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# Invitro Determination of Antioxidant, Antihemolytic and Antibacterial activities of Methanolic Extract and Fractions of Phyllanthus Niruri Leaf

**Ahmad Vandi[1*] & Dr Mujaheed Abubakar PhD.[2]**
**[1,2]Department of Biological Sciences, Faculty of Natural and Applied Sciences, Sule Lamido University, Kafin Hausa, Jigawa State.**
[*]Corresponding Author Email: vandiahmad425@gmail.com

**ABSTRACT**
Oxidative stress arises when the body's defense system fails to maintain physiological balance, necessitating antioxidant supplementation. Hemolysis is a major mechanism by which malaria induces anemia, while the misuse of antibiotics has contributed to increasing resistance among microorganisms. This study investigated the phytochemical composition, antioxidant, antihemolytic, and antibacterial activities of methanol extract and solvent fractions of *Phyllanthus niruri* leaves. Methanol extraction yielded 19.82 g of crude extract, which was fractionated using solvent–solvent partitioning. Quantitative analysis revealed total phenolic and flavonoid contents of 24.05 mg/100 g and 13.81 mg/100 g, respectively, with the aqueous fraction showing the lowest values. Qualitative screening confirmed the presence of alkaloids, anthraquinones, flavonoids, glycosides, saponins, steroids, and tannins. Antioxidant assays (DPPH and FRAP) indicated strong activity in the aqueous fraction, while the n-hexane fraction showed weaker effects. Hemolytic assays revealed that the ethyl acetate fraction had the strongest antihemolytic activity, whereas the aqueous fraction was least effective. Antibacterial testing against *Escherichia coli*, *Staphylococcus aureus*, and *Salmonella typhi* demonstrated inhibitory effects across all fractions, with *E. coli* being most susceptible. Minimum inhibitory concentration (MIC) values ranged from 0.8–1.0 mg/ml, while minimum bactericidal concentration (MBC) results indicated primarily bacteriostatic effects, except for *S. aureus*, which exhibited bactericidal activity. Overall, methanol extract and fractions of *P. niruri* leaves exhibited significant antioxidant, antihemolytic, and antibacterial properties, highlighting their potential as natural therapeutic agents for managing oxidative stress, hemolysis-related conditions, and bacterial infections.

**Keywords:**
Phyllanthus niruri,
DPPH,
FRAP,
MIC,
MBC

## INTRODUCTION

The use of plants for medicinal purpose has assumed important dimension in the past few decades owing, mainly to the discovery that extracts from plants contain not only minerals and primary metabolites but also a diverse array of secondary metabolites with antioxidants potentials (Obichi *et al.*, 2015). Plant used in traditional medicine contain a wide range of substances that can be used to treat chronic as well as infectious disease. All plants produce these chemical compounds as part of their normal metabolites, such as sugars and fats found in all plants and secondary metabolites found in some plants. Some useful ones are found only in a particular genus or species (Sunday *et al.*, 2015). In recent times herbal medicines have become indispensable and are forming an integral part of the primary health care system of many nation (Pan *et al.*, 2014).

with pharmaceutical companies spending a lot of time and money in developing natural products from plants to serve as cost effective remedies that are affordable to the population. *Phyllanthus niruri* plant extract is used as a medicine and is recommended for bronchitis anemia, leprosy, asthma and urinary disorder (Narendra,2012). *Phyllanthus niruri* has extensive medicinal properties and has long history in the health care system of tropical countries (Adebiyi *et al.*, 2017).

The *P. niruri* is used in traditional health care system and is commonly known as stone breaker, however, there is a lot of confusion about this species identification. *Phyllanthus niruri* is used as a folk medicine for treating kidney stones, gall bladder stones, liver related diseases such as liver cancer and jaundice. A part from these, it also shows anti-inflammatory, anti-tumor, anti-noceptive and antioxidant properties (Bagalkotker *et al.*,2011).



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Recent work has indicated potential roles of secondary products at the cellular level as plant growth, gene expression and in signal transduction (Ajavi *et al.*,2016). Secondary products has a variety of functions in plants, they also exert medicinal effect for humans. Secondary products that are found in plant defense through cytotoxicity towards microbial pathogen prove useful as *antibacterial* medicines in humans if not too toxic. Likewise, secondary products involve in defense against herbivores through neurotoxin activity could have beneficial effects in human, through their action on the central nervous system. which promote the ecological survival of plants, structures of secondary product have evolve in the interact with molecular target affecting the cells, tissues and physiological function in compacting microorganisms, plants and animal.

## MATERIALS AND METHODS

### Plant collection

leaf of *Phyllanthus niruri* plant was collected at Sabon Gari, Sangere within Girei Local Government Area of Adamawa State. The area lies between latitude 90.09° and 90.33°N and longitude 120.21° and 12.54°E of the state and has an elevation of 339 meters above sea level. The plant material was transported to the herbarium units in the Department of Plant Science Faculty of Life Sciences, Modibbo Adama University, Yola where it was identified and authenticated.

### Preparation of methanol extract

Methanol extract was prepared according to the method describe by (Adebiyi *et al.,* 2017). Fresh leaf of *Phyllanthus niruri* plant was separated from the undesirable part and shed-dried under room temperature at 30±3ºC for two weeks. The leaf was ground into powder form using blender. The powdered leaf (100g) was extracted with (1200ml) of methanol in an air tight container for 72hrs on a magnetic stirrer untill the major portion of the extracted compound of the plant material was dissolved in the solvent. The mixture was filtered using muslin cloth and the filtrate was concentrated using rotary evaporator at 50ºC to recover back the solvent in the extract.

### Fractionation of extract

(20g) of methanol extract was successfully dissolved in 200ml distilled water, and the mixture was partitioned successively in a separating funnel with 200ml each of n-hexane, Dichloromethane, ethyl acetate, and n-butanol respectively. Fractions were concentrated on hot plate at 40ºC for 48hrs and the final weight of the fractions were determined. The aqueous fraction was equally concentrated using freeze dryer and weighed.

### Qualitative phytochemical screening

The Qualitative phytochemical screening of leaf extract was carried out using standard published procedures to test for the presence of alkaloids, tannins anthraquinones, glycosides, saponins, flavonoids and steroids as described ( Brusce *et al.,* 2019)

### Test for alkaloids

A fraction of extract was treated with 3-5 drops of wagners reagent (1.27g of iodine and 2g of potassium iodide in 100ml of water) and observed for the formation of redish brown precipitate or coloration as positive list for alkaloids. ( Brusce *et al.,* 2019)

### Test for anthraquinones

A small amount of extract was treated with a concentrated hydrochloric acid (HCL) and observed for the formation of yellow precipitate. ( Brusce *et al.,* 2019)

### Test for Flavonoids

To portion of the dissolved extract a few drops of 10% ferric chloride solution were added and a green or blue colour indicates presence of phenolic nucleus. ( Brusce *et al.,* 2019)

### Test for Saponins

Extract (2ml) was added to 6ml of water in a test tube. The mixture was shaken vigorously and observed for the formation of persistence foam that confirms the presence of saponins. ( Brusce *et al.,* 2019)

### Test for Tannins

About 0.5gram of the extract was boiled in 10cm of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and green or blue-black coloration is observed which indicate the presence of tannins. ( Brusce *et al.,* 2019)

### Test for Steroids

Extract (1g) was treated with 2ml of chloroform and equal amount of concentrated sulphuric acid was added, upper layer turns to red indicating the presence of the steroids. ( Brusce *et al.,* 2019)

### Test for Glycosides

Extract (5ml) was treated with 2ml glacial acetic in a test tube and a drop of ferric chloride solution was added to it. This was carefully underlaid with 1ml concentrated sulphuric acid. A brown ring at the interface indicates the presence of deoxy sugar characteristic of cardiac glycoside. ( Brusce *et al.,* 2019)

Quantitative Determination of Total Phenol and Flavonoid Content

### Determination Of *Total Phenol Content*

The aliquots (5ml) of the extract was transferred into a test tube and made up to the volume of 1ml with distilled water then 0.5ml of Folin Cocalteu reagent (1:1 water)

and 2.5ml of sodium carbonate solution (20%) were added sequentially to the test tube. Soon after shaking the mixture, the tubes were placed in the dark for 40mins and the absorbance was recorded at 725mm against the reagent blank. The total phenolic content was calculated and expressed as gallic acid equivalent (Kittakoop *et al.*, 2016).

**Determination Of Total Flavonoids Content**

Extract (1g) was added to 10ml volumetric flask containing 4ml of distilled water, then 0.3ml of 5% $N_aNO_2$ was added for 6 mins then 2ml of 1m NaOH was added and the total volume was made up to 10ml with distilled water. The solution was mixed well and the absorbance was measured against prepared reagent blank at 510nm and the total flavonoids content was expressed as percentage (Kaur *et al.,* 2016).

**Determination of *invitro* Antioxidant Activity**

*invitro* antioxidant activity of methanol extract and fractions of *P. niruri* leaf was determined by DPPH and FRAP assay described by Bagalkotkar *et al*, (2011).

**2,2 Diphenyl -I- Picrylhydrazyl (DPPH) assay Principle**

DPPH (2,2-Diplenyl -l-picrydrazyl), is a stable free radical, when acted upon by an antioxidant is converted into diphenol-picryhydrazine with a color change from deep violet to a high yellow color which was quantified spectrophometrically at 523nm to indicate the extent of DPPH scavenging activity by the extract.

**Procedure**

The antioxidant activity of the crude extract and fractions of *P. niruri* plant was determined using DPPH assay as described by (Bagalkotkar *et al.*, 2011). The DPPH solution (0.04%v/v) was prepared in 95% ethanol. A stuck solution of the crude extract and standard ascorbic acid were prepared in the concentration of 1.0$\mu$g/ml. Different concentration such as 0.2, 0.4, 0.6, 0.8 and 1.00$\mu$g/ml of this extract was taken in five different test tube, and 100ml and 2ml of freshly prepared DPPH (0.04%u/v) of the same solvent was added to each of the test tubes. The mixture was incubated for 15mins in the dark late the optimum density was recorded against the blank at 523nm. For the blank, DPPH solution in ethanol (2ml) was mixed with 10ml of ethanol and after 30mins the density of the solution was recorded, and the decrease in optical density of DPPH in additional to the test samples in relation to the control was used to calculate the antioxidant activity as percentage inhibition (%) of the DPPH radical

Therefore, using the below formula

$$\frac{Abc - Abs}{Abc} \times 100$$

Absorbance of sample = Abs
Absorbance of control = Abc
Scavenging activity = %

***Ferric reducing antioxidant power (FRAP) assay***

Ferric iron ($Fe^{3+}$) is initially reduced by electron donating antioxidants present within the sample to its ferrous form ($fe^{2+}$). The iron-colorimetric probe complex develops a dark blue color product upon reduction which can be measured at 700nn.

**Procedure**

in vitro antioxidant activities of the methanol extract and fractions of *P. niruri* leaf was determined using FRAP assay described by (Bagalkotkar *et al.*, 2011). A stock solution of the extract and standard ascorbic acid were prepared in the concentration of 10mg/ml. ferric reducing antioxidant power assay, 1ml of the sample of the extract in different concentration were mixed with 1ml of 0.2M sodium phosphate buffer (PH 6.6) and 1ml of 1% potassium ferric cyanide in different test tube. The mixtures were incubated in temperature-controlled water bath at 50$^o$C for 20mins and 1ml of 10% trichloroacetic acid. The mixture was centrifuge for 10mins at room temperature. 1ml of the supernatant was added to 1ml distilled water in a test tube and 0.2ml of 1% $FeCl_3$ was added. The blank was prepared using the same analytical procedures except the extract was replaced by distilled water, the absorbance of the reactive mixture was measured at 700nm.

**In vitro Determination of antihemolytic activity**

**Erythrocyte preparation**

Five millimeter (5ml) of the blood sample was collected from a healthy individual with (blood group O positive) in a plastic vacuum sterile test tube and the erythrocytes of the blood sample was collected after repeated washing in a sterilized phosphate buffer saline solution at (pH 6.6), the cells were resuspended in a phosphate buffer saline solution at 0.5%. ***Determination of in vitro antihemolytic***

The hemolytic activity of methanolic and fractions of *Phyllanthus niruri* leaf extract was performed by spectrophotoscopic method (Bain *et al*., 2017); a volume of 0.5ml of the cell suspension was mixed with 0.5ml of the methanol extract and fractions of the plant extract at different concentration such as 02, 0.4, 0.6, 0.8 and 1.0ug/ml respectively in normal saline. The mixture was incubated for 30mins at 37$^o$C. Then, mixture was centrifuge at 1500rpm for 10mins in a refrigerated centrifuge and then the free hemoglobin in the cell suspension was measured in a visible spectrophotometer at 540nm and normal saline was used as minimal hemolytic control while distilled water was used as maximum hemolytic control and each experiment was performed in triplicate for each concentration.

**Therefore, the percentage hemolysis was calculated according to the following formular.**

$$\%\ \text{hemolysiis} = \frac{\text{At} - \text{Ac}}{\text{Ac}} \times 100$$

AT = Absorbance of Test Sample
Ac = Absorbance of control

**In vitro determination of antibacterial activity Microbial culture**

The test organism includes S*taphylococcus aureus (S. aureus), Escherichia coli (E coli)* and *Salmonella typhi* (*S. typhi),* The organisms were obtained from the Department of Microbiology, Modibbo Adama University, Yola. They were sub-cultured on nutrient agar slant and kept at 4ºC.

**Determination of Minimum inhibitory concentration (MIC)**

In vitro determination of antibacterial activity of methanol extract and fractions the of *p. niruri* leaf was determined using the broth dilution method as described by Ewanshia *et al*, (2021). This method was used to determine the minimum inhibitory concentration (MIC). A 2-fold serial dilution of the leaf extract and fractions were prepared to obtain 0.2, 0.4, 0.6, 0.8 and 1.0mg/ml in test tubes and they were well labeled, zero point one millimeter (0.1ml) of the test organism was transferred into the test-tube, both positive and negative control were prepared (positive control tube was inoculated with the test organism while the negative control was left blank without incubated at 37ºC for 24hrs and observed after incubation. The lowest concentration starts inhibiting the organisms when compared with the control.

The test-tube that showed visible growth were examined and recorded as MIC

**Determination of maximum bactericidal concentration (MBC) of methanol extract and fractions of P. niruri leaf.**

Test-tube showing no visible growth from the MIC test were sub cultured on the fresh nutrient agar and incubated at 37ºC for 24hrs. the lowest concentration of the extract and fractions that yield no growth was recorded as the maximum bactericidal concentration (MBC).

**Data Analysis**

All the data in this study were estimated as mean ± standard error of mean (SEM) of three (3) replicates value and was analyzed using two-way analysis of variance (ANOVA) followed by post hock test, for significance between means at $p<0.05$. Statistical Package for Social Sciences (SPSS) version 25 was used for the analysis.

**RESULTS AND DISCUSSION**

**Yield of extract and fractions of *P. niruri* leaf**

The result shows that methanol extract gave a yield of 19.820mg/100g materials. The yield of the fractions are n-hexane 3.474g, dichloromethane 3.241g, n-butanol 2.705g and ethyl acetate 0.47g and aqueous 8.361g. The result is presented in table 1.

**Qualitative phytochemical contents of extract and fractions of *P. niruri* leaf**

The result shows that phytochemical compounds such as alkaloid, anthraquinones, flavonoids, glycoside, saponins, steroids and tannins were present in both extract and fractions of *P. niruri* leaf. The result is shown in Table 2.

**Quantitative total phenols and flavonoids contents of extract and fractions of *P. niruri* leaf**

The result shows that, methanol extract 24.05mg/100g and n-hexane fraction 5.91mg/100g gave the highest phenol and methanol extract 13.81mg/100g and ethyl acetate 4.51mg/100g gave the highest flavonoid content respectively. The result is presented in table .3.

**DPPH scavenging activity**

The ability of *P. niruri* leaf methanol extract and its fractious to scavenge DPPH radical was evaluated and the findings expressed as percentage DPPH scavenging activity is shown in Table 4. The result from this activity shows that Gallic acid which was used as control showed activity in the range between 34.54 ± 2.98 and 55.78 ± 8.69% and is significantly lower than *P. niruri* leaf aqueous fractious ranging between 35.19 ± 9.67 and 68.33±9.78% in order of increasing concentration. Result from the percentage DPPH scavenging activity of aqueous fraction at 80 and 100 ug/ml (67.97±6.22 and 74.12 ±7.63) is significantly ($p<0.05$) higher when compared to the extract and other fractions at the same concentration.

**Total reducing antioxidant power assay**

The result on Table 5 represents total reducing power of methanol extract and fractions of *P. niruri* leaf as compared with Gallic acid used as control where increased absorbance indicate increase in this total reducing power. The result indicate a dose dependent increase in total reducing power. At 20 and 40 ug/ml aqueous fractions shows a significantly ($p<0.05$) higher activity 41.56 + 5.52 and 64.12. + 9.34 when compared with methanol extract, n-hexane, dichloromethane fraction ethyl acetate n-butanol at both concentrations. The result also indicates that at 60 and 80 ug/ml, aqueous fractions shows a significantly high ($p<0.05$) total reducing power 79.87 + 6.82 and 83.18 + 4.23 when compared with methanol extract, n-hexane fraction, n-butanol fraction, ethyl acetate fractions, and n-butanol fractions and Gallic acid at both concentrations (59.58 +

8.33, 45.76 + 8.54, 50.11 + 9.56, 55.43 + 8.11, 50.84 + 7.21 and 54.12 + 2.12) and ( 69.54 + 7.45, 61.13 + 6.82, 68.45 + 7.75, 69.77 + 4.23, 69.61 + 5.21 and 63.98 + 4.18).At the highest concentration (100uglml) aqueous fractions shows a significantly ($p<0.05$) higher activity 89.28 + 3.41 when compared with methanol extract, n-hexane fraction, dichloromethane, ethyl acetate, n-butanol fractions and Gallic acid (78.32 + 0.21, 76.65 + 4.87, 75.7.5, 79.53 + 2.13, 74.03 + 2.13 and 70.42 + 3.14 at the same concentrations.

**Antihemolytic activity**

The antihemolytic assay was performed to ascertain the potency of extract and fractions from *P. niruri* leaf using normal saline as control. The result expressed as percentage antihemolytic activity shown on table .4.6 the methanol extract and fractions demonstrated antihemolytic activity in a dose dependent activity (20,40,60,80 and 100uglml). At the lowest concentration ethyl acetate exhibited a significantly ($p<0.05$) higher antihemolytic activity (56.12. + 9.53) when compared to methanol extract, n-hexane fraction, dichloromethane n-butanol fraction, aqueous fractions and normal saline (2012+1.26, 48.66 + 5.78, 36.61 + 4.31, 7.43 + 0.16, 17.63 + 1.34 and 30.14 + 1.8). However, at the highest concentration (1mg/ml) normal saline control, exhibited a significant ($p<0.05$) higher antihemolytic activity (77.28. +4.98). When compared to methanol extract, n-hexane fraction, dichloromethane fractions, ethyl acetate fractions, n-butanol, fractions and aqueous fraction (67.13. + 6.19, 71.6 + 3.24, 76.12 + 6.18 +, 38.87 + 3.11, 22.76 + 5.12 and 24.03 + 1.42 respectively).

***ANTIBACTERIAL ACTIVITY***

***Minimum Inhibitory Concentration (MIC).***

The result of the minimum inhibitory concentration MIC of methanol extract of *p. niruri* showed that *S. aureus* and *S. typhi* are inhibited at all the concentration. E.coli had MIC at 0.8mg/ml Table4.7.2, table 5 the n-hexane fraction showed that *S. aureus* was inhibited at all concentration, *E.coli* was inhibited at 0.8mg/ml and *S. typhi* was inhibited at 0.8mg/ml. the dichloromethane fractions showed that *S. aureus* and *S. typhi* are inhibited in all concentration while *E.coli* was inhibited at 1.0mg/ml , the Ethyl acetate, n-butanol and Aqueous fraction showed that *S. typhi* are inhibited at 1.0mg/ml in all concentrations of the fractions.

The result of the minimum inhibitory concentration (MIC) of the methanol extract and fractions varied with the type of bacterial space tested. The significant effect was observed on the use of the methanol extract against *E. coli* with MIC 0.8.mg/ml. It was not significant against *S. aureus, S. typhi* because their growth was inhibited in all concentration of the methanol extract and also it was observed that *E. coli* had most significant effect on the use of n-hexane fraction with MIC 0.8mg/ml, it was followed by *S. typhi* with MIC 0.8mg/ml while *S. aureus* was inhibited at all concentration. It was observed that *S. aureus* and *S. typhi* were inhibited at all concentration of the fractions such as dichloromethane ethyl acetate, n-butanol and aqueous fractions while *E. coli* with the list significant effect on the use of this fractions.

***Minimum Bactericidal Concentration (MBC)***

The result of minimum bactericidal concentration (MBC) of methanol extract of *p. niruri* revealed that *S. aureus* and *S. typhi* had a bacteriostatic effect on the test bacteria in all the concentration (0.2mg/ml – 1.0mg/ml) while *E.coli* revealed a lethal bacteriostatic effect at concentration of 0.8mg/ml as seen in table 6 The n-hexane leaf fractions revealed that *S. aureus* and *S. typhi* had bacteriostatic effect on the test bacteria in all concentration (0.2mg/ml – 1.0mg/ml) while *E. coli* revealed a lethal bacteriostatic effect at concentration of 0.8mg/ml. The Dichloromethane leaf fractions revealed that *S. aureus* and *S. typhi* had a bacteriostatic effect on the test bacteria at all concentrations (0.2mg/ml – 1.0mg/ml)/ the Ethyl acetate, n-butanol and Aqueous showed that *S. aureus* and *S. typhi* had a bacteriostatic effect in this fraction at all concentration (0.2mg/ml – 1.0mg/ml) while *E. coli* had a lethal bacteriostatic effect in these three (3) fractions at concentration of 0.8mg/ml respectively.

It showed that, the plant extract and fractions have lethal/bacteriostatic effect on *S. typhi* with MBC 0.8mg/ml and *E. coli* with MBC 1.0mg/ml had the highest lethal concentration. All the concentration had a bacteriostatic effect on *S. aureus.* It was observed that Dichloromethane, ethyl acetate, n-butanol and aqueous fractions had a lethal- bacteriostactic effect on *E. coli* with MBC 1.0mg/ml in all the concentration of the fraction. All concentration of the fractions had a bacteriostatic effect on *S. aureus* and *S. typhi*.

**Table 1. The yield of extract and fractions of *P. niruri* Leaf**

| S/N | Extract and Fractions | Results (g) |
|---|---|---|
| 1 | Methanol | 19.820g |
| 2 | n-hexane | 3.474g |
| 3 | Dichloromethane | 3.241g |
| 4 | Ethyl Acetate | 0.47g |
| 5 | n-butanol | 2.705g |
| 6 | Aqueous | 8.361g |

**Table 2. Qualitative Phytochemical Screening of Extract and Fractions of *P. niruri* leaf**

| Phytochemical | Methanol | N-hexane | E.A | DCM | N-butanol | Aqueous |
|---|---|---|---|---|---|---|
| **Alkaloids** | + | + | + | + | + | + |

| | | | | | | |
|---|---|---|---|---|---|---|
| **Anthraquinones** | + | + | + | + | + | + |
| **Flavonoids** | + | + | + | + | + | + |
| **Glycoside** | + | + | + | + | + | + |
| **Saponins** | + | + | + | + | + | + |
| **Steroids** | + | + | + | + | + | + |
| **Tannins** | + | + | + | + | + | + |

**Key:** (+) = Presence of phytochemical.
E.A = Ethyl acetate
DCM = Dichloromethane

**Table 3. Quantitative Determination of Total Phenolic and Flavonoids Content**

| S/N | EXTRACT/FRACTIONS | TOTAL PHENOL (mg/100g) | TOTAL FLAVONIODS (mg/100g) |
|---|---|---|---|
| 1 | Methanol extract | 24.05 | 13.81 |
| 2 | n-hexane | 5.91 | 1.97 |
| 3 | Dichloromethane | 4.10 | 3.27 |
| 4 | Ethyl acetate | 3.65 | 4.51 |
| 5 | n-butanol | 3.41 | 2.08 |
| 6 | Aqueous | 2.03 | 1.04 |

Table 4. Percentage DPPH Scavenging activity of methanol extract and fractions of *P. niruri Leaf*

| Conc. (mg/ml) | Methanol | N-Hexane | DCM | E.A | n-butanol | Aqueous | Gallic acid (STD) |
|---|---|---|---|---|---|---|---|
| 0.2 | 30.32±0.26$^{d}$ | 20.64±7.99$^{d}$ | 23.47±3.19$^{d}$ | 10.66±8.77$^{d}$ | 28.76±6.81$^{d}$ | 35.19±9.67 | 34.54±2.98$^{d}$ |
| 0.4 | 44.56±2.56 | 31.33±7.99$^{b}$ | 31.72±5.88$^{b}$ | 24.13±3.81$^{b}$ | 38.87±5.81 | 46.93±7.33$^{a}$ | 41.67±6.83 |
| 0.6 | 56.55±6.89$^{a}$ | 40.79±5.91$^{b}$ | 47.19±8.67 | 36.98±6.34$^{b}$ | 50.86±6.44$^{a}$ | 58.88±8.32$^{a}$ | 45.55±±6.11 |
| 0.8 | 61.87±9.68$^{a}$ | 50.12±9.21$^{a}$ | 56.89±6.89$^{a}$ | 49.61±5.59 | 58.79±6.18$^{a}$ | 67.97±6.22$^{a}$ | 45.23±5.57 |
| 1.0 | 74.12±7.63$^{ac}$ | 58.56±7.54$^{ac}$ | 61.33±6.78$^{ac}$ | 59.35±6.97$^{ac}$ | 67.59±7.56$^{ac}$ | 68.33±6.78$^{ac}$ | 55.78±6.69 |
| $IC_{50}$ | 53.35303 | 51.775 | 44.2022 | 65.813 | 62.10289 | 48.92526 | 42.2011 |

*All values are mean ± SEM for 3 determinations a= Significantly (p <0.05) higher compared to gallic acid at the same concentration. b= Significantly (p <0.05) lower compared to gallic acid at the same concentration c= Significantly (p <0.05) higher compared to other values in the same column d= Significantly (p <0.05) lower compared to other values in the same column*

Table 5. *Percentage total reducing antioxidant power activity of P. niruri leaf*

| Conc. (mg/ml) | Methanol | N-Hexane | DCM | E.A | n-butanol | Aqueous | Gallic acid (STD) |
|---|---|---|---|---|---|---|---|
| 0.2 | 41.21±3.26$^{d}$ | 22.45±5.65$^{bd}$ | 26.34±9.78$^{bd}$ | 35.83±7.42$^{bd}$ | 29.68±1.87$^{bd}$ | 41.56±5.52$^{d}$ | 43.7±3.81$^{d}$ |
| 0.4 | 51.63±3.26$^{a}$ | 30.12±4.78$^{b}$ | 42.71±6.23$^{b}$ | 39.18±5.71$^{b}$ | 41.11±3.41$^{b}$ | 64.12±9.34$^{a}$ | 47.12±7.31 |
| 0.6 | 59.58±8.33$^{a}$ | 45.76±8.54$^{b}$ | 50.11± 9.56$^{b}$ | 55.43±8.11 | 50.84±7.21$^{b}$ | 79.87±6.82$^{a}$ | 54.12±2.12 |
| 0.8 | 69.54±7.45$^{a}$ | 61.13±6.82 | 68.45± 7.75$^{a}$ | 69.77±4.23$^{a}$ | 69.61±5.21$^{a}$ | 83.18±4.23$^{a}$ | 63.98±4.18 |
| 1.0 | 78.32±0.21$^{ac}$ | 76.65± 4.87$^{a}$ | 75.75±7.56$^{ac}$ | 79.53±4.13$^{ac}$ | 74.03±2.13$^{ac}$ | 89.28±3.41$^{ac}$ | 70.42±3.14$^{c}$ |
| $IC_{50}$ | 38.17412 | 63.98078 | 49.91356 | 55.7097 | 54.7884 | 22.26725 | 32.417 |

*All values are mean ± SEM for 3 determinations a= Significantly (p <0.05) higher compared to gallic acid at the same concentration. b= Significantly (p <0.05) lower compared to gallic acid at the same concentration c= Significantly (p <0.05) higher compared to other values in the same column d= Significantly (p <0.05) lower compared to other values in the same column*

Table 6. *In vitro* antihemolytic activity of *P. niruri* leave extracts

| Conc. (mg/ml) | Methanol | N-Hexane | E.A | DCM | n-butanol | Aqueous | Control |
|---|---|---|---|---|---|---|---|
| 0.2 | 20.12±1.26$^{bd}$ | 48.66±5.78$^{a}$ | 56.12±9.53$^{ad}$ | 36.61±4.31$^{ad}$ | 7.43±0.16$^{b}$ | 17.63±1.34$^{b}$ | 30.14±1.85$^{d}$ |
| 0.4 | 23.67±2.04$^{b}$ | 8.99±1.12$^{bd}$ | 57.12±8.63$^{a}$ | 41.56±3.23 | 3.23±0.09$^{bd}$ | 13.11± 0.51$^{bd}$ | 43.31±7.11 |
| 0.6 | 67.32±5.44$^{ac}$ | 27.23±1.78$^{b}$ | 83.19±7.67$^{ac}$ | 79.43±6.19$^{a}$ | 16.31±0.41$^{b}$ | 19.16±3.21$^{b}$ | 60.12±5.12 |
| 0.8 | 29.34±4.12$^{b}$ | 48.31±1.96$^{b}$ | 79.35±4.66$^{a}$ | 81.21±7.31$^{ac}$ | 13.11±1.66$^{b}$ | 7.97± 1.03$^{b}$ | 69.32±3.51 |
| 1.0 | 27.13±1.19$^{b}$ | 71.6± 3.24$^{ac}$ | 76.12±6.18 | 38.87±3.11$^{b}$ | 22.76±5.12$^{ac}$ | 24.03±142$^{ac}$ | 77.28±4.98$^{c}$ |
| $IC_{50}$ | 58.81586 | 51.22535 | 5.49807 | 30.92531 | 24.4667 | 43.96356 | 10.609 |

*All values are mean ± SEM for 3 determinations a= Significantly (p <0.05) higher compared to control at the same concentration. b= Significantly (p <0.05) lower compared to control at the same concentration c= Significantly (p <0.05) higher compared to other values in the same column d= Significantly (p <0.05) lower compared to other values in the same column*

**Table 7. MIC AND MBC of Methanol leaf extract of *P. niruri leaf***

| Test Organism | Concentration in (mg/ml) 0.2 | 0.4 | 0.6 | 0.8 | 1.0 | MIC | MBC |
|---|---|---|---|---|---|---|---|
| *S. aureus* | + | + | + | + | + | <1.0 | <1.0 |
| *E. coli* | + | + | + | - | - | 0.8 | 1.0 |
| *S. typhi* | + | + | + | + | + | <1.0 | <1.0 |

**Table 8. MIC AND MBC of n-hexane leaf fraction of *P. niruri* leaf**

| Test Organism | Concentration in (mg/ml) 0.2 | 0.4 | 0.6 | 0.8 | 1.0 | MIC | MBC |
|---|---|---|---|---|---|---|---|
| *S. aureus* | + | + | + | + | + | >1.0 | >1.0 |
| *E. coli* | + | + | + | + | - | 1.0 | >1.0 |
| *S. typhi* | + | + | + | + | + | 0.8 | 1.0 |

**Table 9. MIC AND MBC of Dichloromethane fraction of *P. niruri* leaf**

| Test Organism | Concentration in (mg/ml) 0.2 | 0.4 | 0.6 | 0.8 | 1.0 | MIC | MBC |
|---|---|---|---|---|---|---|---|
| *S. aureus* | + | + | + | + | + | >1.0 | >1.0 |
| *E. coli* | + | + | + | + | - | 1.0 | 1.0 |
| *S. typhi* | + | + | + | + | + | 0.8 | 1.0 |

**Table 10. MIC AND MBC of Ethyl acetate leaf fraction of *P. niruri* leaf**

| Test Organism | Concentration in (mg/ml) 0.2 | 0.4 | 0.6 | 0.8 | 1.0 | MIC | MBC |
|---|---|---|---|---|---|---|---|
| *S. aureus* | + | + | + | + | + | >1.0 | >1.0 |
| *E. coli* | + | + | + | + | - | 1.0 | 1.0 |
| *S. typhi* | + | + | + | + | + | >1.0 | >1.0 |

**Table 11. MIC AND MBC of n-butanol fraction of *P. niruri* leaf**

| Test Organism | Concentration in (mg/ml) 0.2 | 0.4 | 0.6 | 0.8 | 1.0 | MIC | MBC |
|---|---|---|---|---|---|---|---|
| *S. aureus* | + | + | + | + | + | >1.0 | >1.0 |
| *E. coli* | + | + | + | + | - | 1.0 | 1.0 |
| *S. typhi* | + | + | + | + | + | >1.0 | >1.0 |

**Table 12. MIC AND MBC of Aqueous fractions of *P. niruri* leaf**

| Test Organism | Concentration in (mg/ml) 0.2 | 0.4 | 0.6 | 0.8 | 0.1 | MIC | MBC |
|---|---|---|---|---|---|---|---|
| *S. aureus* | + | + | + | + | + | >1.0 | >1.0 |
| *E. coli* | + | + | + | + | - | 1.0 | 1.0 |
| *S. typhi* | + | + | + | + | + | >1.0 | >1.0 |

**Key**

- = No growth

+ = Presence

Plants are naturally rich source of secondary metabolites and novel therapeutic compounds known for their various beneficial effect on human health. This study evaluated the quantitative and qualitative phytochemical constituent and the invitro antioxidant activity of *P. niruri* leaf using DPPH radical scavenging activity, ferric reducing power Frap. It also evaluated the in vitro antihemolytic and antibacterial potential of the methanol extracted and its fractions, the medicinal values of plants lie in their phytochemical constituent. These constituents vary in their solubility in different solvents. Therefore, wide range of extracting solvent has been for the extraction of biologically active molecules from plants (Ptink *et al.*, 2017) these biomolecules from plant extract are been used to treat human disease as an important alternative therapeutic approach and this practice has accelerated in recent years because numerous evidence reveal that plant derived phytochemical compounds are inexperience, readily available and accessible for management of disease (Huang *et al.*, 2010; Ajavi *et al.*, 2016).

The presence of phenols, flavonoids, alkaloids, anthraquinones, glycosides etc. corroborates the work of other researchers Babalola and Alabi, (2015) and Adeleke *et al*, (2016) reported the presence of these phytochemicals in the leaves. Similar result was reported by Grice and Segre, 2012. However, quantitative phytochemical analysis of *P. niruri* leaf using HPLC (Table 4. reveal significant difference in the number of total phenols and flavonoids with various solvents. Total phenols and flavonoids of the methanol extract in this work are higher than those reported by Babalola and Alabi, (2015) using water as the extraction solvents.

The differences in these results could be as a result of the difference in location and season of collection of plant materials. Many researchers have reported altered values for various phytochemicals in plants as seasons and location changes. (Grice and Segre, 2012). changes in water quality which about 6-8 million people die each year due to water related disease such as cancer, skin disease diarrhea (Dauda *et al*.,2026) The quantitative and qualitative phytochemicals studies of this plant suggest that the plant possess significant medicinal properties since phytochemical constituents are known to be responsible for medicinal activity of plant species. (Obichi *et al.*, 2015). Phenol are secondary metabolites of plants and they play an important role in the defense against ultraviolet radiation or aggression by pathogens. They are present in numerous plants and have demonstrated profound antioxidative properties.

ROS Scavenging and lipid Peroxidation inhibition activities (Korkina *et al.*, 2009) increasing evidence suggest that polyphenol protects against the risk of various disease associated with oxidative stress such as cancer, cardiovascular disease, chronic liver related disease. (Pandey *et al.*, 2009) in food, polyphenol contributes to astringency, color, flavor, odor and oxidative stability. A considerable high amount of phenolics was observed in the methanol extracts of the plant. This may explain the wide spread folklore use of the plant (Victor *et al.*, 2019). The n-hexane fractions showed relatively higher phenolic content (8.32g/00g), when compared to the dichloromethane, ethyl acetate, n-butanol and Aqueous fractions of the plant extracts. The difference in the phenolic content may be ascribe to the varying solubility of these phytochemicals in different solvent system (Chandrasekara *et al.*, 2016).

Flavonoids comprise the most studied group of polyphenols and have been shown to possess antioxidant activity (Obichi *et al.*, 2015). They exhibit their

antioxidant properties through several mechanisms such as scavenging of free radical chelation of metal ions such as iron and copper, inhibition of hydrolytic and oxidative enzymes and also act as anti-inflammatory agent (Gholamian-Dehkordi *et al.,* 2017). The flavonoid content of the n-hexane fraction was the highest among all the fractions separated (1.97) compared to the dichloromethane, ethyl acetate, n-butanol and Aqueous fraction. This result is similar to those reported by (Roy *et al.,* 2016). The presence of these active ingredients in *Phyllanthus niruri* corroborates its potential as a medicinal plant.

Consumption of antioxidant has been associated with reduced undesirable effects of reactive oxygen species (ROs) and reduce levels of oxidative damage to lymphocytic DNA by improving endogenous antioxidant of the body (Jafri *et al.,* 2014).

In vitro antioxidant potency of *Phyllanthus niruri* was evaluatated using DPPH radical scavenging activity, ferric reducing power essay FRAP. DPPH is one of the agents of choice for many in vitro studies in evaluating the free radical scavenging activity of natural compounds (Sahreen *et al.,* 2014). Antioxidant activity, determined by DPPH and FRAP assay were high. These results indicated that *P. niruri* possessed substantial antioxidant capacity which varied with different solvent system. Stereo sensitivity of the radicals and the solubility of extract in diverse solvent system have been reported to affect the capacity of extract to react and quench different radicals (Uoki – Assanga *et al.,* 2015) similar results was obtained by Kasote *et al,* (2019). Differences observed in the DPPH free radical scavenging activity of the fractions might likely be due to the difference in the amount of the phytoconstituents. The $IC_{50}$ value of the methanol extracts and n-hexane in this study was found to be higher than the other fractions hence the fractions possess better radical scavenging potentials than the methanol extract. However, at 0.2mg/ml, the methanol extract did not demonstrate any leveling effect on the DPPH radical. The $IC_{50}$ value of the ethyl acetate fraction was found to be lower than that of n-hexane, Dichloromethane, n-butanol and Aqueous fractions hence, the ethyl acetate fraction possesses better radical scavenging potentials than all the fractions. This is probably because water solubility of any therapeutic agent is a fundamental property that plays an important role in the absorption, permeability, dissolution rate, first pass metabolism and overall activity (Recharla *et al.,* 2017).

The reducing properties of medicinal plant and their extract are linked with the presence of compounds which exert their actions by breaking free radical chain by donating a hydrogen atom (Lobo *et al.,* 2010). In the reducing power assay, the antioxidant compounds cause reduction in $fe^{3+}$ by donating one electron. This property in n-hexane, dichloromethane, ethyl acetate, n-butanol and Aqueous fractions were higher than the methanol extract and Gallic acid. The property in aqueous fraction is higher than n-hexane, Dichloromethane, ethyl acetate, n-butanol, methanol extract and Gallic acid standard antioxidant used in this study at the same concentration.

Erythrocyte are very sensitive cells to oxidative stress this sensitivity is related to their rich membrane lipid composition of polyunsaturated fatty acids. The constant interaction with oxygen and the production of reactive oxygen species (ROS) by activated inflammatory cell (Manish Mittal *et al.,* 2014) additionally the presence of hemoglobin in large quantities also exposes red blood cells to high levels of autoxidation (Shead *et al.,* 1992). Ethyl acetate fractions shows high antihemolytic activity as compared with the extract and fractions in all the concentrations.

Antibiotic resistance is a problem that continues to challenge the healthcare sector in a large part of the world in both developing and developed countries. The emergence and spread of multidrug, resistance pathogens have substantially threatened the current antibacterial therapy. This has necessitated research for a new source of antibacterial substance such as plants as they produce a variety of bioactive compounds of known therapeutic properties. This study has been conducted to determine the in vitro anti *antibacterial* activity of *P. niruri* leaf extract. The leaf extract exhibited a good antibacterial activity towards different tested isolates. *P. niruri* leaf extract showed maximum activity against pathogens such as *E. coli* and *S. typhi*, the extract also showed significant MBC and MIC values against *E. coli* and *S. typhi* (Subeki *et al.,* 2005). *P. niruri* leaf extract was found to have anti *antibacterial* activity against 2 out of the 3-test bacterium *E. coli* and *S. typhi* therefore the degree of antibacterial activity of *P. niruri* against the tested gram positive and gram-negative bacterial shows that gram negative bacteria *E. coli* and *S. typhi* were found to be effective. The result from this study also shows that the potency of the extract increased with the increase in concentration. This confirms the findings of Soh *et al.,* (2009) who reported that higher concentrations of antibacterial substance exhibit more growth inhibition of some microbial pathogens. The result of the minimum inhibition concentration MIC of the methanol extract and fractions of the *P. niruri* leaf shows that *E. coli* has significant effect on the use of methanol extract, dichloromethane, ethyl acetate, n-butanol and Aqueous fractions with the exception of n-hexane fraction were *S. typhi* is effective on the use of n-hexane fraction. This is in conformity with the ethno-botanical findings of Yerra *et al.,* (2008), who reported that the plant is used in the treatment of several disease ailments.

The results obtained showed that the methanol extract and fraction of *P. niruri* leaf were potent against the test organisms. The susceptibility of these bacteria agreed with the report of (Ewanshia *et al.,* 2021) that Gram-negative bacteria are more resistant than their gram-

positive counter parts. It was also observed that the fraction established more potency than the methanol extract. This is in line with the used of water as a solvent in the preparation of decoction for used traditionally, because most of the active biochemical present in the leaf are water soluble, fragile and hence, damage in alcohol (Masalha, 2001).

The efficiency of the fractions against the test bacterial agree with Mazumder (2006) who reported that the leaf of *P. niruri* have several uses especially for stomach ailments, dropsy and urinogenital disease in India, Malesia and Philippines.

The result of the investigation revealed that the potency of the extract increased with the increase in concentration. This confirms the findings of Kumar (2004) who reported that higher, concentrations of antibacterial substance exhibit more growth inhibition of some bacterial species. The low efficiency of the methanol extract against test bacteria observed in this study might stem from the difficulty of dissolution of some of the constituents extract in methanol.

This is in conformity with the ethnobotanical findings of Raphael (2006) who reported that the leaf was used in the treatment of several ailment including constipation, flu and typhoid fever course by *S. typhi* in the Bahamas.

The result of the MBC of methanol extract showed that test organism was inhibited but are not killed in all the concentrations. This shows that the methanol extract has a bacteriostatic effect on test organism. The reason is not far-fetched since most of this active biochemical present in *P. niruri* are water soluble and more fragile as reported by Luyindula (2004) the investigation of the MBC of the fractions revealed that most of the concentration of the fractions kill the test organism

## CONCLUSION

In conclusion, this study provides scientific evidence whereby the extract and fractions of p.niruri leaf effectively scavenged or inhibited all the radical tested Among the extract and fractions, of the leaf, aqueous fractions were the most effective scavenger followed by Dichloromethane and n- butanol fractions. ethyl acetate fraction shows the highest antihemolytic activity of the leaf followed by Dichloromethane and n- butanol fractions respectively. the Minimum Inhibitory Concentration (MIC), reveals that E. coli and S. typhi were susceptible to the plant extract and fractions of the leaf, while Maximum Bactericidal Concentration (MBC) reveal that, S. typhi and E. coli had a bacteriostatic effect and S. aureus had a bactericidal effect on the methanol extract and fractions of the leaf in all concentrations. The study reveals that P. niruri leaf show high potency of antioxidant, antihemolytic and antibacterial properties that can be used treating several diseases.

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