 https://dx.doi.org/10.4314/njcr.v26i2.3

# DETERMINATION OF THE RATE OF KILL, MODE OF ACTION, AND THE BIOACTIVE COMPONENTS FROM THE ETHYL ACETATE SUB-FRACTION OF METHANOL EXTRACT OF *Phyllanthus amarus*.

**A. I. Alli*[1], J. O. Ehinmidu[2], Y. K. E. Ibrahim[2] and C. E. Udobi[3]**

*1. Dept. of Applied Science, College of Science and Technology, Kaduna Polytechnic, Kaduna.*
*2. Dept. of Pharmaceutics and Pharmaceutical Microbiology, Ahmadu Bello University, Zaria.*
*3. Dept. of Pharmaceutics and Pharmaceutical Technology, University of Uyo, Uyo.*
[Corresponding Author: allihima@gmail.com]

## ABSTRACT

*The time-kill rate of methanol extract of Phyllanthus amarus was determined in this study which showed that the extract caused a reduction of the viable cells of all the test bacteria after a contact time of 30 mins and there were virtually no surviving cells of all the test bacteria after a contact time of 180 mins. The extract was also found to cause leakages of cellular materials such as potassium ions, sodium ions, protein and nucleic acids from the test bacteria which led to the loss of cell viability. The ethyl acetate sub-fraction of the extract was analyzed by GC-MS and FTIR analysis and the result revealed the presence of Phytochemicals such as 1, 2-Benzenedicarboxilic acid mono (2-Ethylhexyl) ester, Columbin, 2-(6-Methylpyridin-2-ylmethyl) cyclohexane, 2(1H) Naphthalenone,3,5,6,7,8,8a-hexahydro-4,8a-dimethyl-6-(1-methylethenyl) which have all been reported to possess antibacterial activity against both gram-positive and gram-negative bacteria. The result of this study will contribute to the baseline data on the pharmacodynamics of the extract if applied as herbal medicine for human treatment thereby reducing the dosage and period of treatment. The finding also revealed that the ethyl acetate sub-fraction of methanol extract of P. amarus contains antibacterial phytochemicals that may be used to develop more potent, safe and cheap antimicrobial agents using nanotechnology.*

## INTRODUCTION

*Phyllanthus amarus* has a long history of usage all over the world in herbal medicine for the remedy of health-related problems such as cough, diarrhea, dysentery, dropsy, jaundice, intermittent fevers, urinogenital disorders, tuberculosis, sore throat scabies wound healing etc[1,2,3]. Methanol extract of *P. amarus* was earlier found to possess good antimicrobial activity against some microorganisms while its ethyl acetate sub-fraction demonstrated good antibacterial activity relatively higher than the crude extract[4]. The use of the crude extracts of this plant or its phytochemicals (with known antimicrobial properties) for therapeutic treatments has increased significantly in recent times. Several herbal formulations of *P. amarus* are been sold in the market and some of them are currently undergoing clinical trials

so as to determine their optimum and beneficial therapeutic dose ranges. In a recent study[5], the effect of ethanol leaf extract of *P. amarus* on hematological profiles in *Salmonella typhi* infested albino rats was investigated. The results demonstrated that treatment of *S. typhi* infection with ethanol extract of *P. amarus* reverses and ameliorates the hematotoxic effects induced by *S. typhi* infection in rats. The therapeutic effect of methanol extract of *P. niruri (*a close relative of *P. amarus*) was also evaluated at different doses in rats infected with *E. coli* and when compared with Ciprofloxacin as the positive control drug, the extract showed a dose-dependent restorative response comparable to the control drug Ciprofloxacin with no significant difference ($P \leq 0.5$)[6]. In another study, the *in vivo* anti-plasmodia activity of the aqueous and methanolic extracts of *P. amarus* formulated into capsules on *Plasmodium yoelii* (a resistant malaria parasite strain used in animal model studies) infection in mice was evaluated[7]. The results showed that the extracts demonstrated a dose-dependent prophylactic and chemotherapeutic activity compared to the standard drugs used in the treatment of chloroquine-resistant Plasmodium infection. The *in vivo* anti-plasmodia activities of the aqueous and methanolic extracts of *P. amarus* whole plant were also investigated by evaluating the antimalarial activity during established infection using rodent models. In the 5-day curative test, treatment of infected mice with crude extract of *P. amarus* resulted in the inhibition of parasite growth in a dose-dependent manner signifying that the plant is endowed with antimalarial potential[8]. Despite the volume of scientific reports on the use of *P. amarus* extract as herbal medicine, there is still the need for more pharmacological data and phytochemical evaluations before embarking on clinical trials and commercialization. This study was therefore designed to investigate the antibacterial activity of methanol extract of *P. amarus* based on the time-Kill assay, its mode of antibacterial activity and phytochemical evaluation of the most active sub-fraction.

## MATERIALS AND METHODS

### *Collection, identification, and extraction of plant materials*

*P. amarus* was collected from in and around Federal College of Education, Okene. The plant was earlier identified and authenticated at the herbarium of the Department of Botany, A.B.U., Zaria with voucher number 555. The leaves were air-dried under shade at ambient temperature, pulverized into a powder, packed into soxhlet extractor, defatted with n-hexane,

and subsequently extracted with methanol. The extract was concentrated using a rotary evaporator at $40^0C$ and transferred into a clean container and stored in the refrigerator as earlier described in the previous study[4].

***Phytochemical Screening of Extracts***

Screening for carbohydrates, tannins, alkaloids, saponins, flavonoids, steroids/terpenoids, cardiac glycosides and anthraquinone was carried out by standard methods as described by[9,10]. The result of the phytochemical screening has also been presented[4].

***Determination of the rate of kill of the methanol extract of P. amarus against test bacteria*:**

The method of[11] was adopted for the determination of the rate of kill of the extract against the test bacteria used in the previous study[4]. One milliliter (1.0ml) from each culture suspension was added to 9.0 ml of the selected fixed concentrations (MIC and 2 X MIC) of extract in a sterile universal bottle such that the final test suspension contained approximately $10^6$ cfu/ml of the test organism. The test suspensions were kept in a water bath at $37^0C$ and 1.0 ml withdrawn at predetermined intervals of 10, 30, 60, 120, 180 and 240 mins. Ten-fold serial dilutions were carried out with sterile normal saline containing 3% Tween 80. Exactly 0.1ml of each dilution was aseptically plated out in duplicates using the pour-plate method for viable counts after 24 hours at $37^oC$. Colony counts were plotted against time intervals on a semi-log graphing paper to obtain the killing curve for each selected fixed concentration of the various extracts. The Log reduction was calculated as follows:

Log10 reduction = Log10 (initial count) – Log10 ($\chi$ time interval)

***Studies on the leakages of cytoplasmic constituents from the test bacteria by the methanol extract of P. amarus:***

***(a) Determination of the leakages of Potassium and Sodium ions:***

Leakages of potassium and sodium ions from the test bacteria were determined by the methods of[12]. Bacterial cultures (10 ml in nutrient broth) at the exponential growth stage (18 hr) were harvested by centrifugation at 7,000 rpm for 15 min. The cell pellets were re-suspended and washed twice in physiological saline by centrifugation. Each inoculum suspension was standardized to contain approximately $10^6$ CFU/ML as earlier described. One (1) ml of each cell suspension was treated with 9 ml extract (at their respective MBC). After 30, 60, 120, and 180 min of interaction between the cell suspensions and extract, each cell suspension

was centrifuged at 7,000 rpm for 15 min. The supernatant obtained was analyzed for potassium and sodium ions using a flame photometer. Triplicate readings were recorded for each supernatant.

***(b) Estimation of protein leaked from the selected test bacteria***

The method of[11] was also adopted for the determination of the quantity of protein leaked from the cell suspension of the test bacteria. The supernatants obtained from the interaction of washed cell suspension of the test bacteria with the extract at various time intervals were assayed for protein by the Bradford method[13]. Triplicate readings were recorded for each supernatant.

***(c) Estimation of leaked total nucleic acid from the selected test bacteria***

Supernatants obtained after the interaction of the washed cells with the extract at various time intervals were used to determine the total nucleic acid leaked out of the cells, spectrophotometrically at 260nm[14] (Akinpelu *et al*.,2008). Triplicate readings were recorded for each supernatant.

***Identification and Characterization of Bioactive components of the ethyl acetate sub-fraction from the methanol extract of P. amarus***

Four grams (4g) of the ethyl acetate fraction, which was the most active among the sub-fractions obtained from the crude methanolic extract of *P. amarus* in terms of diameter of zones of inhibition and minimum inhibitory concentration was further fractionated on a column (60 x 0.2 cm) packed with silica gel (70–230 mesh). The column was gradient eluted with the following solvent combinations: Hexane (100%), Hexane:Ethylacetate (1:1), Hexane: Ethylacetate (1:2), Hexane:Ethylacetate (2:1) and Ethylacetate (100%). The fraction eluents were collected in 10 ml aliquots, numbered, and the progress of the chromatographic separation monitored by thin-layer chromatography (TLC) using precoated silica gel TLC plates (Merck, silica gel 60 $F_{254}$). The plates were dried and visualized by spraying with 10% sulphuric acid and anisaldehyde in vanillic acid. Fractions showing similar TLC characteristics (no of spots, color and Rf values) were combined and concentrated using a rotary evaporator. The pooled fractions were further assessed for antibacterial activity by both agar diffusion (as earlier described by[4] and bioautographic methods as described by[15, 16] . Bioactive spots/ bands showing inhibition zones on the bioautogram were further purified using multiple development preparative thin-layer chromatography (PTLC x 5). After developing the plates, they were air-dried and

allowed to stand for 24 hours. The straight-line bands that developed on the plates were scraped off using a clean razor blade along with the sorbent into a conical flask. Ethyl acetate was added and the suspension obtained was left to stand for 30 minutes to facilitate leaching of the compound into the solvent and filtered. This process was repeated three times to ensure maximum recovery. The filtrate was left in an open crucible for the ethyl acetate to evaporate. The compounds recovered were scraped off and stored in a desiccator. The compounds obtained from the inhibition spots/ bands were further analyzed on a gas chromatogram(GC QP 2010, SHIMADZU) interfaced to a mass spectrometer (MS) instrument at the Centre for Energy Research and Training, Usman Dan Fodio University, Sokoto. The phytochemical compounds (mixture of compounds) present in the inhibition spots/ bands were identified from the Library Search Report of the GC-MS spectra. The identity of the components in the samples was by comparison of their retention indices and mass spectra fragmentation patterns with those stored in the database of the National Institute of Standard and Technology (NIST) library. The relative % amount of each component was calculated by comparing its average peak area to the total areas. The functional groups present in the obtained compounds were determined by Fourier Transform Infrared Spectrophotometer (FTIR-8400S) at the National Research Institute for Chemical Technology, Zaria.

## RESULTS AND DISCUSSION

***Rate of kill of the test bacteria by the methanolic extract of Phyllanthus amarus:***

The methanol extract of *P. amarus* demonstrated killing activity against the test bacteria within 30 mins of contact at the MIC concentration and after a contact time of 180 mins, the cell population reduction was almost 100% for all the test bacteria cell population as shown in Fig 1a. The kill pattern follows the same trend (Fig 1b) when the extract concentration was increased to 2x MIC against the test bacteria. However, at this concentration, all the test bacteria were killed after an exposure time of 120 mins. This indicated that the bactericidal activity of the extract is both concentration and time-dependent with time been more influential as earlier observed by[17]. The finding of the present study was also supported by the work of [18] who reported that the antimicrobial activity of methanol extract of *P. amarus* against *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* at 100mg/ml, was bactericidal.

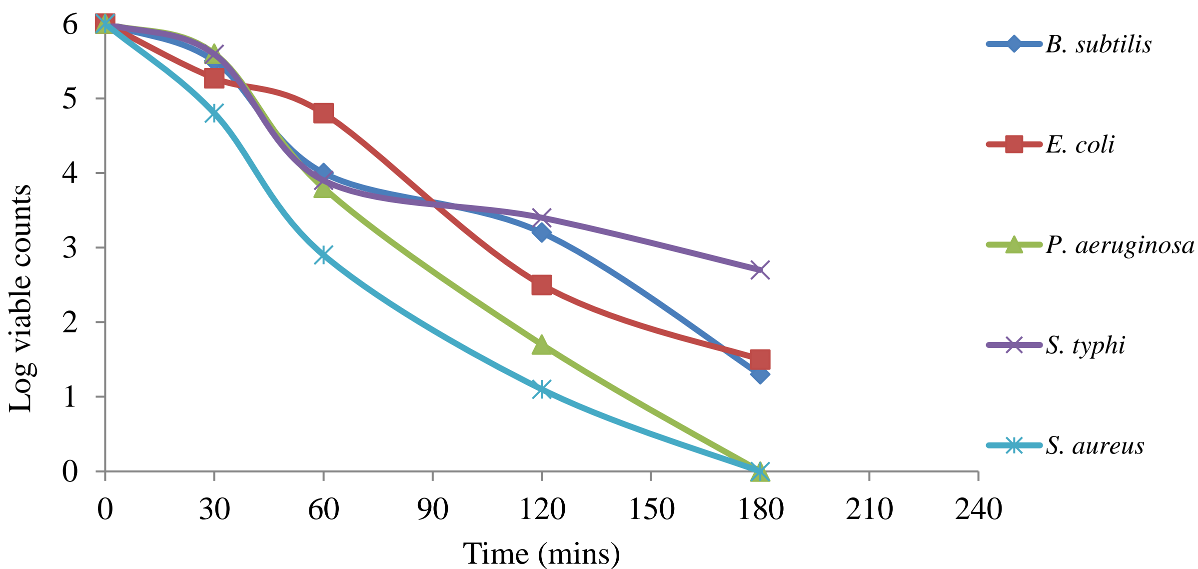



Fig 1a: Survival curve of test bacteria exposed to methanolic extract of *Phyllanthus amarus* against (at MIC)

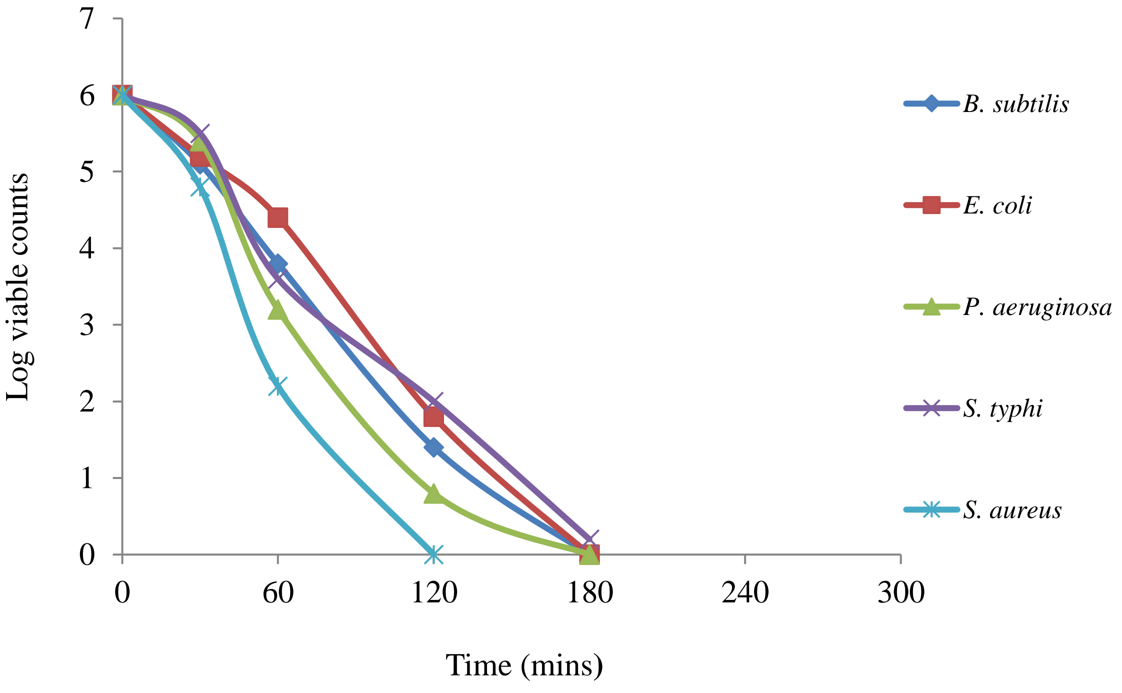



Fig 1b : Survival curve of test bacteria exposed to methanolic extract of *Phyllanthus amarus* against (at 2 X MIC).

***Effect of methanolic extract of P. amarus on the leakages of cytoplasmic materials:***

The methanol extract of *P. amarus* at the MBC concentrations for the test bacteria, induced progressive leakages of both

potassium ions, sodium ions, protein and nucleic acids respectively from the test bacteria cells with an increase in contact times. The concentrations of both potassium ion (K+) and sodium ion (Na+) leaked from the test bacteria cells is as shown in Figure 2a and 2b respectively.

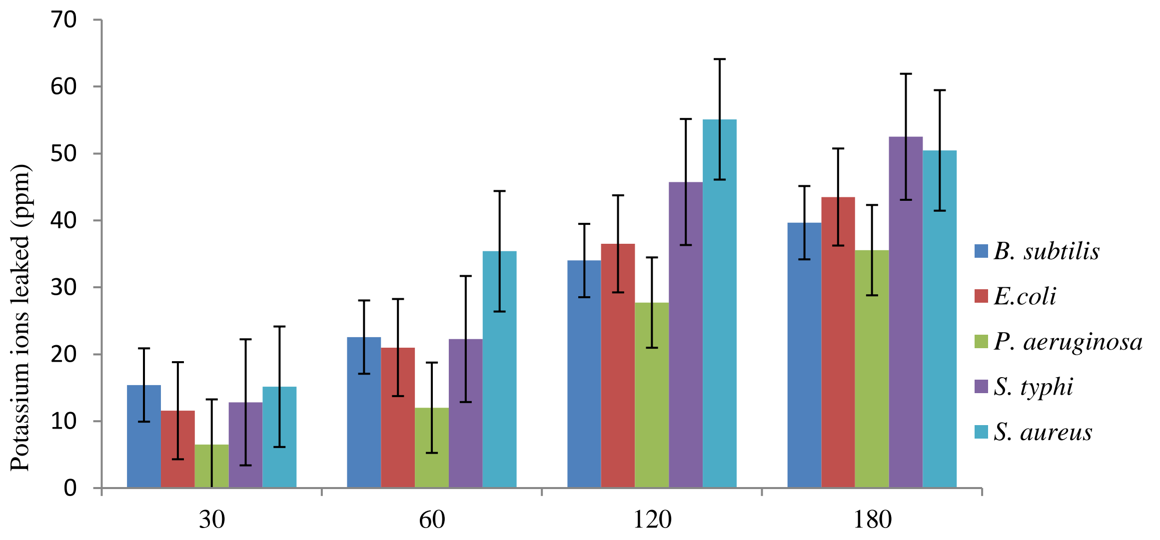



Fig 2a : Concentration of leaked potassium ions from the test bacteria by the methanolic extract of *P. amarus*

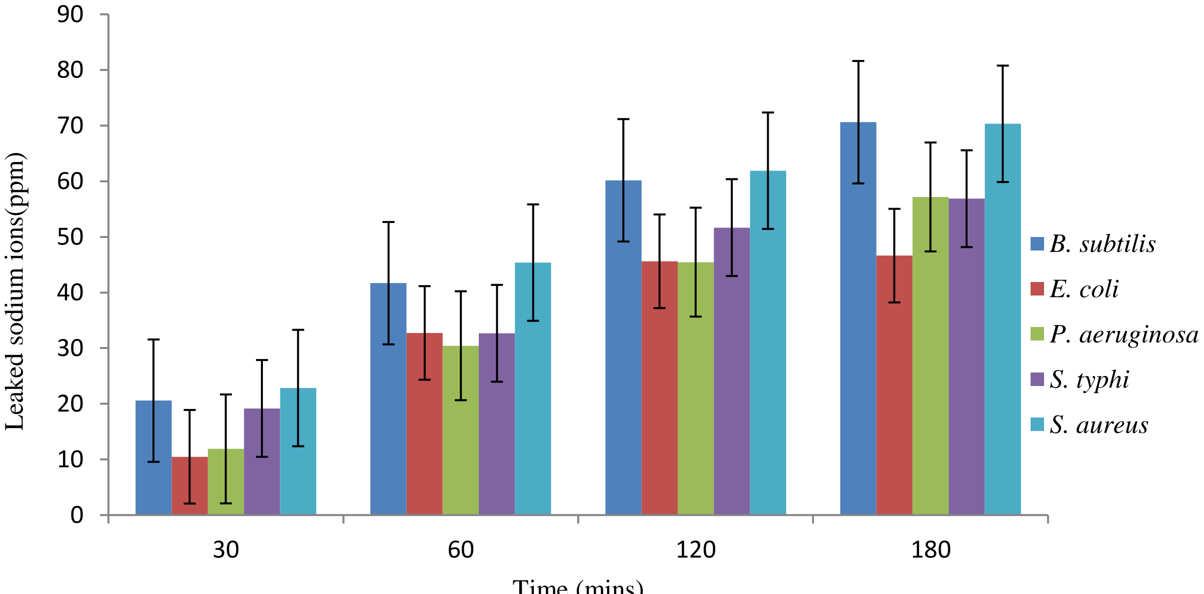



Fig 2b : Concentration of leaked sodium ions (Na+) from the test bacteria by the methanolic extract of *P. amarus.*

The concentration of sodium ions leaked from all the test bacteria was constantly higher than the potassium ion leaked. This may be attributed to the molecular mass of the ions, which might have resulted in a higher concentration of sodium that escaped from the cells than the potassium ions as earlier reported[19]. The result of this study is similar to that of[12] who earlier reported that sodium and potassium ions affect osmotic balances in the cell and their leakages might cause cell lyses and eventual death. The same pattern of leakage observed for potassium and sodium ions was also observed for the loss of protein from the test bacteria cells (**Fig 3**).

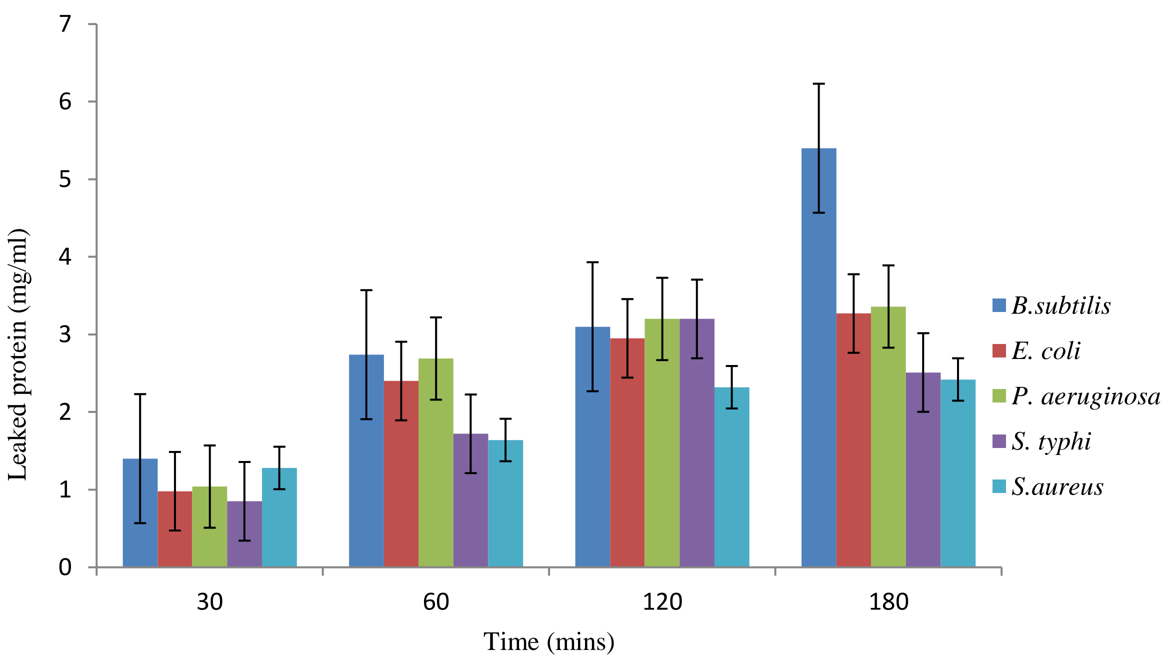



Fig 3 : Concentration of leaked Protein (mg/ml) from test bacteria by the methanolic extract of *P. amarus*

The concentration of protein leaked from *Salmonella typhi* declined from the peak value of 3.2 mg/ml after 120 mins to 2.5mg/ml after 180 mins. This decline may be due to the propensity of some of the phytochemicals such as phenols and tannins to complex with soluble protein molecules as earlier reported[11] who investigated the effect of methanolic extract of *Albizia zygia* on protein leakage from *B. Subtilis*. The results obtained in this study are also similar to those of[12,11,20] who all reported leakages of proteins from bacteria cells which include some of our test bacteria, induced by the

effect of various plant extracts on their cell membrane. Methanolic extract of *P. amarus* induced progressive leakage of 260 nm-absorbing materials, mainly nucleic acids from the cytoplasm of the test bacteria with time as shown in **Fig 4**

Our result is similar to those of[21] who reported that saponins present in *Cissus welwitschii* extract caused nucleic acid leakage in *Bacillus cereus* and *Escherichia coli* after exposure to the extract. Results obtained in this study indicated that methanolic extract of *P. amarus* induced leakages of cytoplasmic materials from the cells of the test bacteria which eventually led to the death of the bacteria cells as confirmed by the bactericidal activity of the extract. There is a fair correlation between the leakages of cellular materials from the test bacteria and their rate of kill pattern. The results of this study are similar to that of[22] who had earlier reported that the cytotoxic effect of methanol extract of *Phyllanthus* species (which include *P. amarus*) was due to the presence of phenols, phenolic acids and flavonoids that disrupted the cell membrane of cancerous cell.

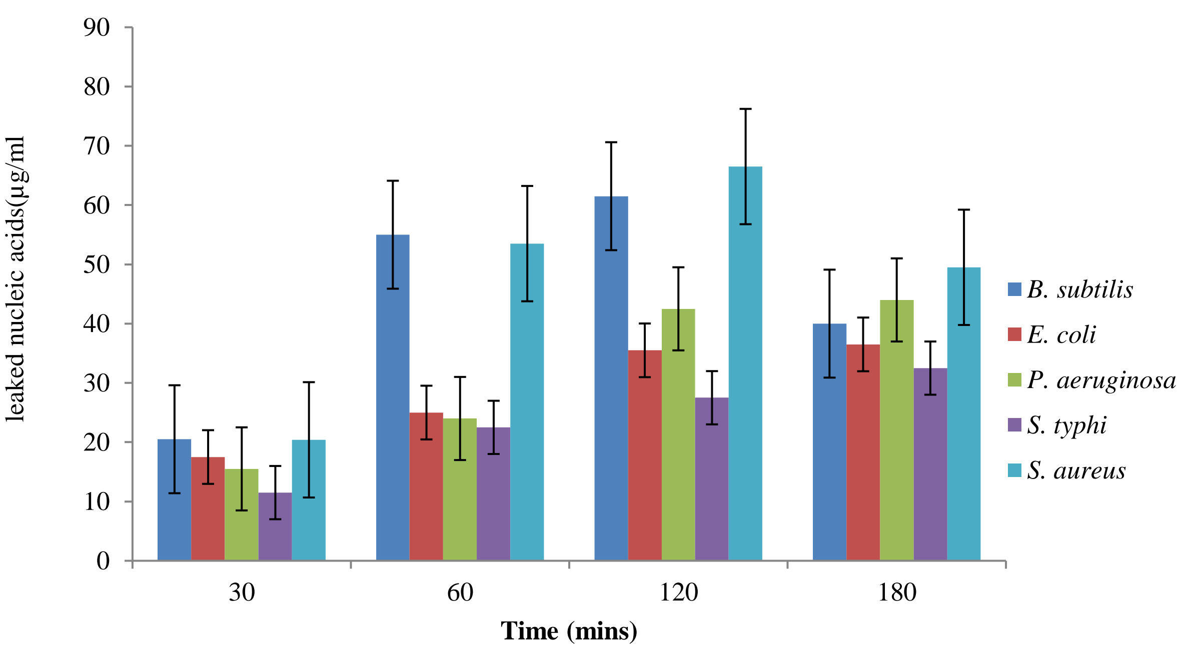



Fig 4 : Concentration of leaked nucleic acids(µg/ml) from test bacteria by the methanolic extract of *P. amarus*

***Column chromatographic studies of the ethyl acetate fraction from the methanolic extract of P.amarus.***

The result of the column fractions obtained from the column chromatographic separation of the ethyl acetate fraction is shown in **Table 1.** Column fractions showing the same TLC analysis (number of spots, color and Rf values) were pooled together to afford four major fractions (I, II, III and IV. Their antimicrobial activity against *S. typhi* and *S. aureus* by the agar-diffusion method is also shown in **Table 1.** Fraction I was found to be the most active and was also found to have similar antibacterial activity with the whole ethyl acetate fraction. The antibacterial activity of the remaining fractions was found to be less than the whole ethyl acetate.

**Table 1: TLC analysis and the antimicrobial activity(zone of inhibitions) of the pooled column fractions from the ethyl acetate fraction of the methanolic extract of *Phyllanthus amarus***

| Pooled Fractions (100µg/ml) | Solvent System | Rf values and the color reaction of major spots/bands | | | | | | Antimicrobial Activity (zones of inhibition in mm) | |
|---|---|---|---|---|---|---|---|---|---|
| | | 0.98 | 0.95 | 0.76 | 0.75 | 0.69 | 0.49 | *S. aureus* | *S. typhi* |
| I (17-19) | Hex:EtOAc (1:1) | Blue | Purple | - | Greenish | Pink | Purple | 23.0 ± 00 | 18.2 ± 0.0 |
| II (20-23) | (1:2) | Blue | Purple | Greenish | Greenish | - | - | 21.0 ± 0.5 | 17.0 ± 0.25 |
| III (34-38) | (2:1) | Blue | Purple | - | - | Pink | Purple | 11.7 ± 0.7 | 8.2 ± 0.7 |
| IV(48-76) | EtOAc (100%) | Blue | Purple | - | | - | - | 8.0 ± 0.0 | N/A |

N/A-No activity

***Bioautographic studies of the pooled fractions (I, II, III and IV) of the ethyl acetate fraction from the methanolic extract of P.amarus.***

The bioautography study of the pooled column fractions revealed clear zones of growth inhibition at the spots/bands with Rf values of 0.49, 0.75 and 0.95 which indicated antimicrobial activity against *S. typhi* and *S. aureus* as shown in **Plate I and II** respectively. However, the Rf values of the antibacterial zones extend over more than one Rf unit, which indicated that there may be overlapping of bioactive compounds in the fractions. These zones of inhibition indicated that the major antibacterial components are located at spots/bands with Rf values of 0.95, 0.75 and 0.49 in all the fractions.

***Identification of bioactive components from column fraction I of the Ethyl acetate fraction and antimicrobial activity of the isolated components or compounds.***

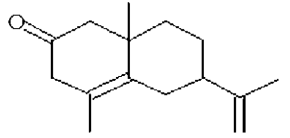
The result of the study revealed three different bioactive components (A, B and C) which have their nuclei at Rf values of 0.95, 0.75 and 0.49 respectively. Although each of the bioactive components showed a single spot on TLC, however, the GC-MS analysis of the bioactive components confirmed that they are a mixture of compounds (Table 2). The major phytochemical compounds identified by GC-MS analysis in bioactive component **A** were 1, 2-Benzenedicarboxilic acid mono (2-Ethylhexyl) ester (13.4%) and 7-chloro-4-methoxy-3-methyl quinoline (0.56 %). The infra-red spectroscopy spectrum of bioactive component **A** (Table 3) displayed characteristic bands corresponding to C-O-C bonds in esters between 1009.77 $cm^{-1}$ and 1246.06 $cm^{-1}$; to amine C-N bond at 1458.23; to CH stretch of aliphatic at peak 2956.7 and characteristic OH bonds of carboxylic acids at peak 3449.8. The antibacterial activity of bioactive component **A** could be partly attributed to the presence of these compounds. 1, 2-Benzenedicarboxilic acid was reported to have been isolated from *Ricinus cumminis* and shown to possess antibacterial activity against *Aeromonas hydrophila* and *Vibrio ordalli*[23]. The presence of this same compound in *Senna podocarpa* extract with antibacterial properties against *Bacillus subtilis, E. coli, Pseudomonas sp*., *Salmonella typhi* and *S. aureus* has also been repored[24]. 7-chloro-4-methoxy-3-methyl quinoline has earlier been reported[25] to have antibacterial activity against *B. subtilis*, E. coli and *S. aureus*.

The major phytochemical compounds identified in bioactive component **B** were Columbin (a diterpenoid sesquiterpene), 2-(6-Methylpyridin-2-ylmethyl) cyclohexanol (an alkaloid) and 2H Naphthol[2,1]pyran-4,7-dione,2-(3-furyl)-1,4a,5,6,6a,10,10a,10b-octahydro-6a,10b-dimethyl (a flavonoid), which comprises of about 42.53%, 25.13% and 13.45% of Component **B**

respectively. Other minor phytochemicals identified in the component include 2, 4-Cycloheptadien-1-one, 2, 6, 6-trimethyl, a eucarvone (2.70%) and 1, 2-Benzenedicarboxilic acid, mono (2-ethylhexyl) ester (1.14%).

**Table 2: Identified bioactive phytochemicals in the ethyl acetate fraction from the Methanolic extract *P.amarus*.**

| Bioactive Component | RT (mins) | % Area | Phyto-constituents | Structure |
|---|---|---|---|---|
| A | 25.47 | 0.56 | 7-chloro-4-methoxy-3-methyl quinoline (Alkaloid) | N, Cl, $H_3C$, O, $H_3C$ |
| | 32.25 | 13.37 | 1,2-Benzenedicarboxilic acid, mono(2-Ethylhexyl)ester (Wax) | O, OH, O, O |
| B | 26.83 | 2.70 | 2,4-Cycloheptadien-1-one,2,6,6-trimethyl (Eucarvone) | O |
| | 32.25 | 1.14 | 1,2-Benzenedicarboxilic acid, mono(2-Ethylhexyl)ester (Wax) | O, OH, O, O |
| | 34.08 | 42.53 | Columbin (Diterpenoid Sesquiterpene) | OH |
| | 35.31 | 25.13 | 2-(6-Methylpyridin-2-ylmethyl) cyclohexanol (Alkaloid) | H C, N, OH |
| | 35.76 | 13.45 | 2H Naphthol[2,1]pyran-4,7-dione,2-(3-furyl)-1,4a,5,6,6a,10,10a,10b-octahydro-6a,10b-dimethyl-(Flavonoid) | O-H |
| C | 32.25 | 7.95 | 1,2-Benzenedicarboxilic acid, mono(2-Ethylhexyl)ester (wax) | O, OH, O, O |

| | 43.68 | 77.78 | 2(1H)Naphthalenone,3,5,6,7,8,8a-hexahydro-4,8a-dimethyl-6-(1-methylethenyl)-<br>(Terpenoid) |  |
|---|---|---|---|---|

The FTIR analysis of Component **B** proved the presence of aromatic rings, alkenes, alcohols, ethers, carboxylic acids, esters, nitro compounds, hydrogen-bonded alcohols and phenols with major peaks at 1015, 1298, 1472, 1729, 2956, 3125 and 3503(**Table 3**) which is consistent with the presence of compounds such as alkaloids, flavonoids and terpenes observed in this component. The antimicrobial potential of columbin (possibly the bitter principle in *P. amarus*) has earlier been reported[26] against chloroquine-resistant *Plasmodium falciparum*. There was an earlier report[27] of columbin been one of the constituents of *Tinospora cordifolia* extract responsible for its antibacterial activity against dental pathogens such as *S. aureus* and *S. mutans*. The antimicrobial potential of 2-(6-Methylpyridin-2-ylmethyl) cyclohexanol has been mentioned in many of the Chinese integrated herbal medicine for treating genital warts[28]. GC-MS analysis of the antibacterial bioactive component **C** identified 2(1H) Naphthalenone,3,5,6,7,8,8a-hexahydro-4,8a-dimethyl-6-(1-methylphenyl) as the major component. The percentage peak area of the compound in the component was 77.78%. 1, 2-Benzenedicarboxilic acid was also identified in the component and comprised of 7.95%. The FTIR spectrum showed peak a small peak at 1008.8 $cm^{-1}$ (CH in the plane bend of aromatics), 1247.02 $cm^{1}$ (OH stretch in aromatic esters) and 1372.4 $cm^{-1}$ (CH bend as in methyl). A strong signal at 1734 $cm^{-1}$ confirmed the presence of C==O bonds usually found in aldehydes, ketones, carboxylic acids and esters. The signal at 2938.65 $cm^{-1}$ confirmed the presence of C==H bonds as in alkenes (**Table 3**). The antimicrobial and antioxidant properties of 2(1H) Naphthalenone,3,5,6,7,8,8a-hexahydro-4,8a-dimethyl-6-(1-methylphenyl) obtained from *Cordia retusa* has earlier been reported[29].

This same compound was also obtained from *Pergularia daemia*[30] and *Lactuca runcinata*[31] and was found to possess antibacterial, antitumor, anti-inflammatory, analgesic, fungicide and sedative properties. The presence of the identified compounds in the extract correlates with earlier reports of their single effective antibacterial activities which confirmed that the observed antibacterial activities seen in this study were due to their combined effects on the test bacteria.

**Table 3: FT-IR Data of bioactive components present in the ethyl acetate fraction of the methanolic extract of *P.amarus*.**

| Peak | Absorption Band | Motion | Functional group |
|---|---|---|---|
| **Bioactive Component A** | | | |
| 1 | 1009.77 | C—O—C stretch | Esters |
| 2 | 1246.06 | C—O—C stretch | Esters |
| 3 | 1458.23 | C—N stretch | Amines |
| 4 | 1734.06 | C═O stretch | Esters |
| 5 | 2936.72 | CH stretch | Aliphatic |
| 6 | 3449.80 | OH | Carboxylic acids |
| **Bioactive component B** | | | |
| Peak | Band | Motion | Functional group |
| 1 | 1015.56 | C—O—C stretch | Esters |
| 2 | 1298.14 | C—O—C stretch | Esters |
| 3 | 1472.70 | C—N stretch | Amines |
| 4 | 1729.24 | C═O stretch | Aldehydes, Esters, Carboxylic acids Ketones, |
| 5 | 2956.97 | CH stretch | Aliphatic |
| 6 | 3125.75 | OH | Carboxylic acids |
| 7 | 3503.81 | N—H stretch | Amines |
| **Bioactive component C** | | | |
| Peak | Band | Motion | Functional group |
| 1 | 1008.80 | C—O—C stretch | Esters |

| 2 | 1247.02 | C—O—C stretch | Esters |
|---|---|---|---|
| 3 | 1459.20 | C—N stretch | Amines |
| 4 | 1734.06 | C=O stretch | Aldehydes, Ketones, Carboxylic acids & Esters |
| 5 | 2938.65 | C—H stretch | Aliphatic |

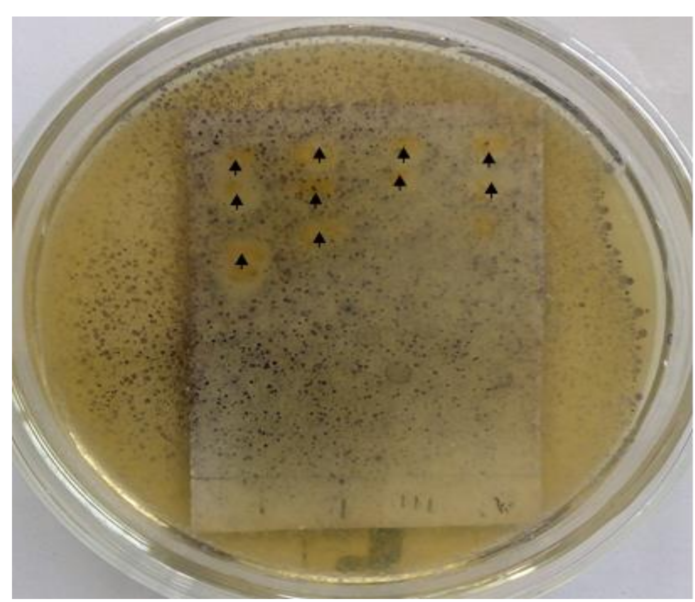


**Plate I: Bioautogram of the pooled column chromatography fractions from the ethyl acetate fraction of the methanolic extract of *P. amarus* against *S. typhi***

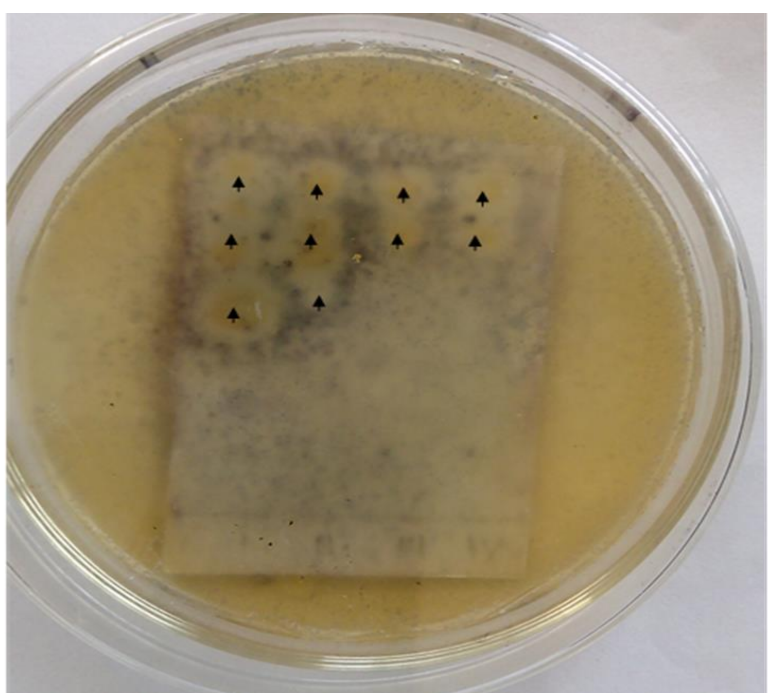


**Plate II: Bioautogram of the pooled column chromatography fractions from the ethyl acetate fraction of the methanolic extract of *P. amarus* against *S. aureus***

## CONCLUSION

The methanolic extract of *P. amarus* is bactericidal to all the test bacteria within three hours of interaction at their respective MBC concentration. The methanolic of *P. amarus* was found to cause leakage of cellular materials and therefore, disruption of the cell membrane is a probable mechanism of action of the extract on the test bacteria. The ethyl acetate sub-fraction from the methanolic extract of *P. amarus* revealed the presence of phytochemicals such as 1, 2-Benzenedicarboxilic acid mono (2-Ethylhexyl) ester, Columbin, 2-(6-Methylpyridin-2-ylmethyl) cyclohexane, 2(1H) Naphthalenone,3,5,6,7,8,8a-hexahydro-4,8a-dimethyl-6-(1-methylphenyl) which may be partly responsible for the antibacterial activity of the extract. The result of this study will contribute to the baseline data on the pharmacodynamics of the methanolic extract of *P. amarus* if applied as herbal medicine for the treatment of human bacterial infections, thereby reducing the dosage and period of treatment. The results of our investigation contributed to the understanding of the mechanism of bacterial inhibition by the methanol extract of *P. amarus*. The findings of this study also showed that the ethyl acetate sub-fraction of methanol extract of *P. amarus* presents new additions of phytochemicals such as 1, 2-Benzenedicarboxilic acid, Columbin and Naphthalenone (which to the

best of our literature search has not been reported from this plant) that may be used to develop more potent, safe and cheap antimicrobial agents using nanotechnology.

## REFERENCES


1. Aparecida M. M. M., Conceição dos Anjos, G., Revoredo, M. S., Padilha D. M. M. and Ramalho, H.M.M.. Annals of Chemical Science Research, 1(2) (2019)ACSR.000510.

2. Devi S., Rashid R., Kumar M. The Pharma Innovation Journal, 6(12) (2017) 169.

3. Oluboyo B. O., Oluboyo A. O, Kalu S. O. African Journal of Clinical and Experimental Microbiology, 17 (3) (2016)166

4. Alli A. I., Ehinmidu J.O. and Ibrahim Y.K.E. Nigerian Journal of Chemical Research,16, (2011)1.

5. Nwankpa P., Agomuo E. N., Uloneme, G. C., Egwurugwu, J. N., Omeh, Y. N. and Nwakwuo G. C. Scientific Research and Essays, 9(1) (2014) 7.

6. Osho I.B., Oyekanmi B.A., Adetuyi F.C. and Oladele A.A. International Journal of Molecular Veterinary Research, 5(3) (2016). 1

7. Ajala T.O., Igwilo, C.I., Oreagba, I.A., Odeku, O.A. Asian Pacific Journal of Tropical Medicine,4 (2011) 283.

8. Alozieuwa U. B., Mann A., Kabiru A. Y. and Ogbadoyi E. O. European Journal of Medicinal Plants, 24(3) (2018)1.

9. Harborne, J. B. Phytochemical methods guide to modern Technique of Plant analysis. 3rd (Ed), 21-72, Chapman and Hall, London, 1998.

10. Trease, G, Evans, W. A Textbook of Pharmacognosy, 5th edition, 603pp, Elsevier Ltd, Edinburgh, 2002.

11. Odeyemi O., Oluduro, A. O., & David, O. M. Journal of Natural Sciences Research,4(19) (2014) 98.

12. Akinyemi A. I., & Ogundare, A. O. European Journal of Medicinal Plants, 4(1) (2014). 75.

13. Bradford M.M. Analytical Biochemistry, 72 (1976) 248

14. Akinpelu D.A., Aiyegoro, O.A. & Okoh, A. African Journal of Biotechnology, 7 (2008) 3665.

15. Udobi C. E., Onaolapo, A. J. and Abdulsalaam I. A. Journal of Pharmacognosy and Phytotherapy, 2(8) (2010) 108.

16. Choma I. & Grzelak, E. M. Journal of Chromatography, A1218 (2015) 2684.

17. Ojo S. K. S., Ejims-Enukwe, O. & Esumeh, F. I. International Journal of Pharmaceutical Science Invention, 2(8) (2013) 9

18. Bharathi, T., Kolanjinathan, K. & Saranraj, P. Global Journal of Pharmacology, 8(3) (2014) 294

19. Ryan K. and Sherris R. C. Medical Microbiology: An Introduction to Infectious Diseases, 4th edition, 992pp, McGraw Hill, 2004

20. Akinpelu D. A., Alayande, K. A., Aiyegoro, O. A., Akinpelu, O. F., & Okoh, A. I. BMC Complementary and Alternative Medicine, 15(116) (2015) 1.

21. Moyo B. & Mukanganyama, S. International Journal of Bacteriology, 10.1155 (2015), 162028.

22. Tang Q., Kang, A. & Lu, C. International Journal of Pharmacology, 12 (2010) 116.

23. Sani U. M. & Pateh, U. U. Nigerian Journal of Pharmaceutical Science, 8(2) (2009) 107.

24. Adebayo M. A., Lawal, O. A., Sikiru, A. A., Ogunwande, I. A. & Avoseh, O. N. American Journal of Plant Sciences, 5 (2014) 2448.

25. Kharb, R. & Kaur, H. International Research Journal of Pharmacy, 4(3) (2013) 63

26. Nok A. J., Sallau, B. A., Onyike, E. & Useh, N. M. Journal of Enzyme Inhibition and Medicinal Chemistry, 20(4) (2005) 365.

27. Dwivedi S.K. and Enespa, A. International Journal of Current Microbiology and Applied Sciences, 5(6) (2016) 446.

28. Hair, L. T. New Chinese, 29 (8) (1997) 37.

29. Amudha M. and Rani, S. Journal of Drugs and Medicines, 6 (1) (2014) 12.

30. Vaithiyanathan V. & Mirunalini, S. Chiov Journal of Biomedical Research, 29 (2015)12.

31. Davie J. A. I. & Muthu, A. K. Asian Journal of Pharmaceutical and Clinical Research, 8(1) (2015) 202.