ANTIBACTERIAL ACTIVITY OF *PHYLLANTHUS AMARUS* (CHAMBER BITTERS) WHOLE PLANT EXTRACT AGAINST *SALMONELLA SPECIES*

BY

BABA, Aishatu Muhammad
MTech/SLS/2017/7367

DEPARTMENT OF MICROBIOLOGY
SCHOOL OF LIFE SCIENCES
FEDERAL UNIVERSITY OF TECHNOLOGY, MINNA

AUGUST, 2021

ANTIBACTERIAL ACTIVITY OF *PHYLLANTHUS AMARUS* (CHAMBER BITTERS) WHOLE PLANT EXTRACT AGAINST *SALMONELLA SPECIES*

BY

BABA, Aishatu Muhammad
MTech/SLS/2017/7367

A THESIS SUBMITTED TO THE POST GRADUATE SCHOOL, FEDERAL UNIVERSITY OF TECHNOLOGY, MINNA, IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTER OF TECHNOLOGY (MTech) IN MICROBIOLOGY (MEDICAL MICROBIOLOGY)

AUGUST, 2021

ABSTRACT

The antibacterial activity of *Phyllanthus amarus* (chamber bitters) whole plant against *Salmonella typhi and Salmonella paratyphi B* isolated from stool samples was investigated using agar diffusion techniques. Phytochemical analysis of the crude extract of *Phyllanthus amarus* revealed the presence of alkaloids (1.54 %), flavonoids (5.12 %), phenol (9.50 %), tannins (16.02 %) and saponins (67.81 %). The zone of inhibition ranged from 6 mm to 22 mm at 300 mg/mL to 1000 mg/mL. The methanolic extract was more effective than the extract obtained using n-Hexane, Ethyl-acetate and Acetone solvents. The minimum inhibitory and bactericidal concentrations of the methanolic crude extract of *P. amarus* whole plant against *Salmonella typhi* were 7.8 mg/mL and 15.6 mg/mL, compared to 9.3 mg/mL and 18.7 mg/mL for *Salmonella paratyphi* B. Therefore, *Salmonella typhi* was significantly ($P<0.05$) more susceptible than *Salmonella paratyphi* B. The histopathology studies show no toxic effect of *P. amarus* used in the study. The presence of Tannin and Saponin at appreciable percentage when compared to other secondary metabolites found in the crude extract suggests that *Phyllanthus amarus* whole plant contains active ingredients that could be used for the development of antibacterial drugs for the treatment of diseases associated with *Salmonella* infections.

TABLE OF CONTENTS

Contents		Page
Cover Page		
Title Page		i
Declaration		ii
Certification		iii
Dedication		iv
Acknowledgements		v
Abstract		vii
Table of contents		viii
List of Tables		xiii
List of Figures		xiv
List of Plates		xv
List of Appendices		xvi
CHAPTER ONE		
1.0	**INTRODUCTION**	1
1.1	Background to the study	1
1.2	Statement of the research problem	2
1.3	Justification for the study	2
1.4	Aim and Objectives of the study	3
CHAPTER TWO		
2.0	**LITERATURE REVIEW**	4
2.1	The Biology of *Phyllanthus amarus*	4
2.2	Phytochemical constituents of *phyllanthus amarus*	5

2.2.1 Flavonoids; an important phytochemical constituent. 5
2.2.2 Alkanoids; an important phytochemical constituent. 6
2.2.3 Saponin; an important phytochemical constituent. 7
2.2.4 Tannins; an important phytochemical constituent. 7
2.2.5 Terpenoids; an important phytochemical constituent. 8
2.2.6 Steroids; an important phytochemical constituent. 8
2.3 Pharmacognosis of Phyllanthus amarus 8
2.3.1 Anti-viral Activity 8
2.3.2 Anti-diabetic Activity 9
2.3.3 Anticancer Activity 10
2.3.4 Anti-oxidative Activity 10
2.3.5 Antimicrobial activity 11
2.4 Salmonella Bacterium 12
2.4.1 Antigenic Structure 13
2.4.2 Pathogenesis Salmonnella infection 13
2.4.3 Epidemiology Salmonnella infection 14
2.4.4 Symptoms Salmonnella infection 14
2.5 Diagnosis Salmonnella infection 15

CHAPTER THREE

3.0 **MATERIALS AND METHODS** 16
3.1 Study area 16
3.2 Collection and Identification of the Plant 18
3.3 Preparation of the Extract (crude extract) 18
3.4 Qualitative Phytochemicals Screening 19
3.4.1 Test for flavonoids 19

3.4.2 Test for alkaloids 19
3.4.3 Test for saponins 19
3.4.4 Test for tannins 20
3.4.5 Test for phenols 20
3.4.6 Test for terpernoid 20
3.4.7 Test for glycosides 20
3.4.8 Test for steroids 21
3.4.9. Test for reducing sugars 21
3.5 Quantitative Determination of Chemical Constituents 21
3.5.1 Alkaloid determination 21
3.5.2 Tannin determination 21
3.5.3 Saponins determination 22
3.5.4 Phenol determination 22
3.5.5 Flavonoid Determination 23
3.6 Bacteria Used in Study 23
3.6.1 Confirmation of the identity of the bacteria used 23
3.6.2 Identification of test organs 23
3.7 Gram's Stanning of the Test Organisms 24
3.8 Biochemical Test 24
3.8.1 Catalase test (Slide or Drop) method 24
3.8.2 Indole formation (Kovac's Method) 25
3.8.3 Voges Proskauer (VP) and Methyl Red (Mr) 25
3.8.4 Motility test (Semi-solid method) 25
3.8.5 Motility test (Semi-solid method) 25
3.8.6 Hydrogen sulphide production 26
3.8.7 Triple iron sugar 26

3.9 Culture Media Preparation 26

3.10 Screening of Plant Extract for Antimicrobial Activity 27

3.11 Determination of Minimum Inhibitory Concentration (MIC) 27

3.12 Experimental Animals 28

3.12.1 Inoculation of *Salmonella* species and administration of plant extract to wister rats 28

3.12.2 Toxicity effect 28

3.12.3 Acute oral toxicity assay in mice (Limit test OECD 401) 28

3.12.4 Sub-acute toxicity testing 28

3.12.5 Mortality and survival rate under observation 29

3.12.6 Haematological investigations 30

3.12.7 Bio-chemical investigations 30

3.12.8 Histopathology investigations 31

3.13 Data Anaylsis 31

CHAPTER FOUR

4.0 **RESULTS AND DISSCUSSION** 32

4.1 Results 32

4. 1.1 Qualitative and quantitative analysis of *Phyllanthus amarus* whole plant 32

4.1.2 Histology of the liver of Wistar rats administered with methanol extract of *P. amarus* 35

4.1.3. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of crude *Phyllanthus amarus* methanolic whole plant against *Salmonella* species. 36

4.1.4 Activity of *Phyllanthus amarus* whole plant extracted using different solvent against *Salmonella* typhi and *Salmonella paratyphi* B. 37

4.1.5 Toxicity studies 38

4.1.6 Activity of sub-acute oral administration of *Phyllanthus amarus* extract on bodyweight variation in rats. 39

4.1.7 Activity of sub-acute oral administration of *Phyllanthus amarus* whole plant *extract* on hematological parameters in Wister rats. 40

4.1.8 Effect of sub-acute oral administration of Methanolic *Phyllanthus amarus* whole plant on lipid profile. 41

4.1.9 Effect of sub-acute oral administration *of* methanolic extract of *P. amarus* whole plant on Liver function. 42

4.1.10 Histology of the liver of wistar rats administered with methanolic extract of *P. amarus* whole plant. 43

4.1.11 Effect of Sub-acute oral administration of P. amarus whole plant methanolic extract on kidney enzymes in rats. 44

4.1.12 Effect of sub-acute oral administration of methanolic extract of *P. amarus* whole plant on serum electrolytes in rats. 45

4.1.13 Histology of the wister rat kidney after 21 days administration of methanol extract of P. amarus. 46

4.2 Discussions 47

CHAPTER FIVE

5.0 **CONCLUSION AND RECOMMENDATIONS** 53

5.1 Conclusion 53

5.2 Recommendations 53

REFERENCES 54

APPENDICES 62

LIST OF TABLES

Table		Page
4.1	Quantitative and analysis of Phytochemicals of *Phyllanthus amarus* wholeplant	32
4.1	Qualitative analysis of *Phyllanthus amarus* whole plant.	33
4.2	Quantitative analysis of *Phyllanthus amarus* whole plant.	34
4.4	Antibacterial effect of *Phyllanthus amarus* methanolic whole plant extract against *Salmonella* species	35
4.5	Minimum inhibitory concentration (MIC) of crude *Phyllanthus amarus* against *Salmonella* species.	36
4.6	Minimum bactericidal concentration (MBC) of crude *Phyllanthus amarus* against *Salmonella* species.	36
4.7	Activity of *Phyllanthus amarus* whole plant extracted using different solvent against *Salmonella* species.	37
4.9	Sub-acute toxicity test (OECD 407)	38
4.8	Acute oral toxicity test (Limit test OECD 401)	38
4.10	Activity of sub-acute oral administration of *Phyllanthus amarus* extract on bodyweight variation in rats.	39
4.11	Activity of sub-acute oral administration of *Phyllanthus amarus* extract on Hematological parameters in Wister rats	40
4.12	Effect of sub-acute oral administration of Methanolic *Phyllanthus amarus* whole plant on lipid profile.	41
4.13	Effect of sub-acute oral administration of methanolic extract of *P. amarus* whole plant on Liver Enzymes.	42
4.14	Effect of sub-acute oral administration of *P. amarus* whole plant methanolic extract on kidney enzymes in rats	44
4.15	Effect of sub-acute Oral Administration of Methanolic extract of *P. amarus* whole plant on serum electrolytes in rats.	45

LIST OF FIGURES

Figures		**Page**
4.1	Map of Niger State Showing the study Area.	17
4.2	*Phyllanthus amarus* whole plant in the field of University Health Services	62

LIST OF PLATES

Plate		Page
I.	Micro graph of liver of normal control of wister rats(A) and liver of rat fed with methanolic extract of *P amarus* whole plant at low dosage 10 mg/kg/broth (B). The moderate dosage of 300mg/kg/broth (C) and higher dosage of 600mg/kg/broth.	43
II.	Micrograph of kidneys of rats treated with methanolic P. amarus whole plant extract at low dosage, moderate dose higher dose and control	46

List of Appendices

Appendix		Page
A	*Phyllanthus amarus*	62
B	Phytochemicals present in *P. Amarus* whole plant using dfferent Solvents	63
C	Anti-bacterial activity of P. *amarus* against *salmonella* species (*salmonella typhi and salmonella paratyphi* B)	66
D	Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC) of *P. amarus* against *Salmonella typhi* and *Salmonella paratyphi* B	69
E	Haematology of wister rats fed with different extract concentration	71
F	Biochemical parameters of liver and kidney functions of wister rats fed with different extract concentrations	72

CHAPTER ONE

1.0 INTRODUCTION.

1.1 Background to the Study

Phyllanthus amarus belongs to the Euphorbiaceae family and is a member of the *Phyllanthus* genus. The plant originated from America (Danladi *et al,* 2018) and is mostly found as a weed (Senjbi *et al*., 2017). It can also be found in Europe, Asia and Africa (Gupta and Vaghela, 2019). In Nigeria, the plant is a perennial herb that reaches a height of 15-38 cm However, some may grow up to the height of 30-60 cm. The plant has angular stem and numerous distichous, elliptic oblong leaves and flowers attached (Danladi *et al*., 2018). It is known among the Hausas as "Geron tsuntsaye", Yoruba as "Eyin olobe", Igbo as "Ite kwonwa nazu" and in English as chamber bitters (Umoh *et al*., 2013).

Phyllanthus amarus is traditionally used in the management of gastro-intestinal disorder, colitis, constipation, dysentery, diarrhea, dropsy, jaundice, kidney problems, urinary tract infection, bronchial infections, appendix inflammation, prostate problems, skin ulcers, swelling, scabies, wound, leucorrhoea, menorrhagia and mammary abscess (Oduola *et al.*, 2018). Therefore, *Phyllanthus amarus* extract is antifungal, antiviral, hepato-protective, anti-diabetic, antihypertensive, anti-inflammatory and antibacterial (Islam *et al.,* 2018). About 80 % of the world's population is reliant on herbal medicine for the treatment of microbial infections, prevention and maintaining well-being (Alebiosu and Yusuf, 2015). Bacterial infections such as Salmonellosis is the most common infection among the world's population with high incidence in developing countries due to poverty and poor sanitary conditions (Andoh *et al*., 2017). Salmonellosis is a bacterial infection which is caused by a bacterium called *Salmonella* species; they are Gram negative, rod shaped bacteria (bacillus) belonging to

the *Enterobacteriaceae* family with two known species, *Salmonella enterica* and *Salmonella bongori* (Ifeanyichukwu *et al*., 2016). The transmission is mostly through food and water, poor sanitation (Nwabor *et al*., 2015).

1.2 Statement of the Research Problem.

Salmonella infection is one of the most serious public health issues of the twenty-first century, with 1.3 billion cases and 3 million deaths worldwide. Non-typhoidal *Salmonella* occur world-wide with high incidence in developed countries while typhoidal is endemic in Africa and accounts for millions deaths annually (Nwabor *et al*., 2015). The typhoidal disease remains endemic in Nigeria and it is responsible for over 18.6% hospital cases annually (Akinyemi *et al*., 2018). The burden of the disease cannot be neglected as the emergence and widespread distribution of drug resistant *Salmonella Species* have imposed serious limitations on successful antibiotic treatment (Bythwood *et al*., 2019). This has led to the loss of many lives attributed to the disease and necessitated the search for therapeutic agents from plant source that are effective, cheap and less toxic.

1.3 Justification for the Study

Different studies by Mzungu *et al*. (2016), Akinyemi *et al*. (2018) and Bythwood *et al.* (2019) had outlined the invasive nature of *Salmonella* infections as one of the leading causes of hospital visits in Nigeria posing a lot of threat to life and economy. Medicinal plants are regarded as safe, non-additive and free of side effects. Therefore, *Phyllanthus amarus* seems to be one of the promising herbs as it has diverse activity on different pathogens (Adegoke *et al.*, 2010). It is in view of this awareness that the research aims to determine the antibacterial properties of *Phyllanthus amarus* whole plant against *Salmonella typhi and Salmonella paratyphi B.*

1.4 Aim and Objectives of the Study

This study was aim at determining the antibacterial activity of *Phyllanthus amarus* extract against *Salmonella typhi* and *Salmonella paratyphi B*. The specific objectives were to:

i. determine the *Phyllanthus amarus whole plant* extract phytochemical constituents.
ii. determine the antibacterial activity of *Phyllanthus amarus* crude extract obtained from various solvents.
iii. investigate minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC) of *Phyllanthus amarus* extracts.
iv. use Wister rats to determine the acute and sub-acute toxicity of the plant extract.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 The Biology of *Phyllanthus amarus.*

Plants have been used as traditional medicines for centuries to treat and prevent illnesses from various infectious diseases or maintain well-being of humans due to the presence of bioactive secondary metabolite embedded in them by nature (Oluwafemi and Dabiri, 2008). Ethno-medicines are used by 80 % of the world's population (Alebiosu and Yusuf 2015). Among the plants of this nature is the family *Euphobiaceae* that consists of about 300 genera including *Phyllanthus* with 800 species (Verma *et al.*, 2014). Most members of the *Euphorbiaceae* family are trees or shrubs and few are herbs growing in many tropical regions. The flowers are unisexual and regular with distichous leaves, the stamens are numerous and are either free or fused (Alebiosu and Yusuf, 2015). The taxonomy of the *Phyllanthus amarus* as disclosed from the studies show Kingdom: Plantae, Division: Angiospermae, Class: Dicotyledoneae, Order: Tubiflorae, Family: euphorbiaceae, Genus: Phyllanthus, and Species: amarus. (Aparecida, 2019),

Phyllanthus amarus is a medicinal plant that is also known as "chamber bitters" or "leaf and flower" because the flower and the fruit that seem attached with the leaf (Umoh *et al*., 2013). In Nigeria, the plant is commonly known as "geron tsutsaye", "eyin olobe", and "Ite kwonwa nazu" among the Hausa, Yoruba and Igbo, respectively. The plant is also found in Europe, Asia, Central and South America and Africa, including Nigeria (Gupta and Vaghela, 2019). The plant is a common weed in Nigeria and it has spread widely through cultivated fields (Senjobi *et al*., 2017).

Phyllanthus amarus as a member of this family is widely employed in folk medicine and closely related to both *Phyllanthus urinaria* and *Phyllanthus niruri* in appearance, structure and constituents (Umoh *et al.*, 2013). *Phyllanthus amarus* possess tiny greenish leaves, stems and fruits and *P. niruri* have larger red leaves and green stems while *P. urinaria* have warlike fruits and bigger than *P. amarus*' plant (Umoh *et al*., 2013). It is an annual herb that grows to be 15 to 38 cm tall as weed in most wasteland. However, some may grow to the height of about 40 - 50 cm with an angular stem and numerous distichous, elliptic oblong leaves and flowers attached (Danladi *et al.*, 2018; Maciel *et al.*, 2019).

2.2 Phytochemicals Constituents present in *Phyllanthus amarus*

Phytochemicals are naturally occurring chemical compounds of specific biological functions that are in medicinal plants (leaves, stem, and roots), responsible for the defense mechanism (Altemimi *et al.,* 2017), or in repulsion to the challenges of the environment. These non-nutritive chemicals have protective properties against bacteria, viruses, parasites and other autoimmune disorders in humans (Ahmad *et al*., 2016). Alkaloids, Saponins, glycosides, tannins, steroids, flavonoids and carbohydrates are examples of secondary metabolites present in *P. amarus* plant (Umoh *et al*., 2013). These phytochemicals have diverse usage in traditional medicine and poisons. Below are some of the constituent phytochemicals in *Phyllanthus amarus.*

2.2.1 Flavonoids; an important phytochemical constituent.

Flavonoids are secondary metabolites in most angiosperm family of phenolic structures with low-molecular-weight. They are commonly distributed in the plant kingdom and are easily recognized as flower pigments (Panche *et al*., 2016). They have antioxidant characteristics linked to cancer, Alzheimer's disease and atherosclerosis among other diseases and hence

promote health. Flavonoids are classified into subgroups based on the position and composition of the carbon ring as its connected to B ring, the degree of unsaturation and oxidation of the ring (Santos *et al.*,2019). Flavonoids are essential component in pharmaceutical industries due to their diverse medicinal and enzymatic functions (Panche *et al.*, 2016).

2.2.2 Alkaloids; an important phytochemical constituent.

Alkaloids are most abundant natural products of plant containing a wide range of chemical constituents (Fett- Neto and Matsuura, 2016). They are low molecular weight nitrogen containing compound. Many radicals (oxygen, morphine, nicotine, quinine, atropine, ephedrine, colchicine and strychnine) are used to replace the hydrogen atom in a peptide ring, which is synthesized from amino acid blocks (Bribi, 2019). Alkaloids are basic in nature, turning red litmus paper blue depending on the structure and location of functional group of the molecule. Most alkanoids are classified into numbers of modes -Biosynthesis, chemical, Pharmacological taxonomy(Kaur *et al.*, 2017) True alkaloids, protoalkaloids and pseudo-alkaloids such are the major types of alkaloids. Important alkaloids are usually present in herbaceous plants and some woody plants (Kaur *et al*., 2017). Plant alkaloids are good sources of foods and have potent pharmaceutical effect for humans (Ahmad *et al.,* 2016) while it protects the plant against animal attack, helps in protein synthesis, as well as in regulation of hormone of metabolism and reproduction.(Fett-Netto and Matsuura 2016).

2.2.3 Saponin; an important phytochemical constituent.

Saponins are bioorganic compounds that contains at least one glysodic linkage (C-O- Sugar bond) at C-3 of sugar chain and aglycone (El-Aziz *et al.*, 2019) with high molecular weight glycosides in complex structure, widely produced by plants and some certain marine

organisms. They have acrid taste and causes irritation to mucus membrane because of their surfactant properties (Vincken *et al.,* 2007), soluble in alcohol and water and insoluble in non-polar organic solvents due to its amorphous nature (Faizal and Geelen, 2013). The sapogenin chemical composition structure defies classification as treterpenoid saponins (30 carbon atoms) which are found primarily in the magnoliopsida class and steroid saonin (27 atoms) with a 6 ringspirostane or 5-ring furostane skeleton. (Faizal and Geelen 2013). Saponin are categorized into 12 according to the carbon skeleton of the aglycon, saponins are sometimes further classified into 12 main groups based on their carbon skeleton; dammarenes, tiricallenas, lupanes, hopanes, oleananes, 2 norolean an estaraxasteranes, ursanes, cycloartanes, anostanes cucurbitanes and steroids (Vincken *et al*., 2007). Saponin are commonly used in the food industry and pharmaceutical purposes (Ustundang and Mazza, 2007).

2.2.4 Tannins; an important phytochemical constituent.

Tannins are water-soluble polyphenols found in the barks and leaves plants (Pizzi 2019). They are large-molecular compounds that form complexes with carbohydrates, alkaloids and gelatins (Khanbabee and Ree, 2001). It is properties are dependent of the phenol ring which has three or two hydroxyl groups. Tannins are divided into two; hydrolysable tannin and Condensed tannin (Singh and Kumar, 2019). Tannins are used in industries as antiseptics, metal chelates, while its biological activities include scavenging, apoptosis, anti-tumor, antiseptic, anti-inflammatory and anti- plasmin (Okuda and Ito, 2011).

2.2.5 Terpenoids; an important phytochemical constituent.

Terpenoid are terpenes that have had different functional groups transferred or removed at different positions (De las Heras *et al.,* 20.03). They are classified based on (C5) units; mono-

terpenes, sesquiterpenes, diterpenes, triterpenes tetraterpenes and polyterpenes are highly used in industries as biofuels, fragrances; medicine in cancer treatment, as drugs, and in agriculture as in food due to its diversity(De las Heras *et al*., 20.03). Terpenoids have a number of essential pharmacological properties, including anti-inflammation, anticancer, antimalarial, antiviral, antibacterial and cholesterol synthesis. Terpenoids exhibit many important pharmacological activities like, anti-bacterial, anticancer, inhibition of cholesterol synthesis, anti-malarial, anti-viral and anti-inflammation activities (Grassmann, 2005).

2.2.6 Steroids; an important phytochemical constituent.

Steroids are organic compounds with four rings arranged in a particular molecular structure that are biologically active as in amarasterol A and Amarasterol –B (Ahmad and Alam, 2003). The two types of steroids are Corticosteroids and anabolic referred to as cardiac glycosides and are mostly used as poisons. Steroids are known to help in the retention of nitrogen in osteoporosis, as well as in accumulation of muscles salt-water balance, development of sexual characteristic and immune function (Sultan and Raza, 2015).

2.3 Pharmacognosis of *Phyllanthus amarus* whole Plant.

Phamacognosy of *Phyllantus amarus* is diverse in nature as it contains different phytochemicals that have different therapeutic effect. *Phyllanthus amarus* has long background in traditional medicine due to its active compounds.

2.3.1 Antiviral activity

Phyllanthus amarus reveals powerful early antiviral activity against Herpes Simplex Virus type-1 and type-2 (Devi *et al*., 2017). In vitro, *P. amarus* binds to the surface antigen of the hepatitis virus to inhibit DNA polymerase by minimizing the transcription, translation process

and suppressed cell line in another study, *P. amarus* extract was discover to have a hepato-protective effect on rat hepatic injury *in-vitro* (Verma *et al*., 2014*).* As the percentage of 4,5 dimethylthiazol 2, 2 diphenyl tetrazolium bromide (MTT) reduction assay increases, the release of aspartate transaminase (AST) alanine transaminase (ALT) high sensitivity human thyroglobin (HTG) and tumor necrosis factor (TNF) decreases returning to normal. It's known that the water/alcohol extract (HIV-1 gp120 with its primary cellular receptor CD4 at 50 % inhibitory concentrations of 2.65) can repel HIV-1 attachment and reverse protease (Khanbabaee and Ree, 2001). As gallotannin components were shown to be the potent mediators. At a concentrations of 150 mg/kg of animal body weight, antiviral activity against WSSV was observed (Dhongade and Chandewaral, 2013).

2.3.2 Anti-diabetic activity

Diabetes is a result of carbohydrate, fat and protein metabolic disorder that is one of the most common endocrine disease (Muktar *et al.,* 2019). The potentials of *Phyllanthus amarus* on diabetes was explored in a study in which rats were fasted and then given a single intra peritoneal injection of 120 mg/kg was given to them in two doses. Oral administration of *P. amarus* hydro-alcoholic extract was compared to a standard control group that received distilled water for 15 days and the outcome reveals a substantial drop in blood glucose level, the treated animal serum showed a rise in insulin and a decrease in Melondi-aldehyde levels (Verma *et al*., 2014). Another trial of *Phyllanthus amarus* extract on human blood sugar level of ages between 35-55 years at dosage of 1 g thrice daily for 90 days of 250 – 400 mg/ mL shows significant reduction in blood sugar level (Joseph and Raj, 2011). The methanolic extract of *Phyllanthus amarus* was discover to inhibit lipid peroxidation and scavenge hydroxyl superoxide radicals in another study, therefore *P. amarus* can be used in the management of diabetes and dropsy (Islam *et al*., 2008). In treated mice an oral dose of 150,

300 and 600 mg/kg of extract from seeds and leaves caused a dose-dependent decrease in the fasting plasma glucose, cholesterol and weights loss (Dhongade and Chandewar, 2013). The presence of dotriacontanyl, docosanoate, and mixture of ofoleanolic acid and ursolic acid in the plant extract inhibits α-amylase implying that the the extract could improve utilization, though the mechanism is unknown (Dhongade and Chandewar, 2013).

2.3.3 Anti-cancer activity

According to Raj and Joseph (2011) aqueous extract of *Phyllanthus amarus* exhibited potent anti-carcinogenic 20-methylcholanthrene induced sarcoma production and increased the survival of mice, same extract was also said to have blocked the function of Dalton's lymphoma ascites, Ehrlich carcinoma as well as the transplant tumor. The extract shows potency against mutant cells of *Saccharomyces cereviciae*. Reduction of aniline hydroxylase enzymes to 50 % is also a potentiality of the extract on cancer activity (Raj and Joseph, 2011). Inhibition of metabolic activities of carcinogen and cell cycle regulators in DNA repair are evidence of protective properties of *P. amarus* on the hemotopoietic system at dosages of 25, 50 and 100 mg/kg bodyweight (Islam *et al*., 2008).

2.3.4 Anti-oxidative activity

Phyllanthus amarus have powerful antioxidant property, as the antioxidant potential of the extract was determined using the Folin- Ciocalteau system and 2, 2-diphenyl-1-picrylhydrazyl (DPPH). The test involved reducing a stable DPPH to yellow colored diphenyl picryl hydrazine, though it was concentration dependent as maximum concentration is 300 g/mL (Verma *et al*., 2014). Due to hydrolysis and cellular degradation of tannin, It was discover that the boiled water extract of the fresh and dried *P. amarus* plant had comparatively greater antioxidant than microwave assisted extraction method. Therefore maximum activity also

depends on the method of extraction (Lim and Murtajaya, 2007; Altemimi *et al.,* 2017). This free radical scavenging activity suggest its potentials in the food industries (Dhongade and Chandewar, 2013).

2.3.5 Antimicrobial activity

Antimicrobial activities derived from plant have a huge therapeutic potential, infectious diseases treatment is effective with them and at the same time alleviating most of the side effects linked with synthetic antimicrobials (Jyoti *et al.*, 2018). *Phyllanthus amarus* has antibacterial properties on both Gram-positive and Gram-negative bacteria (Broad spectrum) (Patel *et al.,* 2011; Danladi *et al.,* 2018). Analysis of various bacterial isolates, including; *Bacillus stearothermophilus, Staphylococcus aureus, Bacillus subtilis, Micrococcus leuteus, Salmonella typhi, Enterobacter aerogens, Proteus mirabilis* and *Proteus vulgaris* revealed that *P. amarus* showed the least MIC on all bacteria tested (Danladi *et al*., 2018). The methanolic extract of *Phyllanthus amarus* as found to have potent inhibitory effects against drug-resistant pathogenic gram-negative bacteria; *Shigella sp., E. coli sp, V .cholera sp, S. aureus sp, S. typhimurium sp, P. aeruginosa spp, B. subtili spp, Klebsiella sp.* and *Streptococcus sp.* (Mazunder *et al*., 2006).

Antbacterial activity of *Phyllanthus amarus* extract was examined using hexane, petroleum ether, chloroform, acetone and methanol against both Gram-negative and Gram-positive bacteria and the result demonstrated high inhibitory activities by methanol extract on the bacteria species (Saranraj and sivasakhivelan, 2012). Similarly, Agar well diffusion method was used in another study to examine *Phyllanthus amarus* extract obtained from ethanol and water against *Salmonella typhi* in which significant result was recorded. Different methods of extraction were compared to standard antibiotics using agar cup diffusion techniques and the result demonstrated high prospects for *P. amarus* viability (Verma *et al*., 2014).

The crude extract and fractions were examine for antimicrobial activity against *P. aeruginosa, E. coli* and *S. aureus* with dichloromethane fraction showing most activity while the hexane, ethyl acetate and aqueous methanol showed no activity .The antibacterial activity of ethanol-extracted *P. amarus* against *E. coli* suggest that they could have therapeutic potential in the treatment of UTI (Sarang and Svasakthi, 2014). Using the bauer disc diffusion process,the antibacterial activity of root and leaves extract was tested against spectrum lactamase (ESBL) generating *E. coli* isolated from immune compromised HIV seropositive patients at various concentrations . The extract inhibit RT- inhibitor resistant HIV strains as wells as wild-type strains (Notka *et al.,* 2003).

2.4 *Salmonella* Bacterium

Salmonella is a rod shaped Gram-negative, facultative bacteria belonging to Enterobactriaceae family (*Eng et al.*, 2015) with two species, *Salmonella enterica and Salmonella bongori* (Popoff *et al.,* 2003) Salmonella enterica is a rod shaped flagellate anaerobic, Gram negative bacterium characterized by O,H and Vi antigens, with a few exceptions, non-spore forming and Salmonella bongori (based on the differences in the 16 rsna sequence analysis) are two main types.(Eng et al., 2015). The species are donated with roman numerals, I and S as enterica subsp.enterica, ii S.enterica subspecies salama (iia,) S. entreica subspecies Diarizonae, (iiib) S.entreica subsp. Diarizonae, (iv) S .entreica subsp houtenae, and S .entreica subspecies indica. Enterica is the most common and contribute to majority infection (Brenner *et al.,* 2000; Eng *et al.,* 2015). They are motile by means of peritrichous flagella with diameters of 0.7-1.5 μm and length of 2- 5 μm (Gianella,1996). There are over 2,500 *Salmonella* serotypes and biotypes with new serotypes being discovered yearly adding to the complexity of the bacterial population of *Salmonella* enterica specie types (Ryan *et al.,* 2017).

2.4.1 Antigenic structure of *Salmonella*

*Salmonell*a species are characterized with three antigens namely somatic (O), flagella (H), virulence (Vi) antigens (Eng *et al.*, 2015). The O antigens is used in serological identification, primarily are heat stable and resistance to alcohol found on the body of the organisms. The H antigens are heat liable and alcohol destroyable proteins found on the flagella which triggers off rapid immune responses and gives high titer during infection. The Vi antigens forms covering layer on the outside of cell wall and is poorly immunogenic which result to low titer production following infection (Ochei and Kolhatkar, 2007).

2.4.2 Pathogenesis of *Salmonella* infections

The pathogenicity of salmonella infection varies depending on the serotype and immune system of the host (Eng *et al*., 2015). Pathogenic salmonella enters the small and large intestine through the gastric acid barrier, invades the mucosa and produces toxins. When epithelial cells invade, they release pro-inflammatory cytokine which cause an inflammatory response and in turn cause diarrhea, ulceration and mucosa damage. It can also spread to intestines and cause systematic diseases (Giannella, 1996). It passes through the mucosa to blood stream and lymphatic resulting to transient bacteremia and dissemination in the reticulo-endothelial system (liver, spleen, and bone marrow), gall bladder and kidneys (Ochei and Kolhatkar, 2007). Both *Salmonella* serotypes are thought to be pathogenic while some serotypes are host-specific, they can affect a variety of hosts. The majority of *Salmonella* serotypes have two phases: a motile phase and a non-motile. In most part of the world, the two most common serotypes of *Salmonellosis* transmitted from animals to human are *Salmonella enteritidis* and *Salmonella typhimur*ium (Crump *et al*., 2015). The most common causes of enteric fever in humans are *Salmonella typhi* and *Salmonella paratyphi* A, B and C.

Salmonella species can be present in faeces, blood, bile, urine, food and other bodily fluids as well as environmental materials (Ifeanyichukwu *et al*., 2016).

2.4.3 Epidemiology of *Salmonella* infections

There are two types of *Salmonella* infections; typhoidal and non-typhoidal almonellosis. Both causes typhoid fever and other enteric fevers spread primarily via fecal-oral channels with no animal reservoir other than asymptomatic human acting as carriers (Typhoid Mary) that spread the disease (Nwabor *et al*., 2015). Non-typhoidal *Salmonellosis* is a worldwide disease that affect both humans and livestock, with animals being the primary source of infection, making it a food borne disease that can be transmitted from person to person and it is self-limiting in nature. The estimated infection in the world is about 21.7 Million with 217, 000 deaths worldwide (Nwabor *et al.*, 2015). In endemic countries, *S. typhi* overweighed *S. paratyphi* in the ratio of 4:1. Asia, Africa and Latin America are highly vindicated with children, elderly and immuno-compromised individuals at a high risk of the infection (Ngogo *et al.*, 2020).

2.4.4 Symptoms of *Salmonella* infections

Enteric fever, bacteremia, gastro-enteritis and persistent carrier state are the four clinical symptoms (Eng *et al*., 2015). Diarrhea, febrile fever, influenza-like symptoms, malaise, anorexia, dry cough, sore throat and headaches are some of the symptoms of salmonella infections. In this case, sample from blood, urine, bile, bone marrow as well as stool are used in diagnosis of the infection (Crump *et al*., 2015).

2.5 Diagnosis Salmonella infections

Diagnosis of salmonella infections is usually based on symptoms and culture. (Eng *et al.,* 2015) In certain cases, the organisms are classified using a combination of serological and biochemical test which are usually in form of strips and antibodies (Andrew and Ryan, 2016).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

The research was carried out at Federal University of Technology Minna, located at Bosso town in Bosso Local Government Area, in Niger State as shown in Figure 1, See Appendix 1. Niger state is situated at latitude of 9.9309^0N and longitude of 5.5983^oE respectively. It has an Area of about 76,363km^2 and was founded on 3rd February, 1976. It has numerous tribes and religions with agriculture as the main backbone of the economy. Niger state is located in the Middle Belt region of Nigeria and it is the largest state in the country by land mass. The state is bounded to the south by river Niger and North with Kebbi and Zamfara States, Kaduna to the North and Northeast, Kogi to the Southeast and Kwara to the South South. The state has an estimated population of about 24,206,644, according to United Nations data the population density of 19 per km2 per a year (Britannica, 2018).

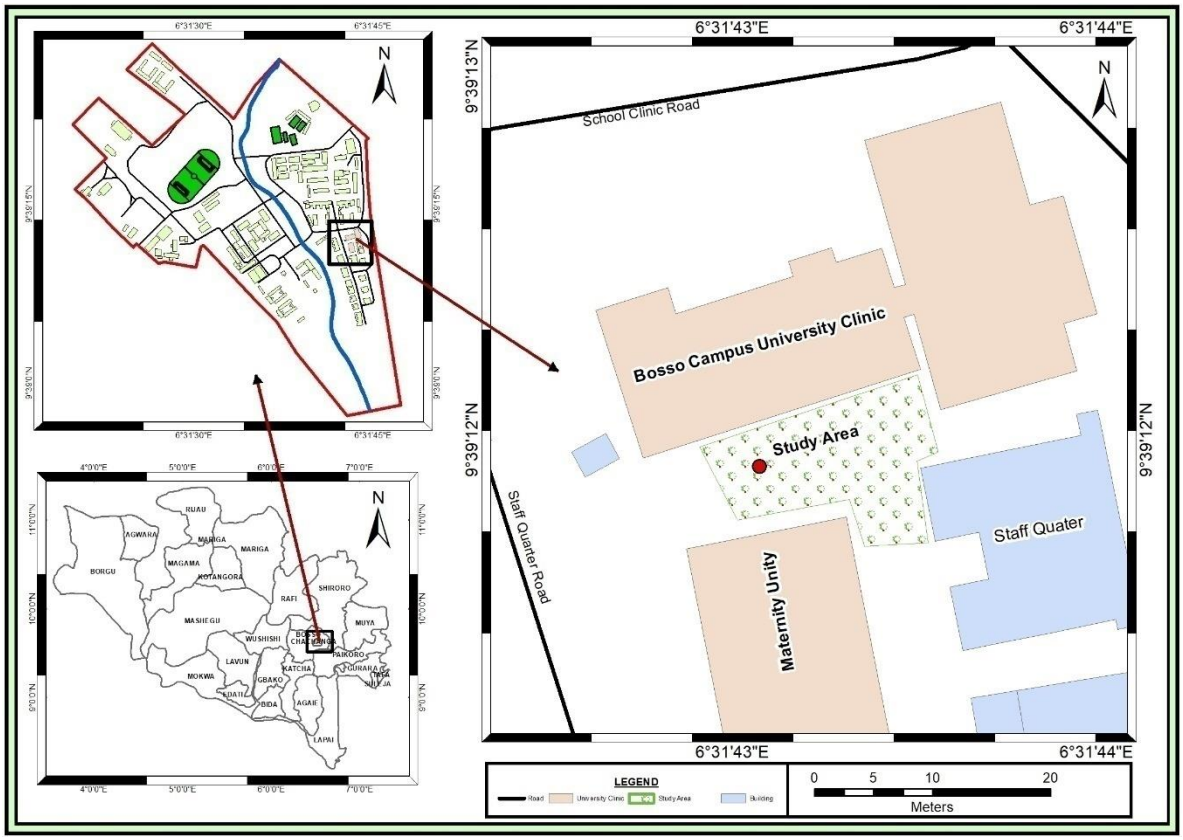


Fig 1 Shows the study Area/ region on a Map of Niger state (Bosso) (Source: GIS Achiever, 2021).

3.2 Plant Material Collection and Identification

The plant materials for the study were collected at the University between April and August, 2019 and were classified by National Institute of Pharmaceutical Research and Development (NIPRD) in Abuja under the voucher number is *Phyllanthus amarus* NIPRD /H/7071**.**

3.3 Preparation of the Extract (Crude Extract)

The plants were thoroughly cleaned, shade dried at temperature of 40 ^{0}C and pulverized using an electric blender and sieved with muslin cloth to obtain uniform texture (Pathak *et al.,* 2017). Hundred grams (100 g) of dried *Phyllanthus amarus* powder (plant material) was mixed with 1000 mL of methanol (1;10 ratio) loaded with condensed fresh solvent from distillation flask in a thimble holder. Distillation was used to separate the solute from the solution in the solvent flask. The operation was repeated until complete extraction was achieved (Egwari and Abah, 2011). The extract yield was 18.48 gw/v and was placed in the water bath at 60 ^{0}C until all the solvent evaporated. The partitioning was carried-out in order of increased polarity. (n-hexane, ethyl acetate and acetone). Exactly 100 mL of n- hexane was poured into the separating funnel containing dissolved extract and swirled, then allowed to stand for 2 hours after which the top layer was deposited into a clean beaker and the process was repeated twice until n-hexane layer became colorless. 100 mL of ethyl -acetate was mixed with the residual fraction and swirled then allowed to stand for 2 hours. The residue was collected into a beaker and the same process was repeated twice. The same amount of 100 mL of acetone was mixed and allowed to settle for same hours. The fractions collected in different beakers were dried on water bath at 45 ^{0}C (Abu *et al.*, 2017).

3.4 Qualitative phytochemical constituents of *Phyllanthus amarus* whole plant.

3.4.1 Test for flavonoids

One gram of the plant powder was heated with 10 mL of ethyl acetate for 3 min. 1 mL of dilute ammonia and 4 mL filtrate were shaken over a steam bath for 5min. Observation for the formation of yellow coloration was made and result recorded (Edeoga *et al.*, 2005).

3.4.2 Test for alkaloids

The defatted sample of P. amarus whole plant was extracted with 5mL aqueous HCl for 20min on a boiling water and the resulting mixture was centrifuged for 10 min at 300 rmp, 1 mL of the filtrate with few drops of Meyers and Wagner's reagents were used to treat the filtrate. Observation for turbidity was done and the result recorded (Saio and Syiem, 2015).

3.4.3 Test for saponins

Using the Edoaga method (2005), 2 g powdered of *P. amarus* whole plant was boiled in distilled water in a test tube in a boiling waterbath and then filtered. A stable persistent froth was obtained of which 10 mL of it with 5 mL of distilled water. Three drops of olive oil were applied to the froth and vigorously shaken. Observation for the formation of emulsion was made and the result recorded (Saio and Syiem, 2015).

3.4.4 Test for tannins

A quantity of 2 g of p. amarus extract was boiled with 5 mL of ethanol for 5 min and allowed to cool then filtered. 1 mL of the filtrate was diluted with distilled water and 2 drops of ferric chloride solution was added. Observation for the formation of dark-blue coloration was done and recorded (Ukoha *et al.,* 2011).

3.4.5 Test for phenols

In a beaker, 1 mL extract was blended with 2 drops of ferric chloride and stirred. Observation for the formation of bluish green solution was recorded (Tripathi and Mishra, 2015).

3.4.6 Test for tepernoid

Edeoga method was used for the test, 5 mL of methanolic extract of *P. amarus* and 2 mL of chloroform were mixed in a test tube with 3 mL of concentrated sulfuric acid carefully added to form a layer. The formation of reddish-brown coloration was observed and recorded (Edeoga *et al.,* 2005).

3.4.7 Test for glycosides

To test for glycosides, the modified Borntrager"s test was used. 5 ml of extract was added to 5 ml of 5 % ferric chloride solution and 5 mL dilute hydrochloric acid .The mixture was heated in water-bath for 5 minute. 3 ml of benzene was added to the cooled solution and shaken to separate organic layer, 10 % ammonium solution was added to the solution and formation of rose red at ammonium layer confirmed the presence of glycosides (BaoDuy *et al*., 2015).

3.4.8 Test for steroids

Two mL acetic anhydride and 0.5 g/mL of methanolic extract was combined with 2 mL of concentrated sulfuric acid in a beaker. The color change from blue to green was recorded (Edeoga *et al.,* 2005).

3.4.9 Test for reducing sugars

According to Sofowora (1993), 2 mL of plant extract was mixed with equivalent amount of Fehling solution A (aqueous solution of copper sulphate) and B (potassium tatrate) placed in a boiling water bath in a test tube. The formation of a red cuprous oxide precipitate was observed (Usman *et al*., 2009).

3.5 Quantitative Determination of Chemical Constituents

3.5.1 Alkaloid determination.

Five grams of sample was weighed into 250 mL beaker, 200 mL of 10 % acetic acid and ethanol were combined and covered, and the mixture was left to stand for four hours. The filtrate and extract were concentrated to a quarter of their original volume using a water bath, drop-wise addition of concentrated ammonium hydroxide were made before the preparation was completed. The solution was allowed to settle and precipitate, collected, washed with ammonium hydroxide and filtered. After drying and weighing the residue, the percentage was determined (Khalid *et al.,* 2018).

3.5.2 Tannin determination

Five hundred milligrams of the sample was weighed into a 100 mL plastic container, 50 mL of distilled water were mixed and shaken for an hour in a mechanical shaker and filtered into a 50 mL volumetric flask. Within 10 minutes, absorbance was measured with a gas

chromatograph at 120nm wavelength using 5 mL of the filtrate combined and with 3 mL of 0.1 Ml of $FeCl_3$ in 0.1 NHCl and 0.008 M potassium ferro-cyanide. A coloured blank sample was prepared and tannin was use as norm to get 100 ppm and was measured (Ajuru *et al.*, 2017).

3.5.3 Saponin determination

Twenty grams of the grinded sample were dispensed into 200 mL of 20 % ethanol, heated for 4 hours at 55 ^{0}C with constant stirring and then another 200 mL of 20 % ethanol, the residue was removed once more. When passed through a water bath at 90 ^{0}C, the combined and filtered. The residue was re-extracted with another 200 mL of

20 % ethanol Combined extract yielding 40 mL. The concentration was shaken with 20 mL diethyl ether and the ether layer was removed and the purification procedure was repeated with 60 mL of n-butanol. The mixture was washed twice with 10 mL of 5 % aqueous NaCl before being heated, evaporated and dried in an oven. Percentage was used to express weight (Harborne, 1973).

3.5.4 Phenol determination

Two hundred microliters of crude extract of *Phyllanthus amarus* whole plant (1 mg/mL) was combined with 3 mL of distilled water were added to 0.5 mL of folin-coicalteu reagent and allowed to stand for 3 minutes. After that, 2 mL of 20 % (w/v) of sodium carbonate was added to the mixture, stirred and allowed to sit for another 1h in the dark. Garlic oil was used as standard for calculating the absorbance at 650 nanometers (Baba and Malik, 2015).

3.5.5 Flavonoid determination

At temperature of 35 ^{0}C, 5 grams of plant sample was weighed into 250 titration flask and 100 mL of 80 % aqueous methanol added and shaken for 4 hours. Whatman filter paper number 42 (125mm) was used to filter the entire solution over a water bath and was evaporated to dryness in a crucible. The weight is express in percentage (%) (Khalid *et al.,* 2011).

3.6 Bacteria used in the Research

Five samples of Stool samples were taken from in-patients at General Hospital Minna and were used to isolate *Salmonella species*. Enrichment media (selenite broth) was used as transport medium for the specimens.

3.6.1 Confirmation of the identity of the bacteria used

The identity of the bacteria used was confirmed by employing an approved technique of gross colony morphology grown and observed in *Salmonella-Shigella* (SS) agar (Andrew and Ryan, 2016). Pure cultures of the bacteria were maintained on stock culture agar medium. The isolates were subjected to serological test, conventional and biochemical tests (Nesa *et al.,* 2011).

3.6.2 Identification of test organism

Isolation and identification of *Salmonella* is on the basis of colony morphology, biochemical and serological characteristics. Samples were inoculated into test tubes of freshly prepared Mueller-Hinton agar and incubated at 37 ^{o}C for 24 hours. Loopful of cultured sample was then streaked into *Salmonella Shigella* agar (SSA) and Macconkey agar (MCA). The colonies on the plates were carefully studied morphologically for *Salmonellae* using gram's staining method described in 1967 by Merchant and Packer (Nesa *et al.,* 2011).

3.7. Gram's staining of the test organisms

The smear of the test organism was flooded with crystal violent stain, allowed to stand for one minute and washed under running water. It was then flooded with iodine, allowed to stand for one minute and washed again under running water. The film was decolorised by acetone between 20-30 seconds and washed. It was again counterstained using safaranin and allowed to stand for one minute before washing until no color was effluent. The film was dried, observed under microscope with oil immersion (X100) objective and result was recorded (Thairu *et al.,* 2014).

3.8 Biochemical Test

S*almonella* colonies were selected from the culture plates and inoculated into the biochemical test tubes for confirmation. In Triple Sugar Iron for hydrogen Sulphide production, citrate test for, urase test and indole test. Colonies are generally non – lactose fermenters, and are facultative anaerobes. They are oxidase negative and produce acid from glucose, with gas production except for the poor producers; *Salmonella Paratyphi* A and *Salmonella typh*i. Majority of bacteria contain hydrogen sulphide.

3.8.1 Catalase test (slide or drop) method

A colony of test organism from a broth of 18 h inoculant was mixed with a drop of 3 % hydrogen peroxide solution on slide in a Petri dish using pipette, the Petri dish was covered with a lid to limit aerosol. Effervescence was produced confirming the presence of the Catalase enzyme. Since catalase is an intracellular enzyme, hydrogen peroxide must enter the cell for bubbles to form (Taylor and Achanza, 1972).

3.8.2 Indole formation (Kovac's Method)

In accordance with the method of Kovac (2014), five grams of p-dimethyl amino-benzel dehyde was mixed with 75 mL of amyl-alcohol and 25 mL of concentrated sulphuric acid to produced Kovac's reagents. A loopful of *Salmonella* pure culture was inoculated into a test tube containing tryphtone broth and was incubated at 37 °C for 48 hours. Five drops of Kovac's reagent were dropped into the test tube directly. Observation of yellow colour was done and result recorded (Collins *et al.,* 2004).

3.8.3 Methyl red (MR)

In 0.5 mL of sterile glucose phosphate broth, a loopful of the test organism was inoculated. Observation of red coloration was done and the result recoded (Nesa *et al.,* 2011).

3.8.4 Voges proskauer (VP)

One mL of 40% potassium hydroxide was added to medium inoculated with test organism for 48 hours at 37 ^{0}C and left to cool at 35 ^{0}C for 1 hour. Observation of yellow brown color was done and the result recorded (Collins *et al.,* 2004).

3.8.5 Motility test (Semi-solid method)

Agar was used in 1.5 to 2.0 % of solid media and makes up about 0.4 % of semi-solid content. A colony of the test organism was selected with sterile bacteriological needle and Stabbed on a semisolid media tube and incubated for 24hrs. The ability of bacteria to move through semisolid media indicates motility (Ralph and Laslie, 1936)

3.8.6 Hydrogen sulphide production

A tube of nutrient broth was inoculated and set on a strip of lead acetate impregnated filter paper on the top of the tube, which was held in place with cotton wool and was incubated at 37 °C for 24 hours. Observation for the formation of the light black color was done and result recorded (Collins *et al.,* 2004).

3.8.7 Triple iron sugar

The triple sugar iron (TSI) test is used to assess micro-organisms capacity to ferment sugars and hydrogen sulphide. A special medium with multiple sugars that acts as a pH sensitive dye on agar slant (phenol red). Lactose is 1 % of the population.One percent sucrose, the test was carried out with 0.1 percent glucose, sodium thiosulphate and ferrous ammonium sulfate. Both of these ingredients were combined and allowed to solidify at a slanted angle in an agar test tube. The slanted shape of this medium offers an assortment of surfaces- that are either exposed to oxygen containing air in varying degrees, resulting in color changes from red to yellow butt. (Collins *et al*.,2004).

3.9 Culture Media Preparation

Muller Hilton Agar was used in the study. In a 1000 mL conical flask, 3.8 g of Mueller Hinton agar was weighed into 100 mL of distilled water. The solution was heated until well dissolved and autoclaved at 121 °C for 15minutes. The media was allowed to cool, Twenty milliliters of the media was dispensed into each plate and allowed to set. The plates were kept in the refrigerator in an inverse position (Balouri *et al*., 2016).

3.10 Screening of Plant Extract for Antimicrobial Activity

The antimicrobial activity of *P. amarus* on *Salmonella* species was determined using agar well diffusion method define By Collins *et al*. (1995) with minor modifications. Extract of different concentrations were weighed (1.5 g, 2 g, 2.5 g, 3 g and 5 g) and reconstituted in 5 mL of both distilled wate r and DMSO. The organisms are calibrated SS for Salmonella typhi and SP for Salmonella paratyphi B. Using a cork borer 5 mm diameter hole was bored on the Mueller Hinton agar seeded with the test organisms and 0.2 mL of the extract was then added into the hole and incubated at 37 ^{0}C for 24 hours, control was set with DMSO (negative control) and ciprofloxacin (positive control) but not with the extract. The procedure was done in triplicate. The development of zone of inhibition was observed and recorded as the antimicrobial activity (Balouiri *et al*., 2016).

3.11 Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration.

The Minimum Inhibitory Concentration (MIC) was determined by measuring 9 mL of sterile Nutrient broth into 11 empty tubes, and a ml of the different concentrations of extract was added into the first tubes which was diluted from tube 1-10 serially, a loop-full of the overnight test organisms was inoculated into the first test tubes and serially inoculated to the nine tube. It was incubated for 24 hours at 37 ^{0}C. Spectrophotometer was used to examine the tubes for visible growth The MIC" tube is the one that showed least/ no growth, the (MIC) tubes is then poured onto a Mueller Hinton Agar plate and incubated for 24 hours at 37 ^{0}C resulting in minimum bactericidal concentration (MBC) (Balouri *et al*., 2016).

3.12 Experimental Animals

The rats were collected from National Veterinary Research Institute Vom (N.V.R.I) Jos with bodyweight of 102-110 kg. They were caged with adequate placebo and water under appreciable environmental conditions in animal house at the Federal University of Technology Minna Biochemistry Department for acclimatization. All conditions met the OEOC standards.

3.12.1 Inoculation of *Salmonella* species and administration of plant extract to Wister rats

A loopful of the test organism stored in Nutrient Agar slant was reactivated using 10 mL of peptone water and was incubated at 37 ^{0}C for 24 hours. Serial dilution was made to obtain $1.0x10^9$ cfu/ml (Itelima and Angina, 2014). Each group of rats was orogastrically fed with the test organism.

3.12.2 Toxicity effect

In Lorke's method, two phases are involved using thirteen rats. The first phase had nine animals. Three animals in each groups of three and extracts were administered at 10, 100 and 1000 mg/kg (body weight) while the second phase involved four groups of animals, given four different dosages of *Phyllanthus amarus* methanolic extract of 1900, 2600 and 5000 mg/kg (bodyweight). These was to determine the dosage range that will give toxic effect of the extract (Lorke, 1983; Enegide *et al*., 2013; Earnest *et al*., 2018).

3.12.3 Acute oral toxicity test (Limit test OECD 401)

The acute oral toxicity research followed OECD test recommendations for acute oral toxicity and used the limit test protocol. The experiment was carried out with rats. Three groups of

animals were chosen at random and given a single gastric intubation of 10, 100 and 1000 mL/kg, respectively. Mortality signs of gross toxicity or excitability, convulsions, lethargy, sleep (behavioral changes) were observed from one hour post dosing and for the entire day (24 hours).

3.12.4 Sub-acute toxicity test (OECD 407)

This is the second phase of the toxicity testing, in this phase longer period is required for the administration of *Phyllanthus amarus* methanolic extract in the ranges of 10, 100, 1000, 1900, 2600 and 5000 mg/kg (bodyweight) of the animals for twenty-one days after which the animals were sacrificed. Body organs were weighed and other Hematological, Biochemical and Histological tests were carried out.

Four groups of 16 animals (randomly selected) were used. Rats were given a regular treatment for 21 days in a row. Oral doses of dissolved extract of 1900, 2600 and 5000 mL/kg were given to the test groups. During the experiment, the control (one group control for each extract) was given distilled water, and each animal was monitored daily for signs of toxicity. Hematological parameters, biochemical levels, and changes in body weight were all tracked on a weekly basis. Animals were anaesthetized with ether and destroyed by cervical dislocation 24 hours after the last dose. For blood analysis, blood was taken from the retro-orbital sinus. The liver and kidneys were taken from two animals in each group for histology test (Enegide *et al.,* 2013).

3.12.5 Mortality and survival rate under observation

The animals were observed for behavioral changes and pathological manifestations during the period of the experiment(Arsad *et al*., 2013). At the end of the study, the rats were sacrificed

and the blood was collected into heparin sample containers for both hematological and biochemical investigations. No death was recorded during the experiment.

3.12.6 Hematological investigations

Hematological investigations are investigations targeting blood disorder that include; the mean corpuscular volume (MCV) is the average number and size of red blood cell circulating in the blood stream. The mean corpuscular hemoglobin concentration (MCHC) is the average concentrations of hemoglobin in the red cells. Mean corpuscular hemoglobin (MCH) the average quantity of hemoglobin present in a single red blood cell. The red blood cells are the measure of size, shape and physical characteristic. The percentage red blood cell is determined by the packed cell volume (PCV) the complete blood count (CBC) deals with the cells that circulate in the blood (white blood cells, red blood cells and platelet) leukocytes or white cell play vital role in protection against inflammation and allergic Reactions, they consist of types; monocytes, neutrophils, lymphocytes, eosinophil and basophiles, each representing peculiar abnormalities.(Asare *et al*., 2011).

3.12.7 Bio-chemical investigations

Biochemical Changes/Markers; Such as Aspartateaminotransferase (AST), Lactatedehydrogenase (LDH) and Alanineaminotransferase (ALT) were investigated for possible changes/alterations in the experimental animals that consumed the *Phyllanthus amarus methanolic* extract, as an aid in predicting Susceptibility and a marker for diagnosis(Aragon and Yonoussi, 2010). Biochemical investigatios were carried out using serum collected from centrifuged blood at 300 rpm for five minutes.(Asare *et al*., 2011).

3.12.8 Histopathology investigations

Liver and kidney from experimental animals were removed after the sacrifice and undergo histological test, to ascertain the level of toxicity or damage caused by the methanolic extract of *Phyllanthus* amarus (Oduola *et al.,* 2010).

3.13. Data Analysis.

Result were expressed as mean ± standard error of mean (STM) of 10 observations.

Statical analysis of data was performed using Anova, Duncan test and student t-test p. value at less than 0.05 were consider significant.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Results

4.1.1 Qualitative and quantitative analysis of *Phyllanthus amarus* whole plant

The result of the phytochemical screening of *Phyllanthus amarus* extracted using four different solvents indicates the presence of Phenols, Tannins, Saponin, Flavonoids and Alkaloids (Table 4.1).

Table 4.1: Qualitative analysis of *Phyllanthus amarus* whole plant.

Phytochemicals	**Solvents (mL)**			
	Methanol	n- Hexane	Ethylacetate	Acetone
Saponin	+	+	+	+
Flavonoids	+	+	+	+
Alkaloids	+	+	+	+
Tannins	+	+	+	+
Seroids	+	+	+	+
Glycosides	+	+	+	+
Volatile oils	-	-	-	-

The concentrations of the phytochemical screened varies in each solvent used (Table 4.2).

Table 4.2: Quantitative analysis of *Phyllanthus amarus* whole plant.

Sample (Gram)	**Phenols**	**Flavonoids**	**Alkaloids**	**Tannins**	**Saponin**
Methanol (crude)	315.3243	131.5734	50.6758	532.8884	2252.64
	315.7541	168.08	51.5647	530.712	2248.416
	315.5392	169.8267	51.12025	531.798	2250.528
N-haxane	206.9703	173.1688	19.0845	852.94	911.076
	206.8622	172.9087	18.0567	852.116	912.9876
	206.9163	173.0388	18.5706	852.528	912.0318
Etylacetate	753.4134	239.9799	37.0978	595.796	306.092
	754.8975	238.0701	37.1734	596.012	305.992
	754.1555	239.025	37.1356	595.904	306.042
Acetone	964.746	138.1112	42.4568	538.144	1052.412
	971.308	136.3644	43.7645	538.424	1049.676
	968.027	137.2378	43.11065	538.284	1051.044

Highest concentration of Phenols (39.03 %), Flavonoids (12.37 %) and Alkaloids (1.92 %) was observed in ethyl acetate. Saponin (67.81 %) had the highest concentration in the methanolic solvent, while Tannin (39.41 %) was highest in Hexane solvent. Least concentration of Phenols (9.50 %) and Tannins (16.02 %) was recorded in the methanolic solvent, Flavonoids (5.01 %) in the Acetone solvent, Alkaloids (0.86 %) in the Hexane solvent and Saponins (15.84 %) in the Ethyl-acetate solvent (Table 4.3).

Table 4.3: Concentration of phytochemicals in 100 g/mL of *Phyllantus amarus*

Phytochemicals	**(g)**	**Solvents (mL)**		
	Crude (%)	**Hexane (%)**	**Ethyl-acetate (%)**	**Acetone (%)**
Phenols	9.50	9.57	39.03	35.36
Flavonoids	5.12	8.00	12.37	5.01
Alkaloids	1.54	0.86	1.92	1.57
Tannins	16.02	39.41	30.84	19.66
Saponins	67.81	42.16	15.84	38.39
Total	100	100	100	100

4.1.2. Methanolic effect of *Phyllanthus amarus* whole plant extract against *Salmonella Species*.

Table 4.4 below shows the methanolic effect of *Phyllanthus amarus* whole plant extract activity against *Salmonella* typhi and *Salmonella* paratyphi B. The measured zones of inhibition for each concentration were significantly different. The extract was more effective at 1000 mg/mL for both isolates as signified by the higher zones of inhibition. Antibacterial activity was concentration dependent as inhibition zones increases with increasing concentration of the extract. See (Appendix III).

Table 4.4: Antibacterial effect of *Phyllanthus amarus* methanolic whole plant extract against *Salmonella* species

	Zones of Inhibition	
Concentration (mg/mL)	***Salmonella typhi***	***Salmonella paratyphi* B**
300	8.33 ± 0.33^{b}	6.00 ± 0.00^{b}
400	11.00 ± 0.57^{c}	9.33 ± 0.66^{c}
500	13.67 ± 0.33^{d}	11.00 ± 0.57^{d}
600	15.33 ± 0.33^{e}	14.00 ± 0.00^{e}
1000	18.33 ± 0.33^{f}	16.33 ± 0.33^{f}
Ciprofloxacin	26.33 ± 0.66^{g}	23.00 ± 1.00^{g}
DMSO	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}

Values are mean ± standard deviation of three replicates
Values with the same alphabet superscript in a column have no significant difference at $p<0.05$

4.1.3. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of crude *Phyllanthus amarus* methanolic whole plant against *Salmonella* species.

In Table 4.5 below, the Minimum Inhibitory Concentration (MIC) of *Salmonella typhi* and *Salmonella paratyphi* B was at 7.8 mg/mL and 9.3 mg/mL respectively. While the Minimum Bactericidal Concentration (MBC) of Crude *Phyllanthus amarus* against *Salmonella typhi* and *Salmonella paratyphi* B was at 15.6 mg/mL and 18.7 mg/mL (Table 4.6).

Table 4.5: Minimum inhibitory concentration (MIC) of crude *Phyllanthus amarus* against *Salmonella* species.

CRUDE EXTRACT (mg)	TUBE 1	TUBE 2	TUBE 3	TUBE 4	TUBE 5	TUBE 6	TUBE 7	TUBE 8	TUBE 9
2.5	500	250	125	62.5	31.25	15.625	7.8125 MIC	3.9063	1.9531
3.0	600	300	150	75	37.5	18.75	9.375 MIC	4.6875	2.3438

Table 4.6: Minimum bactericidal concentration (MBC) of crude *Phyllanthus amarus* against *Salmonella* species.

CRUDE EXTRACT (mg)	TUBE 1	TUBE 2	TUBE 3	TUBE 4	TUBE 5	TUBE 6	TUBE 7	TUBE 8	TUBE 9
2.5	500	250	125	62.5	31.25	15.625 MBC	7.8125	3.9063	1.95313
3.0	600	300	150	75	37.5	18.75 MBC	9.375	4.6875	2.34375

4.1.4 Activity of *Phyllanthus amarus* whole plant extracted using different solvent against *Salmonella* typhi and *Salmonella paratyphi* B.

No activity was recorded for the different solvent used in extractions of *Phyllanthus amarus* whole plant extract on both species of *Salmonella* as presented in (Table 4.7.)

Table 4.7. Activity of *Phyllanthus amarus* whole plant extracted using different solvent against *Salmonella* species.

Concentration	**Solvents (mL)**					
(mg/mL)	**Hexane**		**Ethyl-acetate**		**Acetone**	
	S. typhi	***S. paratyphi***	***S. typhi***	***S. paratyphi***	***S. typhi***	***S. paratyphi***
300	0	0	0	0	0	0
400	0	0	0	0	0	0
500	0	0	0	0	0	0
600	0	0	0	0	0	7
1000	0	0	0	0	0	9
Ciprofloxacin	26	23	26	23	26	23
DMSO	0	0	0	0	0	0

4.1.5. Toxicity studies

No sign of toxicity or death was recorded in the acute oral toxicity test at 10, 100 and 1000 mg/Kg/bw dosage (Table 4.8). Similarly, Table 4.9 shows no signs of toxicity or death as recorded in the experiment as related to sub-acute toxicity test of animals fed with higher dosage (1900, 2600 and 5000 mg/kg/bw) of *P. amarus* methanolic extract.

Table 4.8: Acute oral toxicity test (Limit test OECD 401)

Number Animals	Dosage (mg/Kg bw)	Mortality recorded	Sign of Toxicity
3	10	0	No sign of toxicity
3	100	0	No sign of toxicity
3	1000	0	No sign of toxicity

Table 4.9: Sub-acute toxicity test (OECD 407)

Number of animals	Dosage (mg/Kg bw)	Mortality recorded	Sign of Toxicity
5	1900	0	No sign of toxicity
5	2600	0	No sign of toxicity
5	5000	0	No sign of toxicity

4.1.6. Activity of sub-acute oral administration of *Phyllanthus amarus* extract on bodyweight variation in rats.

In Table 4.10: shown below, there was no significant difference between the bodyweights of experimental animals from all groups in week zero (0), one (1) and two (2). Significant change in the bodyweight was only observed in week three (3), were a decrease in bodyweight was recorded at 600 mg/Kg/bw dosage.

Table 4.10: Activity of sub-acute oral administration of *Phyllanthus amarus* extract on bodyweight variation in rats.

Groups	Dosage (mg/Kg bw)	Bodyweight (g)			
		Week 0	Week 1	Week 2	Week 3
Group 1	10	111.07±6.47[a]	124.32±3.79[a]	133.08±5.59[b]	141.64±6.09[b]
Group 2	300	108.97±7.91[a]	121.73±7.58[a]	131.93±9.83[b]	141.49±11.00[b]
Group 3	600	116.79±4.50[a]	124.01±8.25[a]	119.37±11.44[a]	115.81±8.17[a]
Control	0.5mL Normal Saline	111.30±6.59[a]	118.29±4.99[a]	129.04±6.05[ab]	141.99±5.31[b]

Values are mean ± standard deviation of three replicates

Values with the same alphabet superscript in a column have no significant difference at $p<0.05$

4.1.7. Activity of sub-acute oral administration of *Phyllanthus amarus* whole plant *extract* on hematological parameters in Wister rats.

Table 4.1 below, shows analysis of hematological parameters of the test animals treated with *Phyllanthus amarus* whole plant *extract* at 10, 300 and 600 mg/Kg/bw. The result did not show any significant difference from the control animals.

Table 4.11: Activity of sub-acute oral administration of *Phyllanthus amarus* extract on Hematological parameters in Wister rats

Parameters	*Phyllanthus amarus* extract (mg/Kg BW)			
	10 (Low)	300 (Medium)	600 (High)	Control
Hb (g/dL)	12.67 ± 0.29^{ab}	13.30 ± 1.30^{b}	11.45 ± 0.55^{a}	13.60 ± 0.20^{b}
PCV (%)	38.00 ± 1.00^{ab}	40.50 ± 4.50^{b}	34.00 ± 2.00^{ab}	39.00 ± 1.00^{ab}
MCV (fi)	51.00 ± 2.00^{a}	48.00 ± 2.00^{a}	45.00 ± 5.00^{a}	52.50 ± 5.50^{a}
MCH (pg)	19.50 ± 1.50^{a}	18.00 ± 1.00^{a}	16.33 ± 1.26^{a}	20.00 ± 3.00^{a}
MCHC (g/dL)	39.00 ± 1.00^{a}	40.50 ± 2.50^{a}	39.50 ± 0.50^{a}	38.50 ± 1.50^{a}
RBCs (10^{12}/L)	6.15 ± 0.35^{b}	7.45 ± 0.05^{b}	6.5s0±1.00[b]	4.35 ± 4.00^{a}
PLC (10^{6}/L)	156.00 ± 4.00^{a}	164.50 ± 3.50^{a}	284.00 ± 14.00^{c}	203.00 ± 4.00^{b}
TWBC (10^{12}/L)	5.15 ± 0.05^{ab}	6.20 ± 0.10^{c}	2.65 ± 0.55^{a}	5.70 ± 0.70^{bc}
Neut (10^{9}/L)	41.80 ± 1.80^{b}	47.00 ± 3.00^{c}	10.20 ± 2.20^{a}	55.25 ± 2.75^{d}
Lymph (10^{9}/L)	35.50 ± 0.50^{b}	41.00 ± 1.00^{c}	$22.50\pm.20^{a}$	57.75 ± 2.25^{d}
E/B/M (10^{9}/L)	26.50 ± 1.50^{c}	19.00 ± 1.00^{b}	14.00 ± 2.00^{a}	20.60 ± 2.60^{b}

Keys: Hb= Hemoglobin, PCV= Packed Cell Volume, MCV= Mean Cell Volume, MCH= Mean Cell Hemoglobin, MCHC= Mean Cell Hemoglobin Concentration, RBC= Red Blood Cells Count, PLC= Platelet Count, TWBC= Total White Blood Cell Count, Neut= Neutrophil, Lymph= Lymphocyte, E= Eosinophil, B= Basophils and M= Monocytes.

Values are mean ± standard deviation of three replicates
Values with the same alphabet as superscript in a row have no significant difference at $p<0.05$

4.1.8 Effect of sub-acute oral administration of Methanolic *Phyllanthus amarus* whole plant on lipid profile.

Significant difference in the lipid profile of experimental animals at various concentrations of *Phyllanthus amarus* was observed when compared to the control animals (Table 4.12).

4.12: Effect of sub-acute oral administration of Methanolic *Phyllanthus amarus* whole plant on lipid profile.

Group	Conc mg/kg	Total cholesterol	Triacylglycerol	HDL-C	LDL-C
G1	10	124.36 ± 4.06^{b}	134.22 ± 3.16^{b}	98.26 ± 6.14^{a}	107.60 ± 4.72^{b}
G2	300	112.14 ± 3.76^{a}	124.28 ± 3.97^{a}	115.87 ± 5.45^{b}	71.16 ± 8.78^{a}
G3	600	165.19 ± 6.12^{d}	163.93 ± 7.57^{c}	98.40 ± 4.09^{a}	135.89 ± 4.44^{c}
Normal control	-	142.54 ± 2.86^{c}	131.85 ± 2.45^{ab}	118.59 ± 2.23^{b}	116.44 ± 3.95^{b}

KEYS: Conc: Concentration**,** HDL-C: High-density lipoprotein cholesterol, LDL-C: Low-density lipoprotein cholesterol.

4.1.9. Effect of sub-acute oral administration *of* methanolic extract of *P. amarus* whole plant on Liver function.

In Table 4.13, there was no significant difference in the liver enzymes of tested animals in all the groups when compare to control in this study.

Table 4.13: Effect of sub-acute oral administration of methanolic extract of *P. amarus* whole plant on Liver Enzymes.

Group	Concentration mg/kg	Total protein	A.lbumin	AST	ALT	ALP
G1	10	16.73±1.37^{a}	5.98±0.23^{a}	9.64±0.76^{a}	20.65±0.48^{a}	59.89±0.27^{c}
G2	300	21.39±1.68^{a}	11.62±0.45^{b}	8.54±0.72^{a}	21.71±1.38^{a}	55.65±0.48^{a}
G3	600	33.40±1.68^{b}	15.87±0.60^{c}	20.21±0.51^{c}	20.84±1.28^{a}	66.38±0.78^{d}
NC	-	19.65±0.68^{a}	10.66±1.02^{b}	14.25±1.06^{b}	18.21±1.25^{a}	57.66±0.47^{b}

KEY NC: Normal Control; AST: Aspartate transaminase; ALT: Alanine transaminase; ALP: Alkaline phosphate.

4.1.10. Histology of the liver of wistar rats administered with methanolic extract of *P. amarus* whole plant.

The histology of the liver of Wister rats administered with methanol extract of *P. amarus* at 10, 300 and 600 mg/kg/bw dosages were shown in plate I. The liver sections of the selected experimental animals show largely preserved architecture with viable hepatocytes, though mild dilated sinusoids were observed in the experimental groups that received 600 mg/kg/bw. However, there were no features of acute or chronic damage.

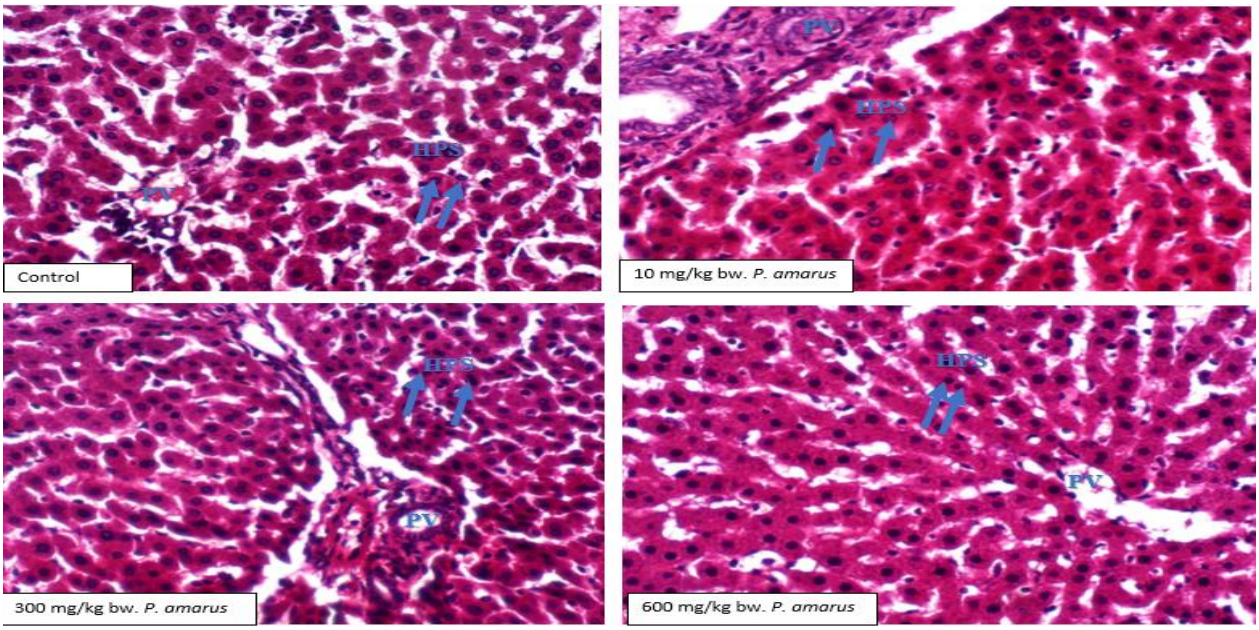


Plate I: Micrograph of liver (A) of normal control rats (B) liver of low dosage (10mg /kg/bwt) (C) moderate dosage of 300mg/kg/bwt liver (D) high dosage of 600mg/kg/bwt liver of experimental rats.

KEYS: HPS; Hepatocytes; PV; Portal vein.

4.1.11. Effect of Sub-acute oral administration of P. amarus whole plant methanolic extract on kidney enzymes in rats.

From the result of biochemical parameters in Table 4.14, there was increased in urea and uric acid in group 1 (6.11±0.21b and 52.62±3.41b) when compare with control while group 2 (51.43±1.05b) increased in urea was observed but exhibit the same direct bilirubin with control. Group 3 (7.02±0.68a and 0.88±0.04d) has high creatinine and total bilirubin when compare to control as shown below in (Table 4.14).

Table 4.14: Effect of sub-acute oral administration of *P. amarus* whole plant methanolic extract on kidney enzymes in rats

Group	Concentration mg/kg	Parameters				
		Creatinine (mg/dL)	Total bilirubin (mg/dL)	Direct bilirubin (mg/dL)	Uric Acid (mg/dL)	Urea (mg/dL)
Group 1	10	5.73±0.69^{a}	0.35±0.02^{a}	0.28±0.03^{c}	6.11±0.21^{b}	52.62±3.41^{b}
Group 2	300	6.83±0.10^{a}	0.51±0.02^{b}	0.22±0.02^{b}	4.93±0.89^{a}	51.43±1.05^{b}
Group 3	600	7.02±0.68^{a}	0.88±0.04^{d}	0.11±0.02^{a}	4.08±0.26^{a}	48.87±1.45^{ab}
Control	0.5mL Normal Saline	6.99±0.91^{a}	0.75±0.60^{c}	0.23±0.04^{b}	8.31±0.51^{c}	46.87±1.49^{a}

The mean ± standard deviation of three replicates

Values with same alphabet as superscript in a column have no significant difference at $p<0.05$

4.1.12: Effect of sub-acute oral administration of methanolic extract of *P. amarus* whole plant on serum electrolytes in rats.

Fluctuations in the heomostastic parameters was observed in the animals treated with *P. amarus* extracts at vaying concentration as seen in Table 4.15.

Table 4.15: Effect of sub-acute Oral Administration of Methanolic extract of *P. amarus* whole plant on serum electrolytes in rats

	Parameter			
Group	**Sodium ion**	**Potassium ion**	**Chloride ion**	**Bicarbonate ion**
Group 1	171.75 ± 1.33^{b}	6.89 ± 0.49^{b}	81.80 ± 1.56^{b}	31.00 ± 0.82^{c}
Group 2	180.40 ± 8.99^{bc}	8.75 ± 0.65^{b}	90.00 ± 2.49^{c}	27.40 ± 1.09^{b}
Group 3	191.34 ± 1.14^{c}	14.06 ± 1.76^{c}	65.73 ± 4.35^{a}	21.86 ± 1.54^{a}
Control	140.38 ± 8.95^{a}	4.80 ± 0.42^{a}	72.44 ± 1.95^{b}	26.11 ± 0.76^{b}

4.1.13 Histology of the wister rat kidney after 21 days administration of methanol extract of P. amarus.

Plate II showed the micrographic of kidney of wister rats treated with *P. amarus* (chamber bitters) methanolic extract. A is a normal control, while B received 10 mg/mL as lower dose, C. a micro graph that receive 300 mg/mL and D. receives high dose of 600 mg/mL.

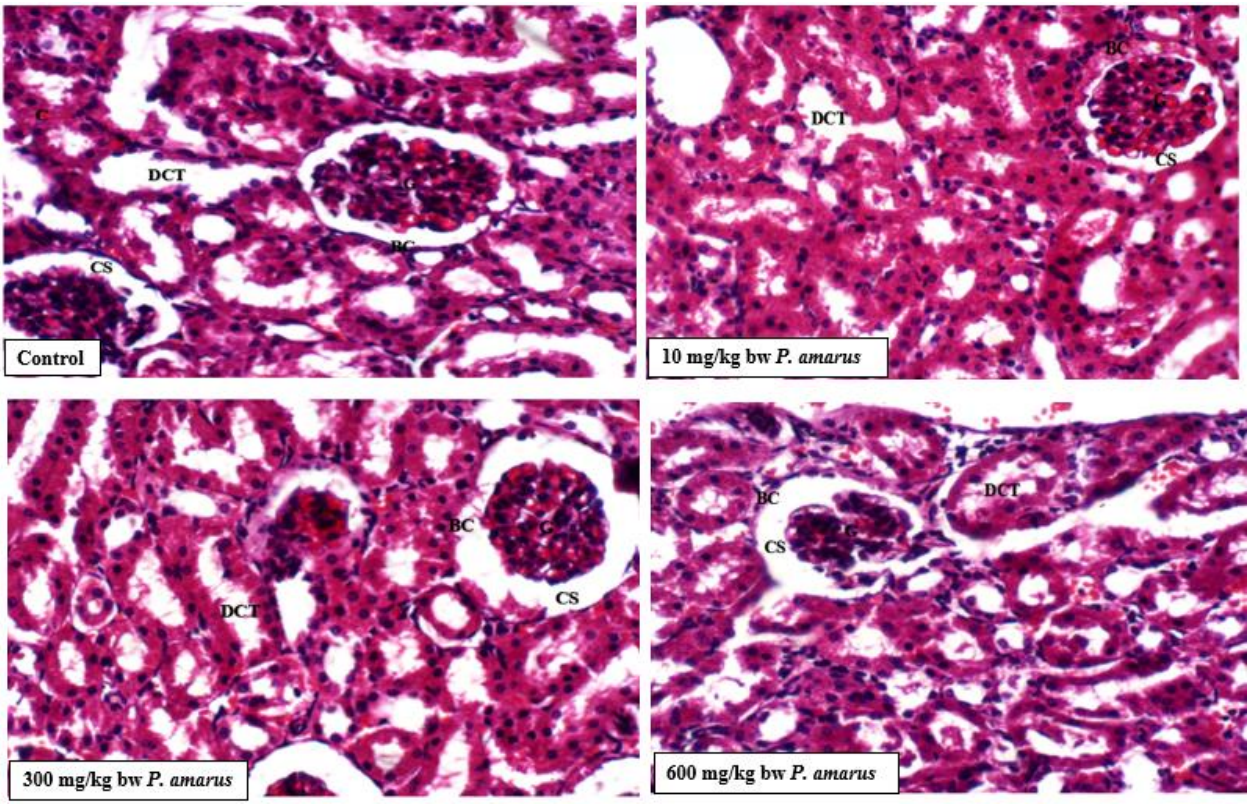


Plate II: Micrograph of kidneys of rats treated with *P. amarus* methanol extract and control, (A) is normal control while (B) is low dosage of 10mg/kg/bwt (C) shows the moderate dosage of 300mg/kg/bwt and finally (D) is high dosage of the extract at 600mg/kg/bwt of the animals.

Key: G= Glomerelus, BC= Bowman's capsule, CS= Capsular space, DCT= Distal covulated tubule.

4.2. Discussions

Phyllanthus amarus is a well-known medicinal plant with health benefit against many ailments. These health benefits are mainly due to the presence of many active phytochemicals in various parts of the plant Adegoke *et al.*, (2010); Ogbuonu *et al.*, *(*2011); Shanmug*am et al*. (2014); Alebiosu and Yusuf (2015); Danladi *et al*. (2018); Vaghela and Gupta (2019). In this study the Extraction yield of the *Phyllanthus amarus* whole plant was determined using four solvents, methanol which was used for extracting crude yield 18.48g and has the highest yield of extract. The n-Hexane has (4.85 kg) yield, followed by Ethyl-acetate (2.79 kg) and Acetone (2.27 kg). From the result it is evident that the recovery yield is dependent on the type of solvent used and its polarity. According to Lolita *et al*. (2012) and Dent *et al*. (2013) the recovery of polyphenols from plant materials is influenced by the solubility of the phenolic compounds in the solvent used for extraction. In the current research four solvents with different polarity were used, and they can be arranged as follows starting from the more nonpolar solvent (Methanol< n-Hexane < Ethyl-acetate <Acetone). Obtained results showed that yield generally increased with decreasing polarity of solvents.

The result of the phytochemical screening indicated the presence of secondary metabolites including Phenols, Tannins, Saponin, Flavonoids and Alkaloids at varying concentrations in all the solvents used for extraction. This shows that the composition of the extract is essentially the same using different methods of extraction. Similar finding was recorded by Patel *et al*. (2011); Verma *et al.* (2014); Egbon *et al*. (2017); Oladosu *et al*. (2019) all reported the presence of similar secondary metabolite in *Phyllanthus amarus* extract. These metabolites are known to have medicinal and physiological activity Alebiosu and Yusuf (2015); Shanmugam *et al*. (2014). Presence of these metabolites suggests great potential for the plant as a source of useful phytomedicines Umoh *et al.* (2013); Egbon *et al*. (2017).

Many investigations and reports highlighted the antimicrobial activity of *P. amarus* in the management of various diseases. Adegoke *et al*. (2010); Saranraj (2012). The result of the antimicrobial activity of *Phyllanthus amarus* extract showed activity against *Salmonella typhi* and *Salmonella paratyphi* B at all concentration used. The efficacy of the plant extract evaluated was concentration dependent; as the antimicrobial activity increases with increasing concentration of the plant extract, indicated by the increasing diameter of each inhibition zone. This result is consistent with the results observed by Adegoke *et al*. (2010); Danladi *et al.* (2018); Oladosu *et al*. (2019) with few exceptions they also reported increasing inhibitory effect of *Phyllanthus amarus* extract against various gram negative pathogens as the concentration of the extract increases. The antimicrobial activity of plant extracts is usually attributed to the phytochemical constituent they possess. Although the specific roles of the various phytochemicals observed were not investigated in this study, previous studies have shown Tannins to have antibacterial activity due to their basic nature that allows them to react with proteins to form stable water soluble compounds thereby inhibiting bacteria by directly damaging its cell membrane Mohamed *et al*, (2010). Flavonoids and Alkaloids have also been reported to have antimicrobial properties Maria *et al*. (2009); Bakri *et al*. (2010).

The MIC and MBC results from this study indicate that the crude *Phyllanthus amarus* extract inhibited the growth of the detected *Salmonela* isolates. The Minimum Inhibitory Concentration (MIC) of *Salmonella typhi* and *Salmonella paratyphi B* was detected at 7.8 mg/mL and 9.3 mg/mL respectively. While the Minimum Bactericidal Concentration (MBC) for both *Salmonella typhi* and *Salmonella paratyphi* B was detected at 15.6 mg/mL and 18.7 mg/mL respectively. This result is similar to the findings of Babatunde *et al*. (2014); Pathmavathi and Thamizhiniyan (2016); Pathak *et al*. (2017); Oladosu *et al*. (2019). who reported lower MIC and MBC values. Plant extract are considered to have a good inhibitory

activity, if they present MICs ≤ 100 mg/ml, a moderate inhibitory activity when MIC ranges between 100 to 500 mg/ml, a weak inhibitory activity if MIC value ranges between 500 to 1000 mg/ml and no inhibitory activity when > 1000 mg/ml Tanaka *et al*. (2005); Natarajan *et al*. (2014). Considering this report the MIC and MBC values recorded from the antibacterial activity of the present investigation is a good inhibitory activity.

The different solvents of *Phyllanthus amarus* extract produced no activity at all, on both species of *Salmonella* at different concentrations This is contrary to the findings of Adegoke *et al*. (2010); Shanmugam *et al*. (2014); Senjobi *et al*. (2017); Oladosu *et al*. (2019). In their various studies antibacterial activity of various fraction *Phyllanthus amarus* and other plant extracts was observed. The differences in the antimicrobial properties of the same plant species may be due to the fact that geographical areas of the plants play a role in the availability of the bioactive secondary metabolites in plants, The active metabolites may also be generated only during specific developmental period of the plant, and also these compounds partition exclusively in particular solvents Wetungu *et al*, (2014).

Toxicity studies of plant extracts in appropriate animal models are carried out to assess its potential health risk to humans Asare *et al*, (2011). Acute oral toxicity test, Limit Test and Sub-acute toxicity test was carried out in accordance with the organization for economic co-operation and development guidelines 401 and 407 respectively.

In the acute oral toxicity study no sign of toxicity of any kind or death was recorded in the test animals at 10, 100 and 1000 mg/kg/bw dosage. Also no signs of toxicity or death was recorded in the test animals during the Sub-acute toxicity phase of the experiment were the animals were fed higher dosage (1900, 2600 and 5000 mg/kg/bw) of *P. amarus* methanolic extract. The LD_{50} value could not be determined because the largest dose recommended in the study (1,000 mg/kg) did not show any toxicity. It is therefore assumed that the LD_{50} value of

Phyllanthus amarus extract in this study was more than 1,000 mg/kg. The present animal study results reveals that *Phyllanthus amarus* does not produce any major acute toxicity. Hence, *P. amarus* could be generally regarded as safe. These results are comparable with animal study done by Lawson-Evi *et al*. (2008); Oduola *et al*. (2018); Umar *et al*. (2019); Ranjita (2020) who all conducted acute oral toxicity study on *Phyllanthus amarus* extract using the limit test procedure according to OECD Test Guidelines in mice which also revealed there were no acute and sub-acute toxicity.

The results obtained for effect of *Phyllanthus amarus* extract on bodyweight of rats indicates that there was no significant difference in bodyweight of test animal at various doses of the extract. Changes in bodyweight have often been used as an indicator of toxicity of substance Adomi *et al*, (2017). Increase in bodyweight is an indication of inflammation, while reduction in the same parameter can be attributed to cellular constriction. As such, lack of any significant change in bodyweight as observed in this study is a likely indication that the extract is non-toxic.

Hematological parameters, Packed Cell Volume, Mean Cell Volume, Mean Cell Hemoglobin and Mean Cell Hemoglobin Concentration did not show significant difference from there controls. While slight difference was observed for other hematological parameters. Similar findings was reported by Nwankpa *et al*. (2014); Adomi *et al*. (2017); Kolawole *et al*. (2019) The non-significant effect of the *P. amarus* extracts on hamatological parameters might be due to the fact that there was no destruction of matured red blood cells Adomi *et al*, (2017).
Liver integrity is often determined by measuring the blood levels of Aspartate transaminase (AST), Alanine transaminase (ALT) and Alkaline phosphate (ALP) Arsad *et al*, (2013). Aminotransferases are markers of hepatocellular damage and injury to the liver, which eventually results in an increase in their serum concentrations. Both aminotransferases are

highly concentrated in the liver. Aspartate transaminase (AST) and Alanine transaminase (ALT) are released into the blood in greater amounts when hepatocytes are damaged (Aragon and Younossi, 2010). In this study, although slight difference in the AST and ALT levels of the treated rats in acute and sub-chronic toxicity study when compared to their respective control was observed; it does not necessarily translates to hepatocellular damage in Wistar rats.

Albumin and proteins involved in secondary haemostasis and fibrinolysis, they are exclusively synthesized by the liver, and their plasma concentrations are, therefore, used as indirect indicators of liver synthetic function. In liver disease, there is a decrease in the synthesis of albumin and coagulation factors (Oduola *et al*, 2018). Although slight fluctuation in plasma total protein and albumin levels of experimental rats was observed in this study ingestion of the extract did not impair the hepatic synthetic function in the animal.

Both liver and kidney histology of wister rats were preserved at the end of the trial. This further confirmed the safety of methanol extract of *P. amarus* on lower doses as this result agrees with the previous results of several researchers such as Adeneye (2006) and Akinjogula *et al*. (2010) which considered the *P. amarus* to be non-toxic at low doses. Though Mamza *et al*, (2015) reports was based on the oral administration of the ethanol extract of *P. amarus* stem. Since there is no mortality rate or injury recorded during this study contradictorily, doses above 2500 mg/Kg bw was reported to demonstrate histo-pathological changes in other study Guha, (2010). Similarly, Adomi e*t al*. (2017) observed minor abnormalities of histopathology of the kidney and liver after both acute and sub-acute administration of aqueous leaves extract of *P. amarus* at doses 2000, 4000 and 8000 mg/kg /bw. This contradictory result is not quite surprising as the dosages chosen are high against recommended dosage for sub-acute toxicity. Based on the above observations. *P. amarus*

extract inhibition on salmonella species (*Salmonella typhi* and *Salmonella paratyphi B*) and its non-toxic effect on both liver and kidney of Wister rats at lower doses. We can conclude that *P. amarus* has the potential to be a novel drug in the treatment of various diseases as reported by Pathak (2017) and Gupta and Vaghela (2019) .

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

Phylathus amarus contains significant phytochemicals that have antimicrobial potential. Phytochemicals such as Saponin (67.81 %) and Tannin (16.92 %) were found to be the major constituents of the plant extract. P *amarus* extract effect was confirmed though in dependent concentrations. Histopathology studies of both liver and kidney shows no toxic effect of *P. amarus* at a low dosage

5.2 Recommendations

i. Upon further confirmation, *p.amarus* can be used in the treatment of *Salmonella* infection as it contains several phytochemical component that are known to exert effect on bacteria and the toxicity effect is low.

ii. Further studies are recommended to investigate, the most effective method of extraction of active phytochemicals of phyllanthus amarus plant.

iii.Since in this present study a whole plant was used, future studies are needed to identify the plant parts that has the highest concentration of the relevant phytochemical that is most effective against *Salmonella* species.

REFERENCES

Abu, F., Taib, M.C.N., Moklas, M.M.A & Akhir M.S (2017). Antioxidant properties of crude extract, partition extract, and fermented medium of *dendrobium sabin* flower. *Journal of Evidence Based Complementary and Alternative Medicine*, 2017(4), 1-9.

Adegoke, A. A., Iberi, P. A., Akinpelu, D. A., Aiyegoro, O. A. & Mboto, C. I. (2010). Studies on phytochemical screening and antimicrobial potentials of *Phyllanthus amarus* against multiple antibiotic resistant bacteria. *International Journal of Applied Research in Natural Products*, 3, 1- 6.

Adeneye, A. A., Benebo, A. S.& Agbaje E. O. (2006). Protective effect of the aqueous leaf and seed extract of *Phyllantus amarus* and alcohol induced hepatotoxicity in rats. *West African Journal of pharmacology and drug resistance*, 42 (50), 22-23.

Adomi, P. O., Owhe-Ureghe, U. B. & Asagba, S. O. (2017). Evaluation of the toxicity of *Phyllanthus amarus* in wistar albino rats. *African Journal of Cellular Pathology*, 8, 27-35.

Ahmad, F. & Alam G. (2003). Saponin and their role in biological process in plants. phytochemical review. *Springer science & business media Dordrecht*, 12, 877-893.

Ahmad, M., Kaloo, Z. A., Ganai, B. A., Ganaie, H. A. & Singh, S. (2016). Phytochemical screening of *meconopsis aculeata royle* an important medicinal plant of kashmir imalaya: A perspective. *Research Journal of Phytochemistry*, 10 (1), 1-9. review paper.

Ajuru, M.G. & Gospel Ajuru, L.F.W. (2017).Qualitative and quantitative phytochemical screening of some plant used in ethnomedicine in Niger delta region of Nigeria. *Journal of food and Nutrition Sciences*, 5(5), 198-205.

Akinjongula, O. J., & Eghafona, N.O. (2010). Antibacterial activity of ethanolic extracts of *phyllanthus amarus* against extended spectrum lactamase producing *Escherichia coli* isolated from ssstool sample of HIV patients. *African journal of pharmacy andpharmacology*, 4 (6), 402 – 407.

Akinyemi, K.O., Oyefolu, A.O., Mutiu, W., Iwalokun, B.A., Ayeni, E.S., Ajose, S.O & Obaru, S. K .(2018).Typhoid fever tracking the Trend In Nigeria. *American Journal of Tropical Medicine Of Hygiene*, 99(3), 41-47.

Alebiosu, C.O. & Yusuf, A.J. (2015). Pyhytochemical screening, thin-layer chromatographic studies and UV analysis of extracts of *citrullus lanatus. Journal of Pharmaceutical, Chemical and Biological Sciences*, 3(2), 214-220.

Altemimi, A., Lakhssassi, N., Baharlouei, A., Watson, D.G. & Lightfoot, D.A. (2017). Phytochemicals: extraction, isolation and identification of bioactive compound from plant extract. *Plants* (Basel), 6 (4), 42.

Andoh, L.A., Shabana, A., John, E.O., Obiri-Danso , K., Mercy, J.N., Japheth ,A.O.,Lisa, B. &Anders, D.(2017).Prevalence and characterization of *Salmonella* among humans in Ghana. *Journal of Tropical Medicine and Health*, 45, 1-3.

Andrews, J. R. & Ryan, E. T. (2016). Diagnostic for invasive salmonella infection currect challenges and future directions. *Vaccine*, 19:33 (3), 8-15.

Aparecida, M. M. (2019). Botanic, phytochemistry and pharmacological aspect of *phyllanthus amarus Schum& Thonn* as powerful tools to improve its biotechnological studies. *Annals of chemical science research*, 1(2), 510.

Aragon, G. &Younossi, Z. M. (2010). When and how to evaluate mildly elevated liver enzymes in apparently healthy patient. *Cleveland Clinic Journal of Medicine*, 77 (3), 195-204.

Arsad, S. S., Esa, N. M., Hamzah, H. & Othman, F. (2013). Evaluation of acute, subacute and subchronic oral toxicity of *Rhaphidophora decursiva* (Roxb.) *Schott* extract in male Sprague Dawley rats. *Journal of Medicinal Plant Research*, 7(41), 3030-3040.

Asare, G. A., Addo, P., Bugyei, K., Gyan, B., Adjei, S., Otu-nyarko, L. S., Wiredu, E. K. & Nyarko, A. (2011). Acute toxicity studies of aqueous leaf extract of *Phyllanthusniruri*. *Journal of InterdisciplinaryToxicology*, 4(4), 206-210.

Baba, A. S. & Mallik, A. S. (2014). Determination of total phenolic and flavonoid content, antimicrobial and antioxidant Activity of a root extract of *arisaema jacquemontii Blume*. *Journal of Taibah University for Science*, 9(4), 449-454.

Babatunde, S. K., Abubakare, A. A., Abdulraheem, Y. J.& Ajiboye, E. A. (2014). Antimicrobial activity of *phyllanthus amarus* on some human intestinal facultatively anaerobic flora. *International Journal of Medicine and Biomedical Research*, 3(1), 52-57.

Bakri, A. (2010). Antiplasmodial activity of some medicinal plants used in sudanese folk-medicine. *Environmental Health Insights*, 4(4),1-6.

Balouri, M., Moulay, S .& Saad, K.I. (2016). Methods for in vitro evaluating antimicrobial activity : *A review Journal of pharmaceutical Analysis,* 6(2),71-79.

BaoDuy, N. L., Trang, D. T. & Trang, M.N. (2015). Preliminary phytochemical analysis of leaf extract of *Thuja orientalis* (L) ENDL. *International Journal of Research Science and Management*, 2(1) ,1-5.

Brenner, F.W., Villar, R.G., Angulo,F.J., Tauxe, R. & Swaminathan, B. (2000). *Salmonella* nomenclature. *Journal of clinical microbiology,y* 38 (7), 2465 – 2467.

Bribi, N. (2018). Pharmacological activity of alkaloids. A review. *Asian journal of Botany*, 1,1-5.

Britannica, T. Editors of encyclopaedia (2018). *Niger Encyclopedia Britannica. https://www.*britannica.com/place/niger-state,nigeria.

Bythwood, T.N., Vivek, S., Karen, L., Harley-Bacon, A., Maggie, D., Lee, C. H., Susan, S. & John, J.M (2019).Antimicrobial resistant *Salmonella* enterica *Typhimirium* colonizing chickens, the plasmid, genotype, bacterial communities and antibiotic administration on resistance. *Froniers Sustanable Food Sytertem,* 3(20), 20.

Collins, C.H., Lynes, P.M., Grange, J.M. & Falkinham, J.O. III. (2004). *Microbiological methods Salmonella* and *Shigella.* London, Oxford University press inc. pp 96-100

Crump, J.A., Sjolund, M. K., Melita, A., Gordon, C. & Perry, M. (2015). *Salmonella* epidimology, clinical presentation, laboratory diagnosis antimicrobial management of invasive salmonella infection Clinical Microbiology review. *Journal of American society for Microbiology*, 28(4), 901-937.

Danladi, S., Idris, M.A. & Umar, II. (2018). Review on pharmacological activities and phytochemical constituents of *phyllanthus amarus*. *Journal of Phytopharmacology*, 7(3), 341-348.

De las Heras, B., Rodrigues, B., Boska, L., & Villar, A.M. (2003). Terpenenoids: Sources,structure elucidation and therapeutic potential in inflammation. *Current Topic in Medicinal Chemistry*,3(1), 56-67.

Dent, M., Dragović-Uzelac, V., Penić, M., Bosiljkov, T. & Levaj, B. (2013). The effect of extraction solvents, temperature and time on the composition and mass fraction of polyphenols in Dalmatian wild sage (*Salvia officinalis L.)* extracts. *Food technology and biotechnology*, 51(1), 84-91.

Devi, S., Rashid, R. & Kumar, M.N. (2017). Phytochemistry and Pharmacological properties of *phyllanthus amarus* schum. A review. *Journal of Pharma innovation*, 6(12), 169-172.

Dhongade, H.& Chandewar, A. (2013). Pharmacognostical and phytochemical studies of *Phyllantus amarus* leaves. *International of Biomechemcial and Advance Research,* 4, 383.

Earnest, O.E., Chibueze,P. I. & Emmanuel, E. II. (2018). Advances in acute toxicity testing: strengths, weeknesses and regulatory acceptance.*Interdisciplinasry toxicology*,11(1), 1-5.

Edeoga, H. E., Okwu, D. E. & Mbaebie, B. O. (2005). Phytochemical constitutes of some Nigerian medicinal plants. *African journal of Biotechnology,* 4(7), 685-688.

Egbon, E. E., Olayioye, E. Y., Olayioye, S. A., Adesuyi, A. T. & Owolabi, T. (2017). Phytochemical screening and proximate composition of *Phyllanthus amarus. International Research Journal of Plant Science*, 8(1), 9-12.

Egwari, L. O. & Abah, S. E. (2011). Method of extraction and antimicrobial susceptibility testing of Plant extracts. *African Journal of Basic and Applied Sciences,* 3(5), 205-209.

El Aziz, M. M., Ashour, A. S. & Melad, A. S. S. G. (2019). A review on saponin from medicinal plants; chemistry, isolation, and determination. *Journal of Nanomedicine*, 8(1), 282-288.

Enegide, C., Davide, A. & Fidelis, S. A. (2013). A new method for determining acute toxicity in animal models. *Internationa*l *Journals of Toxicology*, 20(3), 224 – 226.

Eng, S., Priya, P., Nurul-syakima, A., Hoo-leng, S., Kok-gan, C. & Learn-han, L. (2015). *Salmonella:* A review on pathogenesis, epidemiology and antibiotic resistance,*Frontiersinlifescience*, 8(3), 284-293.

Faizal, A. & Geelen, D. (2013). Saponin and their role in biological processes in plants. phtochemical review. *Journal of Springer,* 12, 877-893.

G.I.S, Achievers. (2021).Spatial distribution of features in Minna. *Study Area Map.* Https:gisachievers.com.

Giannella, R. A. (1996). Salmonella. In: Baron, S. (Ed.) *Medical Microbiology*, 4th Ed., Galveston, University of Texas Medical Branch. Pp 1-7.

Grabmann, J. (2008) .Terpenoids as plant oxidants. *Vitamin and hormones* 72, 505-535.

Guha, G., Rajkumar, U., Ashok, K. R. & Mathew, L. (2010).Aqueous extract of *Phyllanthus amarus* inhibit chromium (vi) induced toxicity in MDA-MB 435S cells. *Journal Food and Chemical Toxicology*. 48, 396-401.

Gupta, M. & Vaghela, J.S. (2019). Recent advances in pharmaceutical and phytochemistry studies on *phyllanthus amarus. Journal of Phamaceutical and Biosciences,* 7(1),1-8.

Harborne, J. B. (1973). Phytochemical methods: *A guide to modern techniques of plant analysis*. London, Champam and Hall Ltd. Pp. 279.

Ifeanyichukwu, I., Chika, E., Ogonna, A., Chidinma, I., Monique, A., Ikechukwu, M., Stanley, E., Emmanuel, N., Ngozi, A. & Agabus, N. (2016). Prevalence and antibiogram of *Salmonella* species isolated from poultry products. *Journal of Advanced Veterinary and Animal Research*, 3(4), 353-359.

Islam, A., Selvan, T., Mazunder, U.K., Gupta, M.& Sibnath, G.(2008).Antitumor effect of *Phyllanthin* and *Hypophyllanthin* from *Phyllanthus amarus* against ehrlich ascites carcinoma in mice .*Journal of pharmacological online*, 2, 796-807.

Itelima, J.U. & Agina, S.E. (2014). Invo anti-microbial activity of plant species on *Escherichia Coli* 0157:h7 inoculated into albino rats. Research article, *Journal of world microbiology,* 1(1), 2-9.

Joseph, B. & Raj, S.J.(2011). An overview pharmacognostic property of *Phyllanthus amarus Linn. International Journal Pharmacolog*,1(41), 1-6.

Jyoti, S., Meena, R. A. & Rashmi, R. (2018). A review on the phytochemical properties of *phyllantus amarus. schum and thons. Journal of pharmaceutical sciences and research* , 9(4),1377-1386.

Kaur, R., Tushar, M. & Harpreet, K. (2017). Plant derived alkaloids. *Haya the Saudi Journal Life Science, 2* (5), 158-189.

Khalid, S., Adil, S., Neelam, B., Muhammad, A. & Pervaz, A.(2018). Phytochemical screening and analysis of selected medicinal plant in gujirat. *Journal of phytochemistry and biochemistry, 2*(1),108.

Khanbabaee, K., & Ree, T. V. (2011). Tanins; classification and definition. *Journal of natural product reports,* 18(6), 641-649.

Kolawole, A., Ndubusi, I., Ajiboye, O. & Oluwole, F. (2019). Effects of aqueous leaf extract of *Phyllanthus amarus* on liver function and blood parameters in male wistar rats. *Journal of Biological Innovation*, 8(4), 471-483.

Lawson-Evi, P., Eklu-Gadegbeku, K., Amegnona, A., Kodjo, A., Serge, M., Edmond, E. C. & Messanvi, G. (2008). Toxicological assessment on extracts of *Phyllanthus amarus Schum and Thonn. Academic Journal of Scientific Research Essay*, 3, (41),1–5.

Lim ,Y.& Murtijaya, J. (2007). Antioxidant properties of *Phyllanthus amarus* extract as effected by different drying methods. *Food science and technology,* 40(9), 1664 -1669.

Lolita, T., Zanda, L. & Ruta, G. (2012). Comparison of different solvents and extraction methods for isolation of phenolic compounds from horseradish roots (*Armoracia Rusticana*). *World Academy of Science, Engineering and Technology*, 64, 903-908.

Lorke, D. (1983). A new approach to practical acute toxicity testing. *Achieves of Toxicology*. 54(4), 275 –2 87.

Maciel, B., Maria, R. F. L., Conserva, L., Andrade, V., da Rocha, E. and Lemos, R.s (2009). Studies on the antimicrobial activity and brine shrimp toxicity of *Zeyheria tuberculosa* (Wall.) Bur. (*Bignoniaceae*) extracts and their main constituents. *Annals of Clinical Microbiology and Antimicrobials*, 8, 1-16.

Mamza, U. T., Sodipo, O. A., Abdulrahman, F. I. & Khan, I. Z. (2015). Evaluation of phytochemical, antimicrobial and toxicity studies of ethanolic stem extract of *Phyllanthus amarus thonn and schum* (Euphobiaceae) *International journal of science and Technology,* 4(.1), 21 – 50.

Mazunder, A., Mahato, A. & Mazunder, R. (2006*).* Antimicrobial potentials of *phyllanthus amarus* against drug resistance pathogens. *Natural Product Research*, 20(4), 323-326.

Mohammed, H., Hansi, P. D. & Kavitha, T. (2010). Antimicrobial activity and phytochemical analysis of selected Indian folk medicinal plants. *International Journal of Pharmaceutical Sciences and Research*, 1(10), 430-434.

Muktar, Y., Galalain, A. M. & Yunusa, U. M. (2019). A modern overview on diabetes *mellitus*; A chronic endocrine disorder. *European journal of Biology*, 4 (1), 11-14.

Mzungu, I., Helen, I., Stephen, O. O. & Maryam, A. (2016).Antibiotic suscepbilities of *Salmonella* species prevalent among children of 0-5years with diarrhea in Katsina state Nigeria .*Achives of Medical and Biomedical research*, 3(1), 39-51.

Natarajan, D., Srinivasan, R. & Shivakumar, M. S. (2014). *Phyllanthus wightianus Müll. Arg*.: A Potential Source for Natural Antimicrobial Agents. *International Journa of lBioMedical Research*, 2014 (135082), 1-9.

Nesa, M.K., Khan, M.S.R.& Alam, M.(2011). Isolation,iIdentification and characterizations of *Salmonells Serovars* from diarrheic stool samples of human. *Bangladesh journal of vetenary*, 9(1), 85-93.

Ngogo, A. F., Agricola, J., Ahmed, M., Abade, S. F., Runusha, M. M. Mizinduko. & Mtebe, V.M. (2020). Factors asssociated with *Salmonella* in patients with gastro intestinal complaints seeking health care at reginal hospital in southern high land of Tanzania. *Journal of Biomedical and Infection diseases*, 20, 135.

Notka, F., Meier, G. R. & Wagner, R. (2003). Inhibition of wild type human immune-defieciency virus and reverse transcriptase in hibitor resistant variants by *Phyllanthus amarus*. *Journal of Antiviral Research*, 58 (2), 175 - 186.

Nwabor, O. F., Ihenriochi, D. D. & Queensley, C. A. (2015). Epidemiology of *Salmonella* and *Salmonelosis. International Journal of Letters of Natural Science,* 47, 54-73.

Nwankpa, P., Agomuo, E. N., Uloneme, G. C., Egwurugwu, J. N., Omeh, Y. N. & Nwakwuo, G. C. (2014). Effect of *Phyllanthus amarus* leaf extract on alterations of haematological parameters in *Salmonellae typhi* infested wistar albino rats. *Scientific Research and Essays*, 9(1), 7-12.

Ochei, J. & Kolhatkar, A. (2007). *Medical Laboratory Science: Theory and Practice*. New York: Tata McGraw-Hill.pp 730.

Oduola, T., Abubakar, A. M., Fatimah, A., Mardiyya, T., & Shehu, O. O. (2018). Hepatotoxic Assessment of *Phyllanthus amarus* Leaf extract in Wister rats. *European Journal of Medicinal Plants*, 23(4), 1-11.

Oduola, T., Bello, I., Idowu, T., Avwioro, G., Adeosun, G. & Olatubosun, L. (2010). Histopathological changes in wister albino rats exposed to aqueous extract of unripe carica papaya. *North American journal of* Medical *Science*, 2(5), 234.

Okuda, T. & Ito, H. (2011). Tanins of constact structure in medicinal and food plants hydrozable tannin's and polyphenols related to tannins. *Journal of Molecules,* 16, 2191-2217.

Oladosu, S. A., Coker, A. O. & Nwaokorie, F. (2019). Antibacterial effects of *Phyllantus amarus* on urinary tract pathogens. *International Journal Clinical Pathology*, 7(1),1-10.

Oluwafemi, F.& Dabiri, F. (2008). Antimicrobial effect of *phyllanthus amarus* and *paerquentina nigrescence* on *Salmonella typhi. African Journal of Biomedical Reseach*, 11(2), 215-219.

Panchea, A.N., Diwan, A.D. & Chandras, S.R. (2016). Flavonoids: An Overview. *Journal of Nutritional Science*,5(47),1-15.

Patel, J. R., Priyanka, T. B., Vikas, S., Nagendra, S. C.& Vinod, K. D. (2011). *Phyllanthus amarus*: Ethnomedicinal uses, phytochemistry and pharmacology: A review. *Journal of Ethnopharmacology*, 138, 286–313.

Pathak, M., Singh, U. K. & Upadhyay, G. (2017). Antibacterial activity of *phyllanthus amarus* plant extract against resistant pathogenic bacterial strains: an ethnomedicinal plant. *Asian Journal of Science and Technology*, 8(9), 56725674.

Pizzi, A. (2019). Tannin: prospective and actual habit application. *Journal of Biomolecules*, 9(8), 344.

Popoff, M. Y., Bockemüh, J. & Gheesling, L. L. (2003). Supplement 2001 (No. 45) to the Kauffman white scheme. *Journal of Research in Microbiology*, 154 (3), 173-174.

Ralph, P. T. & Leslie, A, S.H. (1936). The use of semi-solid agar for the detection of bacteriasl motility. *Journal of Bacteriology, 31*(6), 573-580.

Ranijita, (2020). Acute toxicity study and phytochemical screening of *Phyllanthus amarus* ethanolic extract on wistar albino rats. *International Journal of Comprehensive and Advanced Pharmacology*, 5(2),84-86.

Ryan, M. P., O'Dwyer, J. & Chatherine, C. A. (2017). Evaluation of the complex nomenclature of the clinically and veterinary significant pathogenof *Salmonella*. *International Journal Biomedical Research*, 2017(37), 821-826.

Saio, V. & Donsyiem, S. (2015). Phytochemical analysis of some traditionally used medicinal plants of North east India. *Journal of science and Environmental today*. 1(2015),1-8.

Santos, L., Beatriz, H.L.N., Sales, M., Aurea, P.F. & Sirlei, D.T. (2019). *Flavonoids: classification, biosynthesis and chemical ecology*. Flavonoids- from Biosynthesis to Human Health, London UK. Intechopen. pp 14.

Saranraj, S. & Sivasakhivelan, P. (2012). Screening of activity of the medicinal plants of *Phyllanthus amarus* against urinary tract infections causing bacterial pathogens.*AppliedJournalofHygien*,1(3),19-24.

Senjobi, C. T., Ettu, A. O. & Otujo, C. O. (2017). Antibacterial and antifungal activities of leaf extracts of *Phyllanthus amarus Schum and Thonn. Journal of Pharmacognosy and Phytotherapy*, 9(1), 6-10.

Shanmugam, B., Shanmugam, K. R., Ravi, S., Subbaiah, G. V., Mallikarjuna, K. &Reddy, K. S. (2014). Antibacterial activity and phytochemical screening of *Phyllanthus niruri* in ethanolic and aqueous extracts. *International Journal of Pharmaceutical Sciences Review and Research*, 27(2), 85-89.

Sing, P. A. & Kumar, S. (2019). *Applications of tannin in industry;* Tannins structural properties,biologicalproperties and current knowledge.London, U.K. Intechopen. Pp 20.

Sofowora, A. (1993). *Medical plants and traditional medicine in Africa*. Ibadan, Spectrum Books. Pp191-289.

Sultan, A. & Raza, A.(2015). Steroids*:* A diverse class of secondary metabolites. *Journal Medicinal Chemistry* 5, 1-7.

Tanaka, J. C. A., Da silva, C. C., Filho, B. P. D., Nakamura, C. V., de Carvalho, J. E. & Foglio, M. A. (2005). Chemical constituent of *Luehea divaricate* Mart. (Tiliaceae*). Journal of Quimica Nova*, 28(5), 834-837.

Taylor, W. I. & Achanza, D. (1972). Catalase test protocol: The identification of *Enterobacteriacea. Journal of Applied Microbiology,* 24,58-61.

Thairu, Y., Nasir, Y. & Usman, I. A.(2014). Laboratory perspective of grams staining and its significance in investigations of infectious diseases. *African journal of medicine*. 1 (1), 68-74.

Thamizhiniyan, P. & Pathmavathi, M. (2016). Antimicrobial activity of various extracts of *Phyllanthus ambionicus* and *Phyllanthus amarus. Jo 1-urnal of Applied and Advanced Research*, 1(2), 29–35.

Tripathi, I. P. & Mishra, C. (2015). Phytochemical screening of some medicinal plants of chitrakoot region. Research paper. *Indian journal of applied research,* 5(12), 94.

Ukoha, P. O., Egbuonu, A., Cemaluk, C., Obasi, L. N. & Paul, M. E. (2011). Tanin and other phytochemicals of the *Samanaea Saman* pod and their antimicrobial activities. *African journal of pure and applied chemistry*, 5(8), 237-244.

Umar, I. S., Yusuf, A.A., Alawode, A. R., Obiekezie, C. I., Okunlola, B. M., Abdulrazaq, O. M., Ariyeloye, S. D. & Lawal, B. (2019). Phytochemical compositions and biochemical effect of *Phyllanthus amarus* in albino rat. *GSC Biological and Pharmaceutical Sciences*, 8(1),128–133.

Umoh, D., Ukana. D., Itoro, E.(2013). Phytochemical screening of nutrient analysis of *Phyllanthus amarus*. *Asian Journal of Plant Science and Research,* 3(4), 116 – 122.

Usman, H., Abdulrahman, F. I. & Usman, I. (2009). Qualitative phytochemical screening and its invitro antimicrobial effect of methanol stem bark extracts of *ficus thinning* (moraceae). *African journal of traditional complementary and alternative medicines,* 6(3), 289-295.

Ustandang, O. G. & Mazza, G. (2007). Saponin: properties, applicaton and processing critical review. *Journal of Food Science and Nutrition*, 47 (3), 231-258.

Verma, S., Sharma, S. H. & Garg, M. (2014). *Phyllanthus amarus:* A Review. *Journal of Pharmacognosy and Phytochemistry*, 3(2), 18-22.

Vincken, J. P., Heng, L . Y. & Cruppen, A. G. H. (2007). Saponin, classification of occurance in the plant kingdom. *Journal of photochemistry* ,68 (3),275-297.

Wetungu, M. W., Matasyoh, J. C. & Kinyanjui, T. (2014). Antimicrobial activity of solvent extracts from the leaves of *Tarchonanthus camphoratus.* (Asteraceae). *Journal of Pharmacognosy and Phytochemistry*, 3(1), 123-127.

Appendix A: *Phyllanthus amarus*

Figure I. *Phyllanthus amarus* whole plant in the field of University Health Services, Federal University of Technology Minna.

Appendix B: phytochemicals present in *P. Amarus* whole plant using dfferent solvents

Methanol

Methanol (Crude)	%	%	%	%	Average
Phenols	315.1243	315.7541	9.483419	9.52637	9.504895
Flavonoids	171.5734	168.08	5.163367	5.07101	5.117189
Alkaloids	50.6758	51.5647	1.525049	1.555718	1.540383
Tannins	532.884	530.712	16.03673	16.0117	16.02421
Saponin	2252.64	2248.416	67.79144	67.8352	67.81332
	3322.898	3314.527	100	100	100

Hexane

Hexane	%	%	%	%	Average
Phenols	206.9703	206.8622	9.567609	9.563975	9.565792
Flavonoids	173.1688	172.9087	8.005068	7.994184	7.999626
Alkaloids	19.0845	18.0567	0.882219	0.834825	0.858522
Tannins	852.94	852.116	39.42883	39.39635	39.41259
Saponin	911.076	912.9876	42.11628	42.21066	42.16347
	2163.24	2162.931	100	100	100

Ethylacetate

Ethylacetate	%	%	%	%	Average
Phenols	753.4134	754.8975	38.9889	39.07044	39.02967
Flavonoids	239.9799	238.0701	12.41888	12.32154	12.37021
Alkaloids	37.0978	37.1734	1.919799	1.923945	1.921872
Tannins	595.796	596.012	30.83225	30.84717	30.83971
Saponin	306.092	305.992	15.84016	15.83691	15.83853
	1932.379	1932.145	100	100	100

Acetone

Acetone	%	%	%	%	Average
Phenols	964.746	971.308	35.26286	35.45519	35.35902
Flavonoids	138.1112	136.3644	5.048164	4.977644	5.012904
Alkaloids	42.4568	43.7645	1.551857	1.597515	1.574686
Tannins	538.144	538.424	19.66994	19.65383	19.66189
Saponin	1052.412	1049.676	38.46718	38.31582	38.3915
	2735.87	2739.537	100	100	100

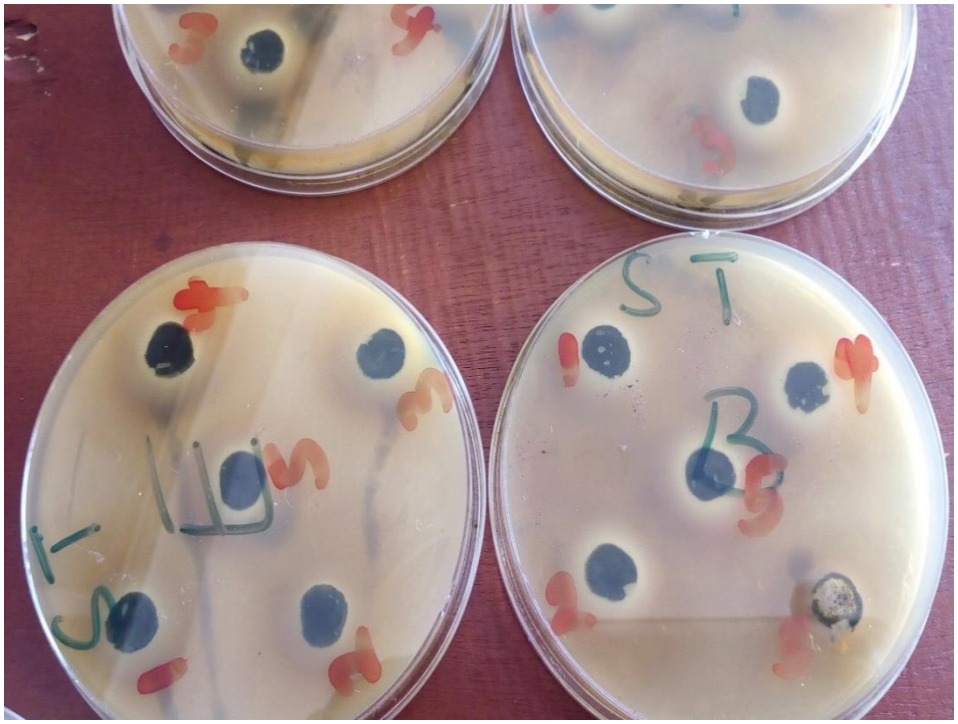
Summary of phytochemicals present in *P. amarus* whole plant in four different solvents *(100 g/mg).*

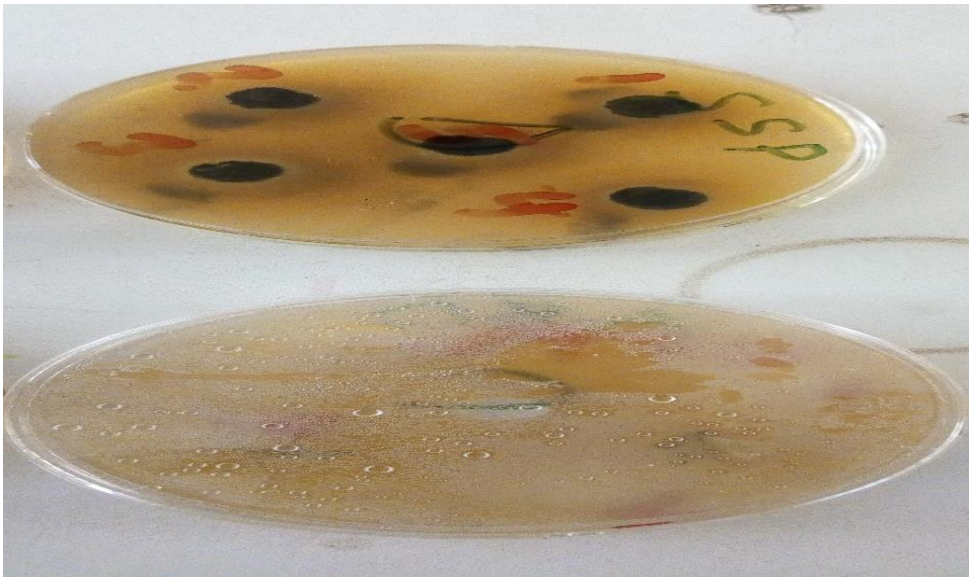
Sample	Phenols	Flavonoids	Alkaloids	Tannins	Saponin
CRUDE	315.3243	171.5734	50.6758	532.884	2252.64
	315.7541	168.08	51.5647	530.712	2248.416
	315.5392	169.8267	51.12025	531.798	2250.528
HEXANE	206.9703	173.1688	19.0845	852.94	911.076
	206.8622	172.9087	18.0567	852.116	912.9876
	206.9163	173.0388	18.5706	852.528	912.0318
ETHYLACETATE	753.4134	239.9799	37.0978	595.796	306.092
	754.8975	238.0701	37.1734	596.012	305.992
	754.1555	239.025	37.1356	595.904	306.042
ACETONE	964.746	138.1112	42.4568	538.144	1052.412
	971.308	136.3644	43.7645	538.424	1049.676
	968.027	137.2378	43.11065	538.284	1051.044

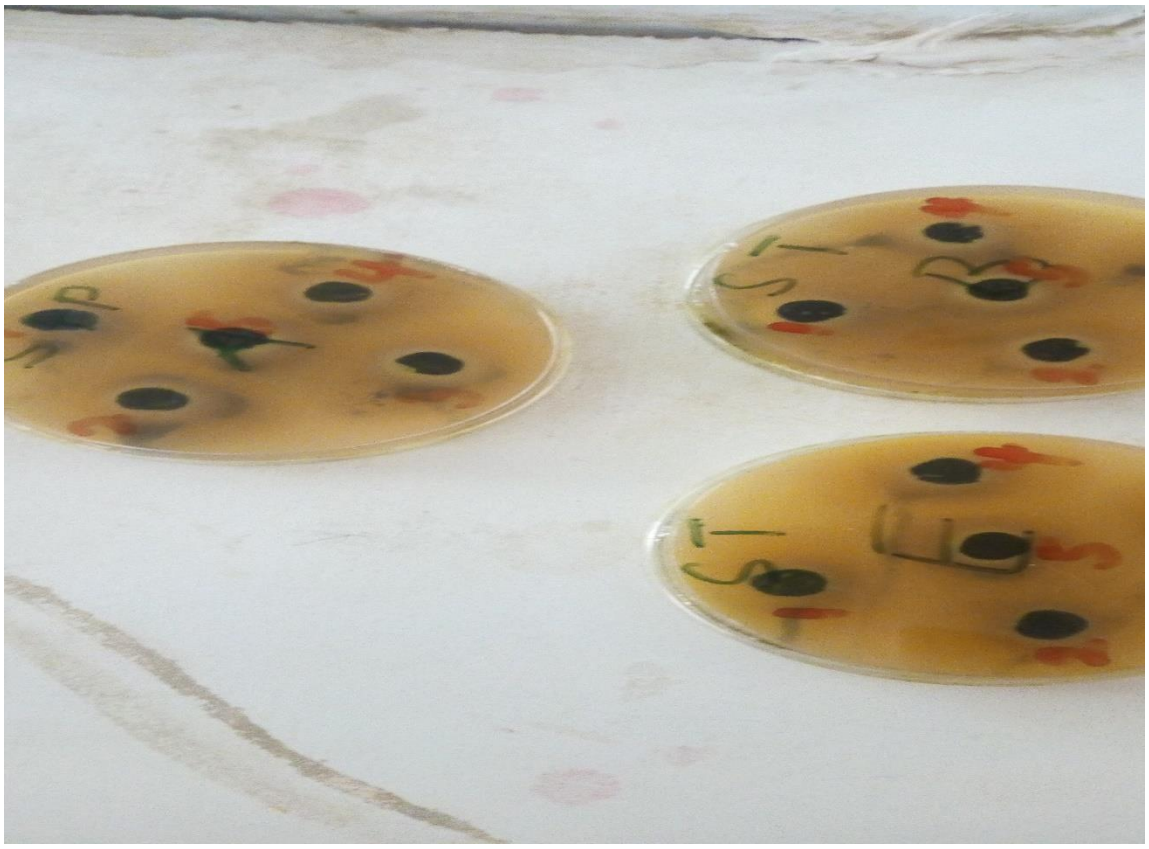
Percentage of phytochemicals present in different solvent

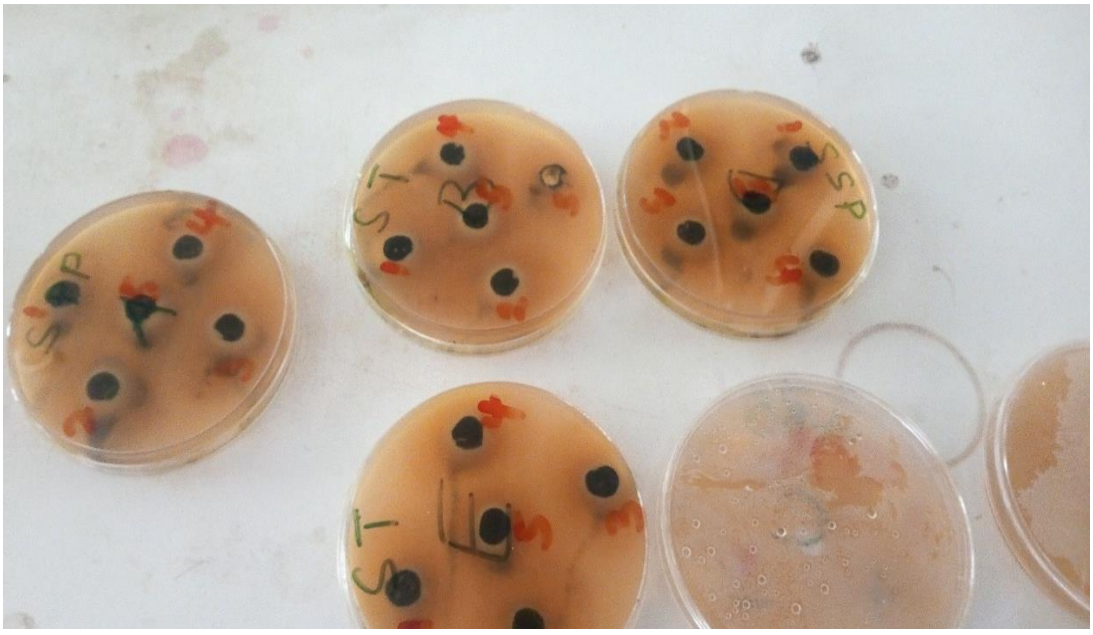
Phytochemicals	Crude	Hexane	Ethylacetate	Acetone
Phenols	9.5	9.57	39.03	35.36
Flavonoids	5.12	8	12.37	5.01
Alkaloids	1.54	0.86	1.92	1.57
Tannins	16.02	39.41	30.84	19.66
Saponin	67.81	42.16	15.84	38.39

Appendix C: Anti-bacterial activity of P. *amarus* against *salmonella* species (*salmonella typhi and salmonella paratyphi* B)







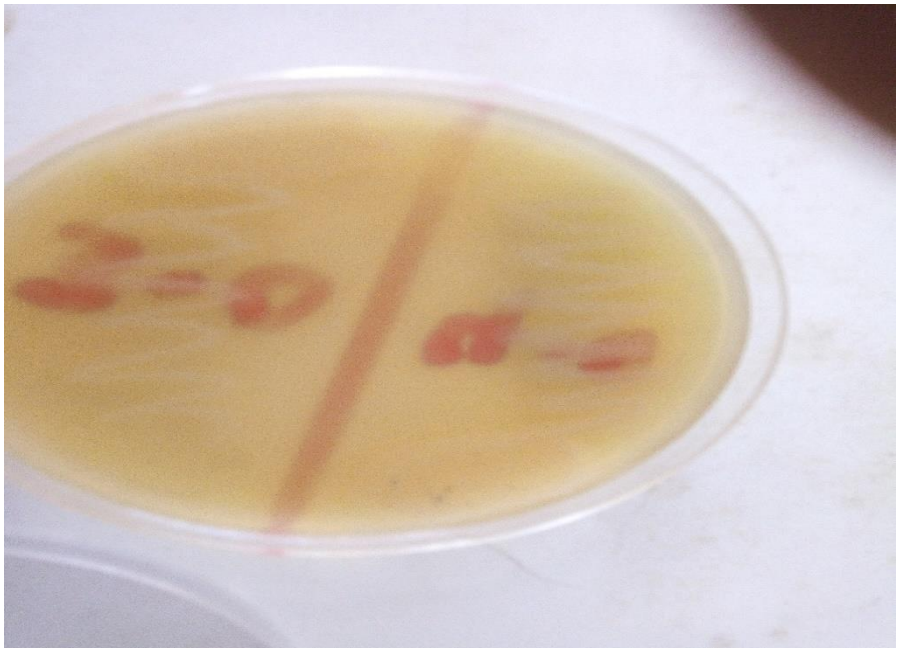


Key:

ST: *Salmonella typhi*, SP: *Salmonella paratyphi* B

1- 1.5 g, 2 – 2.0 g, 3- 2.5 g, 4-3.0 g and 5-5.0 g

Appendix D: Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC) of *P. amarus* against *Salmonella typhi* and *Salmonella paratyphi* B at different extract concentration



Appendix E: Haematology of wister rats fed with different extract concentrations

	Hb	PCV	MCV	MCH	MCHC	RBCs	PLC	TWBC	N	L
G1	13	39	49	18	38	6.5	160	5.1	40	35
G1	12.5	37	53	21	40	5.8	152	5.2	43.6	36
G1	12.5	38	51	19.5	39	6.15	156	5.15	41.8	35.5
G2	14.6	45	46	19	43	7.5	168	6.1	44	40
G2	12	36	50	17	38	7.4	161	6.3	50	42
G2	13.3	40.5	48	18	40.5	7.45	164.5	6.2	47	41
G3	10.9	36	40	15	39	7.5	298	2.1	8	70
G3	12	32	50	17.5	40	5.5	270	3.2	12.4	75
G3	11.45	34	45	16.5	39.5	6.5	284	2.65	10.2	72.5
N	13.6	39	52.5	20	38.5	4.35	203	5.7	55.25	57.75
N	13.8	38	58	23	40	5.9	199	6.4	58	55.5
N	13.4	40	47	17	37	2.8	207	5	52.5	60

Key: G 1= Group 1, G2= Group 2, G3= Group 3 and N=Normal control. n

Keys: Hb: Hemoglobin, PCV: Packed Cell Volume, MCV: Mea

Appendix F: Biochemical parameters of liver and kidney functions of wister rats fed with different extract concentrations

Liver Function Tests

	Cholesterol	Triglycerides	HDL	LDL	Total protein	Albumin	AST	ALT
G1	128.4	137.38	92.48	102.88	14.36	6.37	8.31	21.48
G1	120.31	131.06	104.71	112.32	19.1	5.58	10.96	19.82
G1	124.36	134.22	97.6	107.6	16.73	5.98	9.64	20.65
G2	108.38	128.24	110.42	62.38	24.3	12.39	7.29	19.32
G2	115.9	120.31	121.32	79.93	18.48	10.84	9.78	24.09
G2	112.14	124.28	115.87	71.16	21.39	11.62	8.54	21.71
G3	159.07	156.36	102.49	131.45	30.49	14.82	21.09	23.06
G3	171.3	171.49	94.31	140.32	36.31	16.91	19.32	18.62
G3	165.19	163.93	98.4	135.89	33.4	15.87	20.21	20.84
N	142.54	131.85	118.59	116.44	19.65	10.66	14.25	18.21
N	139.68	134.3	116.36	112.49	18.47	8.89	12.41	20.38
N	145.39	129.4	120.82	120.38	20.82	12.42	16.09	16.04

Key: G 1=Group 1,G2=Group 2,G3=Group 3 and N=Normal control.

Kidney Function Tests

	Creatinine	Total bilirubin	Direct bilirubin	Uric acid	Urea	Sodium	Potassium	Chloride
G1	5.04	0.32	0.25	5.9	56.02	170.42	6.4	80.24
G1	6.41	0.38	0.3	6.31	49.21	173.08	7.38	83.36
G1	5.73	0.35	0.28	6.11	52.62	171.75	6.89	81.8
G2	6.93	0.49	0.24	5.81	50.38	171.41	9.39	70.41
G2	6.73	0.53	0.2	4.04	52.48	189.38	8.1	75.38
G2	6.83	0.51	0.22	4.93	51.43	180.4	8.75	72.9
G3	6.34	0.92	0.09	4.33	50.31	190.2	12.3	70.08
G3	7.7	0.84	0.12	3.82	47.42	192.47	15.82	61.38
G3	7.02	0.88	0.11	4.08	48.87	191.34	14.06	65.73
N	6.99	0.75	0.23	8.31	46.87	140.38	4.8	72.44
N	7.89	0.81	0.19	7.8	45.38	149.32	4.38	74.38
N	6.08	0.69	0.26	8.81	48.36	131.43	5.21	70.49

Key: G 1=Group 1,G2=Group 2,G3=Group 3 and N=Normal control.