give credence to the use of *P. niruri* in folk medicine and serve as a guide in potential therapeutic applications.

MATERIALS AND METHOD

Plant Sample Collection and Identification

Specimens of the aerial parts of *Phyllanthus niruri* were obtained from the wild in Benin City, Edo State, and were subjected to authentication and subsequently validated in the Herbarium unit of the Department of Plant Biology and Biotechnology, Faculty of Life Science, University of Benin, Benin City, Edo State by Prof. H. A. Akinnibosun, with the voucher number UBH-P406.

Preparation of Plant Extracts

The extracts of the plant were prepared by maceration - a procedure that was clearly explained by Tiwari *et al*. (2011). Aerial parts of *Phyllanthus niruri*, were obtained and dried at ambient temperature in the laboratory till the weight became stable, with no fluctuations. Then, a Silvercrest blender (SC-9880 model) was used to blend the dried plant materials, and an airtight container was used to store the resulting powder till it was time to use it.

In the extraction process, two solvents - water and ethanol - were utilized. A total of 100 g of the resulting powder was macerated in 1L of each solvent (water and ethanol) respectively, and shaken with a cycling vibrator (HY-4C model) for five (5) days at room temperature. The resulting mixtures were subjected to filtration using a muslin strainer and thereafter, it was passed through Whatmann no.1 filter paper. The solvent was completely evaporated with a digital water bath (model no. DK420, Lincoln Mark Medical, England) at 70°C. The dry residue obtained was kept in a sealed container at 4°C for later use.

Phytochemical Evaluation of Plant Extracts

This research involved qualitative and quantitative phytochemical evaluation of the plant extracts, utilising established procedures to identify the presence and concentrations of alkaloids, saponins, flavonoids, terpenoids, phenolic acids, glycosides, tannins, eugenols and steroids. The phytochemical analysis was done in the Department of Chemistry in the University of Benin.

Qualitative Phytochemical Assessment of Extracts

The qualitative phytochemical assessments of the plant extracts were conducted following standard procedures explicitly narrated by Tiwari *et al*., 2011 and Egharevba *et al*., 2025, with little modifications, as outlined below:

Detection of Alkaloids: This was done by first evaporating 2.0ml of the plant extract to dryness. Subsequently, the resulting residues were dissolved in 5ml of HCl ($2mol/dm^3$). The solution was filtered and separated into two test tubes, labelled 1 and 2. Few drops of Mayer's reagent was introduced into test tube 1 and the appearance of a yellow precipitate signified that alkaloids are present. Wagner's reagent was introduced in drops into test tube 2 and the appearance of a brownish-red precipitate signified that alkaloids are present.

Detection of Glycosides: The detection of glycosides was performed by dissolving 0.5 mg of the extract in about 1 ml of water, after which aqueous NaOH solution was introduced. The appearance of a yellow colour suggests that glycosides are present.

Detection of Tannins: To 1.0 ml of the extract, 1.0 ml of a 1% gelatin solution which contained sodium chloride was incorporated. The appearance of a white precipitate signified that tannins are present.

Detection of Phenols: This procedure involved the treatment of 1.0 ml of the plant extract with 4 drops of ferric chloride solution. The manifestation of a bluish-black hue signified the existence of phenols in the extract.

Detection of Saponins: The detection of saponins was done using the foam test and froth test methods. In the foam test, 0.5g of the plant extract was vigorously mixed

with 2.0 ml of distilled water. The presence of foam that lasted for 10 minutes indicated that saponins are present. In the froth test, distilled water was used to dilute 5.0 ml of the extract to achieve a final volume of 20.0 ml and agitated in a 50 ml measuring cylinder for a duration of 15 minutes. The development of a 1cm layer of foam signified that saponins are present.

Detection of Flavonoids: The detection of flavonoids was done using the alkaline reagent test and the lead acetate test. In the alkaline reagent test, some drops of a $2mol/dm^3$ sodium hydroxide solution was added to the extract. The development of a vibrant yellow hue that changed to colourless upon the introduction of dilute hydrochloric acid ($2mol/dm^3$) signified that flavonoids are present.

The lead acetate test involved treating the plant extract with several drops of lead acetate solution. The appearance of a yellow precipitate signified that flavonoids are present.

Detection of Eugenols: Approximately 2 ml of the extract was combined with 5 ml of a 5% KOH solution. The aqueous layer was separated and subjected to filtration. A small quantity of HCl was introduced into the filtrate, resulting in the appearance of a pale-yellow precipitate, which confirmed that eugenols are present.

Detection of Steroids: To 0.5 g of the extract, 2 ml of acetic anhydride was added, and the next step was the addition of 2 ml of H_2SO_4. A colour change from violet to blue or green signified that steroids were present.

Detection of Terpenoids: The detection of terpenoids involved mixing 0.2 g of the plant extract with 2 ml of chloroform ($CHCl_3$). The next step involved adding 3 ml of concentrated H_2SO_4 to the solution carefully, to create a distinct layer. A reddish-brown colouration at the interface signified that terpenoids are present.

Quantitative Phytochemical Evaluation of Extracts

The quantitative phytochemical evaluations of the plant extracts were performed by following standard procedures which are detailed below:

Determination of total phenolic contents

The Folin–Ciocalteu reagent was used to assess the plant extract's total phenolic content (with minor adjustments), with tannic acid serving as standard. In summary, a test tube containing 1.0 ml of the extract solution (250 µg/ml) was used. After that, 1.0 millilitre of Folin-Ciocalteu reagent was incorporated, and the solution was well combined. Five minutes later, 15.0 ml of 20% Na_2CO_3 was also introduced into the mixture and left to stand for two hours. Using a UV-Vis spectrophotometer (Jenway 6100, Dunmow, Essex, U.K.), the absorbance was measured at 760 nm. An algorithm based on the standard tannic acid calibration graph was used to estimate the total phenolic content in µg of tannic acid equivalent (TAE).

Determination of Alkaloids Content

The approach described below was used to determine the total alkaloid content. 100 ml of 20% acetic acid in ethanol was introduced into a 250 ml beaker that contained 5g of the extract. The solution was then covered and left to stand for two hours, then filtered. After filtering, the solution was concentrated to a fourth of its initial volume with a waterbath. To complete the precipitation process, drop-wise addition of concentrated ammonium hydroxide was done. The precipitate was filtered, cleaned with a 1% ammonia solution, dried, and weighed when the mixture had fully settled.

$$\text{Alkaloid (\%)} = \frac{\text{Weight of residue}}{\text{Weight of sample}} \times 100$$

Determination of Flavonoid Content

Triplicate aliquots of the homogenous plant extract (1.5 g) was used to measure the flavonoid content. For flavonoid analysis, the ethanol extract was divided into 30 µl

aliquots. 90 µl of ethanol, 6 µl of 10% aluminium chloride ($AlCl_3$), and 6 µl of 1 mol/L sodium acetate (CH_3COONa) were added to the diluted samples. Lastly, 170 µl of ethanol was added. At 415 nm, the absorbance was measured following a 30-minute incubation period. Quercetin was the standard used to measure the flavonoids content.

Estimation of Total Saponins Content

A technique based on a vanillin-sulfuric acid colorimetric reaction was slightly modified and used to quantify the total saponin content, with some modifications. 250 µL of distilled water was mixed with approximately 50 µL of the plant extract. Then, 250 µL of vanillin reagent was added (which is 800 mg of vanillin in 10 ml of 99.5% ethanol). After that, 2.5 ml of 72% sulphuric acid was added, and the mixture was shaken vigorously. Next, the solution was placed in a water bath for ten minutes at 60°C. After that, it was chilled in ice-cold water and the absorbance was read at 570 nm. From a saponin stock solution, standard saponin solutions with concentrations ranging from 0 - 25 ppm were made, and they were handled similarly to the test samples. The results were expressed in PPM.

Estimation of tannin content

To quantitatively measure the tannin levels of the extracts, 0.20 mL of the extract was mixed with 20 mL of 50% methanol and put in a water bath (temperature range between 77˚C and 80˚C) for an hour while being stirred. Whatman No. 1 filter paper with two layers was used to quantitatively filter the resultant extract. Then, 20 mL of distilled water, 2.5 mL of Folin-Denis reagent and 10 mL of 17% Na_2CO_3 were added and well-mixed. The resulting solution was allowed to rest for twenty minutes. Using a UV/Visible spectrophotometer set at 760 nm, a series of standard tannic acid solutions were prepared in methanol, and the absorbance of the standard, as well as that of the samples were measured after colour formation. The calibration curve was used to calculate the total tannin content.

Isolation and characterization of bacterial isolates

Eleven (11) *Escherichia coli* strains used in this study were isolated and characterized (using standard microbiological methods) from stool samples of patients who presented with gastritis at the Edo Specialist Hospital, Benin City.

Antibiotics Susceptibility testing of Bacterial Isolates

The Kirby Bauer Disc Diffusion technique was used to evaluate the susceptibility of the *E. coli* srains (EC01 – EC11) to standard antibiotics. The medium used was Mueller Hinton agar (MHA). To prepare the agar, 38g of (MHA) powder was measured into a conical flask and mixed with 1000ml of distilled water to dissolve the powder. After thoroughly mixing the mixture to ensure it is properly dissolved, it was sterilized by autoclaving at 121^{o}C for 15 minutes. It was allowed to cool to around 45^{o}C. Following this, the medium was aseptically dispensed into properly labeled petri dishes in the media-pouring room and left to solidify. Then, the MHA plates were inoculated with the isolates. Using sterile forceps, each set of antibiotic discs was firmly positioned on the surface of the inoculated plates and incubated upside down (to prevent water of condensation from dropping on the inoculated plates), at 37^{o}C for 18-24 hours. Measurements of the clear zones around the discs (which depicted inhibition) were taken with a metre rule (in mm) and interpreted as sensitive, intermediate, or resistant (using the standards provided by CLSI, 2020). The experiment was performed in triplicate.

Antibacterial Activity Assessment of the Plant Extracts

The evaluation of the antibacterial efficacy of the plant extracts was performed using the agar well diffusion technique. Using a sterilized cork borer, 6mm wells were aseptically created in Mueller Hinton agar plates which has been inoculated with standardized inoculums of the test bacteria (adjusted to 0.5 McFarland turbidity

standards, equivalent to 1.5 x 10^8 CFU/ml). An aliquot (0.1ml) of the plant extract concentration intended for testing was introduced into each appropriately labeled well. The plates were allowed to stand for 30 minutes, then incubated right side up at 37°C for 24 hours. Measurement of the clear zones that developed around the wells were taken using a metre rule (in mm). The experiment was conducted in duplicate.

Minimum Inhibitory Concentration (MIC)

For each concentration of the extract, 1ml of nutrient broth was placed in a test tube. Into each test tube containing the nutrient broth, 0.1ml of the test bacterial suspension (McFarland turbidity standard) was incorporated. Then, 1ml of the various extract concentrations were added to each tube. Sterile balls of cotton wool were used to seal the mouth of the tubes and incubated at 37°C for 24 hours, after which they were examined for turbidity. The MIC was determined and recorded as the lowest concentration that completely inhibited growth.

Minimum Bactericidal Concentration (MBC)

This involved the preparation of sub-cultures from the MIC tubes (tubes with the lowest concentration that completely inhibited growth) onto nutrient agar plates. Following an 18-24hours period of incubation, plates without any bacterial growth were recorded as the MBC and MBC indicates that the plant extract has exhibited bactericidal effect at that concentration.

Statistical Analysis

A three-way analysis of variance (ANOVA) was conducted to evaluate the effects of the bacterial isolates (EC01 – EC11), extraction solvents (ethanol and water), and concentrations (0, 125, 250, 500, 1000 mg/mL) on inhibition zone diameters (mm). The dataset included 11 isolates × 2 extracts × 5 concentrations. Normality and homogeneity of variances were verified using Shapiro-Wilk and Levene's tests. The ANOVA assessed main effects and interactions, followed by Tukey's HSD test for 2556 pairwise comparisons to identify significant group differences (p-tukey < 0.05). Analyses were performed in Python (version 3.8) using pandas, numpy, statsmodels, and bioinfokit libraries.

RESULTS

Phytochemical evaluation of the ethanol and aqueous extracts

The outcomes of the qualitative phytochemical screening of the ethanol and aqueous extracts of *P. niruri* are displayed in Table 1 while that of the quantitative phytochemical evaluation of the ethanol and aqueous extracts of *P. niruri* are shown in Figure 1. The qualitative phytochemical evaluation detected that alkaloids, flavonoids, phenolic acids and other phytochemical constituents were present in the extracts. However, steroids were only present in the ethanol extracts. It was also observed that the quantity of saponins, flavonoids, tannins and phenolics and were higher in the ethanol extracts, than in the aqueous extracts (Figure 1).

Antibiotics Sensitivity Tests and Antibacterial Activity Results

The sensitivity testing of bacterial isolates to reference antibiotics revealed that a majority of the *E. coli* strains demonstrated resistance to these antibiotics, with three of the *E. coli* strains exhibiting the highest MAR index of 0.5 (Table 2). Both extracts (ethanol and aqueous) displayed antibacterial efficacy against the eleven *E. coli* strains tested, with MIC values varying between 500 and 1000 mg/ml (Tables 3 - 6). The results of the statistical analysis are reported in Table 7a (ANOVA) and Table 7b (selected comparisons of tukey results).

Table 1: Qualitative Phytochemical Assessment of Ethanol and Aqueous Extracts of *P. niruri*

Phytochemical constituents	Ethanol extract	Aqueous extract
Alkaloids	+	+
Phenolics	+	+
Terpenoids	+	+
Glycosides	+	+
Steroids	+	-
Saponins	++	++
Eugenols	-	+
Flavonoids	+	+
Reducing sugar	+	+
Tannins	++	+

Key:

+ present ++ abundantly present - absent

Table 2: Antibiotics sensitivity test and Multiple Antibiotics Resistance (MAR) Index of Test isolates

Test isolates	Antibiotics										MAR Index
	OFX	AU	PEF	CTZ	CN	CPX	CEP	TRX	S	CEF	
EC01	R	R	R	R	I	R	R	R	I	R	0.8
EC02	R	R	R	R	R	R	R	R	R	R	1.0
EC03	R	R	R	R	R	R	R	R	R	R	1.0
EC04	I	R	R	R	R	I	R	R	R	R	0.8
EC05	R	R	R	R	I	R	R	R	R	R	0.9
EC06	I	R	R	R	I	I	R	R	R	R	0.7
EC07	R	R	R	R	R	R	R	R	R	R	1.0
EC08	I	R	R	R	I	R	R	R	R	R	0.8
EC09	S	R	R	R	R	I	R	R	R	R	0.8
EC10	I	R	R	R	I	I	R	R	R	R	0.7
EC11	S	R	R	R	R	R	R	R	R	R	0.9

OFX-OFLOXACIN (10MCG), AU-AUGMENTIN (30MCG), PEF-PEFLACINE (10MCG), CTZ-CEFTAZIDIME (30MCG), CN-GENTAMYCIN (10MCG), CPX-CIPROFLOXACIN (10MCG), CEP-CEPOREX (10MCG), TRX-CEFTRIAXONE (30MCG), S-STREPTOMYCIN (30MCG), CEF-CEFFUROXINE (30MCG), MAR-Multiple Antibiotic Resistance Index, EC- *Escherichia coli*, EC01 – EC011 represents the 11 isolates used, S- Sensitive, I- Intermediate, R-Resistant

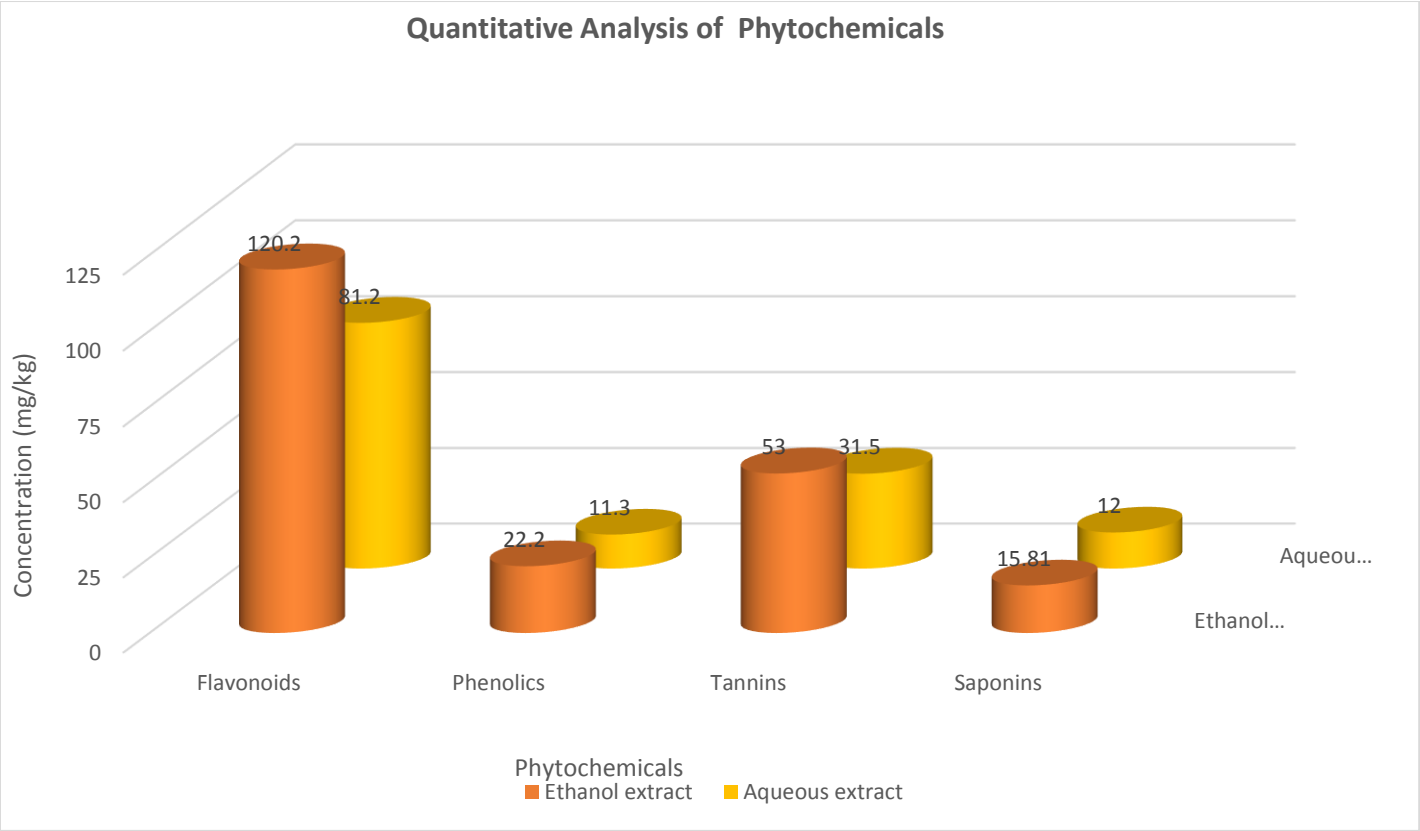

Figure 1: Quantitative Phytochemical Screening of Ethanol and Aqueous Extracts of *P. niruri*

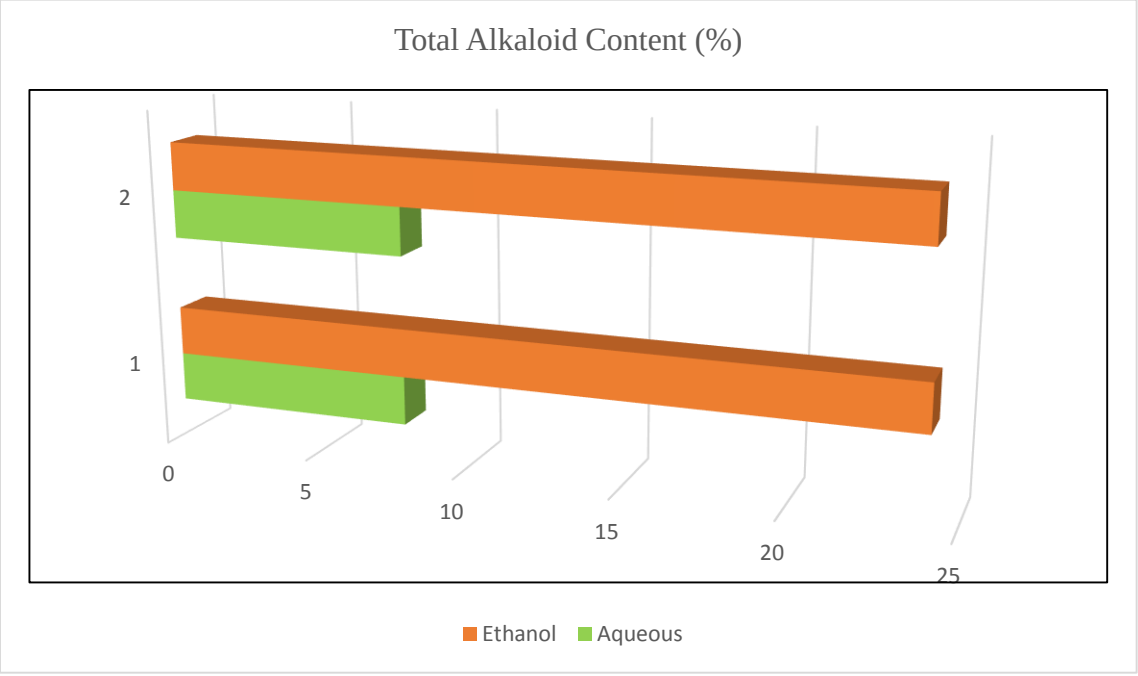

Figure 2: Quantitative Phytochemical Analysis of Alkaloid Content (%) of Ethanol and Aqueous Extracts of *P. niruri*

Table 3: Zone of inhibition (mm) of ethanol extracts of *P. niruri* on test isolates

Test Isolates	**0 mg/ml (Control)**	**125 mg/ml**	**250 mg/ml**	**500 mg/ml**	**1000 mg/ml**
EC01	0	09 ± 4.1	13 ± 1.6	14 ± 4.7	16 ± 2.0
EC02	0	10 ± 2.4	11 ± 3.2	12 ± 1.8	16 ± 5.1
EC03	0	12 ± 4.3	13 ± 3.0	15 ± 4.0	17 ± 2.8
EC04	10 ± 0	10 ± 2.3	12 ± 5.0	14 ± 3.4	18 ± 2.7
EC05	9 ± 0	08 ± 3.6	13 ± 2.1	13 ± 4.5	17 ± 1.9
EC06	7 ± 0	10 ± 5.2	11 ± 2.9	10 ± 2.5	15 ± 4.9
EC07	0	09 ± 4.4	10 ± 3.9	11 ± 2.2	16 ± 3.1
EC08	0	10 ± 2.6	10 ± 5.0	13 ± 3.5	16 ± 1.7
EC09	11 ± 0	08 ± 3.3	10 ± 4.2	10 ± 1.8	13 ± 2.7
EC10	0	09 ± 4.9	10 ± 2.0	13 ± 3.6	15 ± 4.8
EC11	10 ± 0	10 ± 3.7	13 ± 1.9	15 ± 2.2	15 ± 3.8

Table 4: Minimum Inhibitory Concentration - MIC (mg/ml) and Minimum Bactericidal Concentration – MBC (mg/ml) of Ethanol Extracts of *P. niruri* on Test Isolates

Isolates	Concentration of ethanol extract (mg/ml)				MIC	MBC
	125	250	500	1000	(mg/ml)	(mg/ml)
EC01	+	+	-	-	500	Nil
EC02	+	+	+	-	1000	Nil
EC03	+	+	-	-	500	Nil
EC04	+	+	-	-	500	Nil
EC05	+	+	-	-	500	Nil
EC06	+	+	+	+	Nil	Nil
EC07	+	+	-	-	500	Nil
EC08	+	+	-	-	500	Nil
EC09	+	+	+	+	Nil	Nil
EC10	+	+	+	+	Nil	Nil
EC11	+	+	+	+	Nil	Nil

Key: + = Growth; - = No Growth; Nil = No activity

Table 5: Zone of inhibition (mm) of aqueous extracts of *Phyllanthus niruri* on test isolates

Test Isolates	**0 mg/ml (Control)**	**125 mg/ml**	**250 mg/ml**	**500 mg/ml**	**1000 mg/ml**
EC01	0	13 ± 1.9	13 ± 2.8	12 ± 3.0	15 ± 5.0
EC02	0	08 ± 2.5	10 ± 3.1	11 ± 4.4	09 ± 4.6
EC03	0	11 ± 3.3	12 ± 2.1	14 ± 3.9	18 ± 4.2
EC04	0	14 ± 2.0	15 ± 1.6	16 ± 3.5	15 ± 2.9
EC05	0	10 ± 4.8	10 ± 3.7	11 ± 2.7	12 ± 4.7
EC06	0	14 ± 5.1	15 ± 4.3	16 ± 2.3	16 ± 3.6
EC07	0	13 ± 4.9	12 ± 4.1	16 ± 2.2	15 ± 1.8
EC08	0	08 ± 3.4	08 ± 2.0	10 ± 5.2	11 ± 3.0
EC09	0	11 ± 2.4	12 ± 1.7	10 ± 4.0	10 ± 1.6
EC10	0	05 ± 4.1	10 ± 3.2	10 ± 2.5	12 ± 3.8
EC11	0	07 ± 3.5	11 ± 2.6	12 ± 2.4	09 ± 4.3

Table 6: Minimum Inhibitory Concentration - MIC (mg/ml) and Minimum Bactericidal Concentration – MBC (mg/ml) of Aqueous Extracts of *P. niruri* on Test Isolates

Isolates	Concentration of aqueous extract (mg/ml)				MIC	MBC
	125	250	500	1000	(mg/ml)	(mg/ml)
EC01	+	+	+	-	500	Nil
EC02	+	+	+	+	1000	Nil
EC03	+	+	-	-	500	Nil
EC04	+	+	-	-	500	Nil
EC05	+	+	+	+	500	Nil
EC06	+	+	-	-	Nil	Nil
EC07	+	+	-	-	500	Nil
EC08	+	+	+	-	500	Nil
EC09	+	+	+	+	Nil	Nil
EC10	+	+	+	+	Nil	Nil
EC11	+	+	+	+	Nil	Nil

Key: + = Growth; - = No Growth; Nil = No activity

Table 7a. Three-Way ANOVA Table for Zone of Inhibition

Source of Variation	Sum of Squares	df	F-Value	p-Value
Isolate	1750.23	10	13.51	<0.001 (***)
Solvent (Ethanol vs Aqueous)	124.51	1	9.61	0.002 (**)
Concentration (0–1000 mg/mL)	2490.04	4	48.06	<0.001 (***)
Isolate × Solvent	224.09	10	1.73	0.079 (ns)
Isolate × Concentration	387.50	40	0.75	0.873 (ns)
Solvent × Concentration	133.31	4	2.57	0.039 (*)
Isolate × Solvent × Concentration	279.27	40	0.54	0.995 (ns)
Residual	2854.67	220	–	–

Significance codes: ***p < 0.001, **p < 0.01, *p < 0.05, ns = not significant

Table 7b. Sample of Tukey HSD Pairwise Comparisons

Group 1	Group 2	Mean Diff	Std Error	t-Value	p-Value	Adj. p (Tukey)	Significant?
E1_Ethanol_0	E1_Ethanol_1000	-16.0	1.70	-9.43	<0.001	<0.001	Yes
E1_Ethanol_0	E1_Ethanol_250	-13.0	1.70	-7.66	<0.001	<0.001	Yes
E1_Ethanol_0	E1_Aqueous_250	-13.0	1.70	-7.66	<0.001	<0.001	Yes
E1_Ethanol_0	E1_Aqueous_125	-13.0	1.70	-7.66	<0.001	<0.001	Yes
E4_Ethanol_1000	E9_Ethanol_1000	5.0	1.70	2.94	0.004	0.011	Yes
E2_Ethanol_1000	E2_Aqueous_1000	7.0	1.70	4.12	<0.001	<0.001	Yes
E9_Aqueous_500	E11_Ethanol_0	-1.0	1.70	-0.59	0.556	1.000	No
E9_Aqueous_500	E10_Ethanol_0	11.0	1.70	6.48	<0.001	<0.001	Yes
E9_Aqueous_500	E10_Aqueous_0	11.0	1.70	6.48	<0.001	<0.001	Yes

Discussion

In times past, plants have served as the creative stimulus for the formulation of relatively new therapeutic molecules because plant-based medicines have greatly improved human health and well-being. Presently, their relevance is still increasing (Amengialue *et al*., 2013). The study of plants as potential sources for new therapeutic agents to address different ailments is important and requires an extensive search for resources, so as to obtain plant extracts containing a wide array of phytochemical compounds (Shanmugam, 2014).

This investigation was to identify and quantify the phytochemicals contained in the extracts of aerial parts of *Phyllanthus niruri*, following extraction with two (2) suitable solvents - ethanol / water (aqueous) and to determine their antibacterial activity.

The phytochemical profiling of the ethanol extract from the aerial parts of *P. niruri* demonstrated the occurrence

of alkaloids, glycosides, saponins, phenols, terpenoids, steroids, flavonoids, reducing sugars, and tannins in the sample.

However, it was a bit different for the aqueous extract, as all the phytochemicals were seen, except steroids. In addition, eugenols were present (Table 1). The results agree with the ones published by Adebisi *et al*. (2021), which revealed that the analysis of phytochemicals in the extract obtained from *Phyllanthus niruri* leaf detected the presence of alkaloids, tannins, glycosides, anthraquinones, saponins, flavonoids, steroids and terpenoids. It also agrees with the results of Divya *et al*. (2018) in which the presence of alkaloids, flavonoids, tannins, diterpenes, polysterols, glycosides, and carbohydrates was unravelled by the phytochemical profiling of the plant's ethanol extracts. However, saponin was detected in the current study.

From the valuable reports obtained in this research and presented in Tables 3 and 5, it is evident that *Phyllanthus niruri* extracts have potential as alternative antibacterial agents. The antibacterial effect displayed against *E. coli* isolates could be attributable to the presence of the phytochemicals which have shown biological activity in similar studies (though their specific functions were not directly studied in this research). It is widely acknowledged and documented that the pharmacological activities of plants are embedded in their phytochemical constitution (Amengialue *et al*., 2016), and the compounds can demonstrate antimicrobial (microbicidal and microbistatic) effects via various pathways (Shanmugam *et al*., 2014).

Tannins have been noted for their antibacterial characteristics, and the mechanisms underlying their antibacterial action include the prevention of enzymatic functions, inhibition of nucleic acid synthesis, iron chelation, disruption of cell wall formation, impairment of cell membrane integrity, and inhibition of fatty acid biosynthesis pathways (Farha *et al*., 2020; Huang *et al*., 2024). The distinctive antibacterial efficacy of tannins have been effectively displayed against both Gram-positive and Gram-negative bacteria, like *Staphylococcus aureus*, *Streptococcus pyogenes*, *Escherichia coli*, *Yersinia enterocolitica*, and *Listeria innocua*, among others (Kaczmarek, 2020). According to Tiwari *et al*. (2011), flavonoids, terpenoids, polyphenols and alkaloids possess antimicrobial and antidiarrhoeal properties; polyphenols, alkaloids and saponins possess antihelminthic properties while glycosides possess antidiarrhoeal properties. In addition, saponins possess anticancer properties. Shanmugam *et al*. (2014) also documented that saponins are useful in treating wounds / ulcers (due to their ability to coagulate red blood cells), phenol components have cancer activity, and alkaloids have antispasmodic, analgesic and antibacterial effects.

The antibacterial effect of ethanol and aqueous extracts of *P. niruri* aerial parts which was tested against selected *Escherichia coli* isolates, using the agar well diffusion technique, displayed varied degree of antibacterial activity (Tables 3 and 5). Oyekanmi *et al*. (2023), Akinjogunla *et al*. (2010), Amin *et al*. (2012) and Shanmugam *et al*. (2014) have also reported the antibacterial efficacy of *P. niruri* on *E. coli* and other bacterial pathogens. Zones of inhibition of *P. niruri* against the *E. coli* isolates displayed the efficacy of the extracts on all the test bacteria. However, the ethanol extract demonstrated higher zones of inhibition (8 ± 3.3 – 18 ± 2.7 mm) than those of the aqueous extract (5 ± 4.1 – 18 ± 4.2 mm). This may be attributable to higher amounts of phytochemicals such as alkaloids, flavonoids, phenolics, tannins and saponins in the ethanol extracts, than in the aqueous extracts (see Figures 1 and 2). The work of Shanmugam *et al*. (2014) also highlighted a

higher quantity of phytochemicals in ethanol extract, than in the aqueous. The higher zones of inhibition demonstrated by the ethanol extract may also be due to the higher soluble properties of active components in ethanol. Amengialue *et al*. (2013) noted that ethanol is a better extraction solvent than water and bioactive components of plants are basically more soluble in organic solvents such as ethanol, than in aqueous – an inorganic solvent. Tiwari *et al*. (2011) also opined that although water is a universal solvent, plant extracts from organic solvents exhibit more consistent antimicrobial activity, in comparison to water extracts. Furthermore, the increased efficacy of ethanol extracts in comparison to aqueous extracts, may be due to the higher polyphenol content in the former.

The antibacterial efficacy of *P. niruri* extracts were tested at various concentrations. The ethanol extract exhibited a dose-dependent response, as the higher doses showed wider zones of inhibition against the test organisms. However, this was not so for the aqueous extract. Also, the MIC values ranged between 500 and 1000mg/ml for both extracts but there was no MBC value recorded (see Tables 4 and 6). This could be due to the potency of the crude extracts, which could only create bacteriostatic effect on the test isolates.

These antibiotics sensitivity of the test isolates was evaluated with commercially available antibiotics which include ofloxacin, augmentin, peflacine, ceftazidime, gentamycin, ciprofloxacin, ceporex, ceftriaxone, streptomycin and cefuroxine. The doses and the results are indicated in Table 2. The multiple antibiotic resistance (MAR) indexes for all *E. coli* strains tested in the current research ranged from 0.7 to 1.0. A MAR index of 0.2 or greater indicates that the origin is a high-risk source of contamination, which involves utilization of many antibiotics (Afunwa *et al*., 2020) and indicates a high likelihood of the bacteria being resistant to multiple antibiotics. Despite the high MAR indexes of *E. coli* isolates in this study, the isolates demonstrated remarkable susceptibility to both extracts of *Phyllanthus niruri*. This is an indication that new therapeutic substances that can exhibit activity against multidrug resistant bacteria can be developed from *Phyllanthus niruri*.

The ANOVA revealed significant main effects for all factors (Table 7a). Bacterial isolates had a highly significant effect ($F(10, 220) = 13.51$, $p < 0.001$), indicating varied responses among isolates. The extraction solvent also had significant effects ($F(1, 220) = 9.61$, $p = 0.002$), with the ethanol extracts generally producing larger inhibition zones than aqueous extracts. Concentration showed the strongest effect ($F(4, 220) = 48.06$, $p < 0.001$), confirming a dose-dependent increase in antibacterial activity.

The solvent × concentration interaction was significant ($F(4, 220) = 2.57$, $p = 0.039$), suggesting that the effect of concentration on inhibition zones varied by extraction solvent. Other interactions (isolate × solvent, isolate × concentration, and isolate × solvent × concentration) were not significant ($p > 0.05$), indicating largely independent effects of these factors.

Tukey's HSD test (2556 pairwise comparisons) identified significant differences between treatment groups (p-tukey < 0.05; Table 7b). For example, EC04_Ethanol_1000 vs. EC09_Ethanol_1000 showed a significant mean difference of 5.0 mm (p-tukey = 0.011), and EC02_Ethanol_1000 vs. EC02_Aqueous_1000 showed a 7.0 mm difference (p-tukey < 0.001), highlighting the superior efficacy of ethanol extracts at higher concentrations. Non-significant comparisons, such as EC09_Aqueous_500 vs. EC11_Ethanol_0 (p-tukey = 1.000), were also observed.

Ethanol extracts of *Phyllanthus niruri* demonstrated greater antibacterial efficacy than aqueous extracts, particularly at 500 – 1000 mg/ml.

Conclusion

This research demonstrates the phytochemical makeup and antibacterial efficacy of *Phyllanthus niruri* extracts against strains of *Escherichia coli*. Additional investigations are needed to explore the capabilities of *Phyllanthus niruri* extracts as alternative antibacterial agents, as the findings indicate that these ethanol and aqueous extracts will be relevant for the isolation of new/unique biomolecules in ethnomedicine and the development of prospective pharmaceuticals. The dose-dependent response and isolate-specific susceptibility (e.g., EC03, EC04, EC11) underscore the need for targeted testing in developing plant-based antimicrobials. The significant extraction solvent × concentration interaction suggests that formulation strategies should prioritise ethanol extracts at higher concentrations for optimal efficacy.

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