PROPHYLACTIC ACTIVITY OF *PHYLLANTHUS AMARUS* CRUDE EXTRACTS ON SWISS ALBINO RATS

BY

NORMO, JOY IFEANYICHUKWU

MCB/11/0338

DEPARTMENT OF MICROBIOLOGY

FACULTY OF SCIENCE

FEDERAL UNIVERSITY OYE EKITI

EKITI STATE

OCTOBER, 2015

PROPHYLACTIC ACTIVITY OF *PHYLLANTHUS AMARUS* CRUDE EXTRACTS ON SWISS ALBINO RATS

BY

NORMO, JOY IFEANYICHUKWU

MCB/11/0338

A RESEARCH PROJECT SUBMITTED TO THE DEPARTMENT OF MICROBIOLOGY, FACULTY OF SCIENCE, FEDERAL UNIVERSITY OYE EKITI IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF BACHELOR OF SCIENCE (B.Sc.) DEGREE IN MICROBIOLOGY

UNDER THE SUPERVISION OF DR S.K OJO

OCTOBER, 2015

CERTIFICATION

We certify that this project work was carried out by NORMO, JOY IFEANYICHUKWU of the Department of Microbiology, Faculty of Science, Federal University Oye Ekiti under the supervision of DR S.K.OJO

DR S.K.OJO

Supervisor

03-12-2015

Date

PROF BRYAN OGENEH

Head of Department

04/12/2015

Date

DEDICATION

This work is dedicated to the Almighty God, the creator of heaven and earth, lover of my soul who gave me sound health to carry out this research work.

ACKNOWLEDGEMENTS

First and Foremost, I want to acknowledge my wonderful and amiable supervisor DR S.K.OJO for his immense help and contribution and for being able to go through this work.

My heartfelt gratitude also goes to the most loving and wonderful parents ever, Pastor and Mrs Ndidi Normo for their concern and financial contribution. I love you so much, you are the best. To someone special Daniel Izuchukwu Obah thanks for your love and concern in every way. To my loving siblings; Steven Normo, Blessing Normo, Stella Normo and Micheal Normo, I love you all.

To every members of Gospel student fellowship (GSF) FUOYE, I appreciate your love and concern, God bless you. I am grateful to Mr Abimbade, Mr Dayo, Mr Ayo of the Department of Industrial chemistry for their and the rest for their help and contribution during the period of this research, I also appreciate the Department of Biochemistry, Mr Adeoye and other lecturers in the Department of Biochemistry, God bless you all. To Corper Shola, I say a big thank you. I am indebted to all technologists Mr odun, Mrs Adebiyi and others in Department of Microbiology.

To the Head of Department of Microbiology, Prof Bryan Ogeneh and to all the lecturers in Microbiology Department, I say thank you. To all my colleagues and all members in Class 15 of the Microbiology Department, I love you all. Lastly to all my wonderful friends: Aduragbemi, Pastor Segun, Bishop, Iyanuoluwa, Samuel, Bose, Kazeem, Gabriel, Tunrayo, Jummy, Dolapo, Kemisola, Asegun and to the best group members ever; Orire, Atitebi, Victoria and Damilola for their support and contribution. I say a big thank you may the Good Lord bless and keep you for me.

TABLE OF CONTENT

LIST OF TABLE

Table **Page**

LIST OF PLATES

Plates **Page**

ABSTRACT

Herbal medicines have been part of traditional health care in most parts of the world for ages and there is increasing interest in them as sources of agents to fight microbial diseases. The aim of this study was to evaluate the prophylactic activity of *Phyllanthus amarus* plant extracts on Swiss albino rats challenged with *Staphylococcus aureus*. The methanol and ethyl-acetate extracts were administered orally to each group of the Swiss albino rats according to their body weight. Each group received a dose of 0.5ml of the sterile extracts at two different concentrations of 50mg/kg and 100mg/kg respectively which serve as pre-treatment before challenging them with *S. aureus* for 4days. The results revealed that the concentrations used as pre-treatment on the Swiss albino rats, had no toxic effect. There was no weakness, no inflammation, no fever, no discharge from the eye, no diarrhea, no redness of the eye throughout the period of the pre-treatment and no death was recorded. After challenging the Swiss albino rat with *Staphylococcus aureus*, the non-treated group (control group) and the treated group showed signs of weakness after 1hour but after 4hours, the treated group adjusted to their normal state of health while it took a long period of time before the non-treated group were able to adjust to their normal state of health. Thus, the crude extracts of *Phyllanthus amarus* possess antibacterial and prophylactic activity against *Staphylococcus aureus* on the animal subjects.

CHAPTER ONE

1.0 INTRODUCTION

Medicinal plants have been used for the treatment of several human diseases over the century and have been very important in health care delivery of every nation at one stage or the other (Oluma *et al.*, 2004). Recent research has centered on natural plant product as alternatives to the existing drugs for disease remedy in developing countries (Aiyegoro *et al.*, 2007). In most parts of the world, plant derived medicines have been part of traditional health care for ages and there is increasing interest in them as sources of agents to fight microbial diseases (Ajayi and Akintola., 2010).

All plants produce a diverse range of bioactive molecules, making them a rich source of different types of medicines. Over 50% of all modern clinical drugs are of natural product origin and natural products play an important role in the development of drugs for use in the pharmaceutical industry. Herbal drugs have gained importance in recent years because of their efficacy and cost effectiveness (Sen and Batra, 2013). Medicinal plants have been used for ages as remedies for human diseases because they contain components of therapeutic value (Ushie *et al.,* 2013). Secondary plant metabolites also known as phytochemicals have been extensively investigated as a source of medicinal agents. It is expected that phytochemicals with good antibacterial activity will be used for the treatment of bacterial infections, fungal infections and viral infections in the nearest future (Ushie *et al.,* 2013). Scientific investigation of medicinal plants used in traditional remedies, especially in a bid to finding lasting solutions to the problems of multidrug resistance to the existing conventional antimicrobials (Ojo *et al.*, 2013). Today in Africa, many resort to the use of locally made herbal medicines prepared as infusions in hot water, decoction in cold water, concoction with food and as tinctures with alcohol as an alternative therapy for bacterial infections (Oluduro and Omoboye, 2010). Plant parts such as the roots, leaves, shoots, barks, fruit peels, immature and unripe fruits have been used in most herbal preparations. Infectious diseases

still remain an important cause of morbidity and mortality in man, especially in developing countries (Oluduro and Omoboye, 2010).

The *Phyllanthus* genus contains over 600 species of shrubs, trees and annual or biennial herbs distributed throughout the tropical and subtropical regions of both hemispheres (Ushie *et al.,* 2013). *Phyllanthus amarus* is a herb that belongs to the family Euphorbiaceae, and it is commonly used in India and is found in other countries including China, Philippines, Cuba, Nigeria and Guam. It is traditionally used in the treatment of malaria-related symptoms, jaundice, constipation, diabetes, kidney ailments, and chronic dysentery, frequent menstruation, ringworm, ulcers, genitourinary tract infections, hemorrhoids, and gonorrhea, hepatic and urolitic diseases. It is reported to possess antimalarial, anti-inflammatory and anti-amnesic activity (Sen and Batra, 2013). *Phyllanthus amarus* is an annual, glabrous herb widely spread throughout the tropics and subtropics as a weed in cultivated and waste land with much availability in India. It is commonly called 'hurricane weed', 'stonebreaker', 'windbreaker', 'gulf leaf flower' or 'gala of wind'. In Nigeria it is called 'Eyin olobe' in Yoruba, "Oyomokeisoamankedem" in Efik, Ngwu in 'Igbo' and 'Ebebenizo' in Bini (Etta, 2008). *Phyllanthus amarus* is known to block DNA polymerase in hepatitis B virus during replication, it also blocks reverse transcriptase in HIV infection, and in diabetes complication. The leaves and whole plant are usually used for the treatment of gonorrhea, jaundice, rickets and asthma and can be used in the management of HIV/AIDS (Adegoke *et al.*, 2010).

Herbal medicine is readily available in our diverse vegetation, cheap and above all carries the potential for introducing new templates into modern medicine (Akinyemi *et al.*, 2005). There are multitudes of potential; useful bioactive substances to be derived from these plants. *Phyllanthus amarus* is reported to have healing properties and not toxic to either the kidney or liver. The plant also contains several

phytochemical elements including glycosides, flavonoids, alkaloids, phenylpropanoids, sterols, saponins, limonine, among others.

The emergence and spread of microbes that are resistant to a cheap and effective first-choice drugs have become a common occurrence. The problem is even more evident in bacterial infections which contribute most of the global infectious disease burden such as diarrhea, respiratory tract infections, meningitis, sexually transmitted infections and tuberculosis (Adegoke *et al.*, 2010). The emergence of resistant microorganisms in hospitals and the community is causing problems for the treatment of patients and infection control. Organisms of particular concern include methicillin resistant *Staphylococcus aureus* (MRSA), glycopeptides resistant enterococci (GRE), gentamicin-resistant and extended spectrum β-lactamase producing *Klebsiella* and multi-resistant pseudomonads (Ojo *et al.*, 2013). At present, most clinical isolates of *Staphylococcus aureus* are multiple drug resistant, showing resistance to three agents such as ciprofloxacin, erythromycin and clindamycin (Adegoke *et al.*, 2010).

1.1 RESEARCH PROBLEM

The disbelief often encountered in the use of herbal medicines for treatment of various pathological and physiological diseases owing to the undetermined toxic level of such herbal medicines has plaqued both the primary and tertiary health care system in Nigeria.

1.2 AIM OF THE STUDY

This study is designed to evaluate the prophylactic activity of *Phyllanthus amarus* extract on Swiss albino rats challenged with *Staphylococcus aureus*.

1.3 OBJECTIVES OF THE STUDY

The objective of this study includes:

- To perform a confirmatory test on the reference strains using culturing and biochemical methods.
- To carry out crude extraction of bioactive ingredients using hot (soxhlet) and cold extraction method in different solvents.
- To determine prophylactic activity of *Phyllanthus amarus* on swiss albino rats using different crude extract concentrations for pre-treatment.
- To carry out organism challenge test on the Swiss albino rat.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Botanical Description of *Phyllanthus amarus*: *Phyllanthus amarus* is a Monoecious, annual, erect, glabrous herb up to 60cm tall, reddish; branchlets flattened, often slightly winged and sparsely hairy. Leaves alternate, and are crowded along lateral branchlets, simple and entire, sessile; stipules ovate-lanceolate to lanceolate; blade oblong to elliptical-oblong, and slightly unequal, apex rounded, often pointed, deltoid acuminate; leaf 3.0-11.0 x 1.5-6.0 mm, elliptic oblong to obvate, obtuse or minutely apiculate at apex, obtuse or slightly inequilateral at base; Flowers axillary, proximal 2-3 axils with unisexual 1-3male flowers and all succeeding axils with bisexual cymules. calyx lobes 5, 0.6 x 0.25 mm, ovate-oblong, acute at apex, Capsule 1.8 mm in diameter, oblate and rounded, seeds about 0.9mm long, triangular with 6-7 longitudinal ribs and many transverse striations on the back flowers 1–2 in the axils of leaves, unisexual, pale green, often flushed red; male flowers at the base of branches, other leaf axils with 1 female flower and 1 male flower; pedicel. 1 mm long; perianth lobes 5(–6), 0.5–1 mm long; male flowers with 5-lobed disk, stamens 3, filaments fused, anthers free; female flowers with cup-shaped, 5-lobed disk, ovary superior, ovoid, warty, 3-celled, styles 3, free. Fruit an obtusely 3-lobed capsule 2–2.5 mm in diameter, smooth, hanging, 6-seeded. The seeds are 1mm long, with transverse ridges (Oudhia *et al.*, 2008).

Plate 1.0: *Phyllanthus amarus* (Source: Federal University Oye Ekiti environment)

2.2 Origin and Distribution of *Phyllanthus amarus*

Phyllanthus belonging to the family Euphorbiaceae is widely distributed in most tropical and subtropical countries. It is a very large genus consisting of approximately 550 to 750 species and is subdivided into 11 subgenera which includes *Bothryanthus, Cicca, Conani, Emblica, Ericocus, Gomphidiu, Isocladus, Kirgenilia, Phyllanthodendron, Phyllanthus and Xylophyla* (Unander *et al.*, 2005), with about 150 species in mainland tropical Africa and about 60 in Madagascar and other Indian Ocean Islands (Oudhia *et al.,* 2008). A subgeneric classification of *Phyllanthus* is in preparation. Until 20 years ago taxonomists placed a number of species, including *Phyllanthus amarus*, under *Phyllanthus niruri* L. Where the name *Phyllanthus niruri* has been applied in older literature to African or Asian specimens, usually *Phyllanthus amarus* is intended, but sometimes also *Phyllanthus debilis* Klein ex Willd., *Phyllanthus fraternus* G.L.Webster, *Phyllanthus maderaspatensis* L. or *Phyllanthus rotundifolius* Klein ex Willd. Specimens of true *Phyllanthus niruri* have actually never been confirmed from outside the Americas (Oudhia *et al.*, 2008). The branching pattern of *Phyllanthus amarus* is 'phyllanthoid', i.e. the spiralled leaves on the main axes are strongly reduced to 'cataphylls', which subtend a deciduous branchlet with distichous leaves, the branchlet resembling a compound leaf (Oudhia *et al.*, 2008). *Phyllanthus amarus* occurs in open localities, waste ground, grassy scrub vegetation and dry deciduous forest, usually on humid, sandy soils, from sea-level up to 1000 m altitude. It is reported as a troublesome weed in pulses, soya bean, groundnut, cereals, sugar cane, cassava, taro, sesame, sunflower and cotton (Oudhia *et al.*, 2008).

2.3 Traditional Use of *Phyllanthus amarus*

Phyllanthus amarus is widely used as a medicinal plant. An infusion is considered a good tonic, diuretic and antipyretic. A decoction of the aerial parts or only of the leaves is taken to treat gonorrhoea, diarrhoea, dysentery, and stomach-ache, pain in the sides, haemorrhoids and absence of menstruation or female sterility. A suppository of the leaf paste is applied to the vagina to treat absence of menstruation and polyps. Leaf sap, mixed with palm oil or not, is applied as ear drops to treat otitis and applied to abscesses, sores and wounds (Oudhia *et al.*, 2008). In Côte d'Ivoire a plant decoction is taken to facilitate childbirth, to treat oedema and pain caused by fever or a sore throat. In Mali a leaf decoction is drunk to treat jaundice. In Benin a decoction of leafy twigs is drunk to treat palpitations. A root decoction mixed with other plants or not, is taken to treat colic and rectal prolapse (Oudhia *et al.*, 2008). *Phyllanthus amarus* is said to have sand-binding properties. In West Africa it is used in medico-magical ceremonies. Adeneye *et al.* (2006) reported that *P.amarus* was used in traditional medicine for its hepatoprotective, anti-diabetic, anti-hypertensive, analgesic, anti-inflammatory and antimicrobial properties. *Phyllanthus amarus* which is otherwise called Eyin olobe in South-Western Nigeria has healing effects on hypertensive patients. It was equally found efficacious for treating malaria, diabetes, kidney stones and jaundice. Chaudhury (2007) reported that the plant is effective for treating gonorrhoea, genito-urinary diseases, asthma, diabetes, typhoid fever, jaundice, stomach-ache, dysentery, ringworm, and hypertension. *Phyllanthus amarus* herb has found its traditional usefulness in several health problems such as diarrhoea, dysentery, dropsy, jaundice, intermittent fevers, urinogenital disorders, scabies and wounds. Further, these are used in the treatment of kidney problems, urinary bladder disturbances, pain, gonorrhea, diabetes and chronic dysentery. Topically, it is used for several skin problems ranging from skin ulcers, sores, swelling and

itchiness, wounds, bruises, scabies, ulcers and sores, edematous swellings, tubercular ulcers, ringworm, scabby and crusty lesions. Its effect in excretory system is due to its anti-urolitic property and is used in the treatment of kidney/gallstones, other kidney related problems, appendix inflammation and prostate problems (Ushie *et al.,* 2013). Because of its efficacy in the field of gastro-intestinal disorders it is used in the treatment of disorders like dyspepsia, colic, diarrhea, constipation and dysentery. The herb has found use in several female problems such as in leucorrhoea, menorrhagia and mammary abscess and can act as galactagogue (Chandewar and Dhongade, 2013). The young shoots of plant are administered in the form of an infusion for the treatment of chronic dysentery. Fresh leaf paste has wound healing capacity and used to cure white spots on skin and jaundice. The stem juice is also used as wound healers. The whole plant extract is used in urinary problems and swelling of liver. The root extract is used to cure stomach pain. The flower paste of plant is applied externally as antidote against snake bite (Sonia *et al.,* 2014). *P. amarus* can be taken for weight loose and help to increase male fertility. It has widely been reported to offer good treatment for leprosy, hiccup, and peptic ulcer. It is anti-spasmodic, good laxative, blood tonic, treatment of itch, flu, fever, dyspepsia, blennorrhagia, tenesmus, gonorrhea, malaria, uterus complaints, constipation, anorexia, carminative, tumor, colic; it has HIV inhibitory activity, good anti –inflammation of appendix, bladder disorder (Obianime and Uche, 2009).

2.4 Phytochemical Properties of *Phyllanthus amarus*

Phytochemistry is regarded as the heart of herbal therapy and the phytochemical research plays an important role in the development of herbal medicines. The following chemical constituents have been isolated from *Phyllanthus amarus* which include: lignans (phyllanthin, hypophyllanthin, phyltetralin, nirtetralin, niranthin), ellagitannins (phyllanthusiin D, amariinic acid, elaeocarpusin, repandusinic acid A and geraniinic acid B), flavonoids (quercetin-3-O-glucosides), tannins (geraniin, amariin, gallocatechin, corilagin and 1,6-digalloylglucopyranoside), alkaloids of the quinolizidine type [phyllantine (methoxy-securinine), securinine, norsecurinine, isobubbialine, epibubbialine], phenolic compounds (gallic acid, ellagic acid, dotriacontanyl docosanoate, triacontanol, oleanolic acid and ursolic acid) and a chroman derivative (4,4,8-trimethoxy chroman). The alkaloid phyllanthine must not be confused with the lignan phyllanthin. The leaves were found to contain the highest amounts of phyllanthin (0.7%) as compared to the whole plant: phyllanthin (0.4%), hypophyllanthin (1.2%), gallic acid (0.4%) and ellagic acid (0.2%) (Oudhia *et al.,* 2008). *Phyllanthus amarus* extracts were found to significantly inhibit DNA polymerase of the hepatitis B virus and other hepatitis-DNA-viruses, such as the woodchuck hepatitis virus, together with in-vitro activity against the enzyme reverse-transcriptase of retroviruses (Oudhia *et al.,* 2008). The activity of the extracts in test animals as well as in clinical studies was controversial: both success and failure have been reported. Callus induced from *Phyllanthus amarus* showed less activity against viral DNA polymerase and reverse transcriptase than extracts from field-grown plants. In a clinical trial, a plant extract had a remarkable effect for chronic viral hepatitis B in recovery of liver function and inhibition of the replication of hepatitis B virus (Oudhia *et al.,* 2008). Phyllanthin and hypophyllanthin have protective activity in rat hepatocytes against cytotoxicity induced by CCl_4 and galactosamine,

and it has been suggested that phyllanthin is responsible for antigenotoxic effects reported for the extracts. However, phyllanthin has also been reported to be toxic to the nervous system and liver. A crude extract given orally to rats showed significant liver regenerative effects against alcohol-induced liver cell injury. An ethanolic extract administered orally to mice possessed a potent protective effect against aflatoxin B-1-induced hepatic damage. A crude aqueous extract of *in-vitro* cultured roots caused a dose-dependent reduction of bovine viral diarrhea virus with no cytotoxic effect. In another test, extracts of *in-vitro* grown hairy or adventitious roots showed about 85% inactivation of hepatitis B virus surface antigen. A chroman derivative, 4,4, 8-trimethoxy chroman, isolated from the dichloromethane fraction, exhibited very little *in-vitro* cytotoxicity (Oudhia *et al.*, 2008). *Phyllanthus amarus* aqueous extracts show potent anticarcinogenic activity against development of different tumor types. Administration of the extract after tumour development increased survival of rats and mice up to 1 year. An alcoholic extract was found to significantly reduce cytochrome P450 enzymes both *in vitro* as well as *in vivo* when orally administered to mice. A hexane extract, the lignans-rich fraction and the lignans nirtetralin, niranthin and phyllanthin exerted cytotoxic effects in 2 human leukaemia cell lines, as well as multidrug resistance reversing properties, mainly due to their ability to synergize with the action of conventional chemotherapeutics. An ethanolic extract showed significant preventive effect against benign prostatic hyperplasia in rats (Oudhia *et al.,* 2008). Aqueous and alcohol-based extracts potently inhibit HIV-1 replication in human cell lines. A gallotannin enriched fraction showed enhanced activity, and the purified gallotannins geraniin and corilagin were most active. A concentration-dependent inhibition of HIV-1 reverse transcriptase and protease could be demonstrated *in vitro*. A potent anti-HIV activity was demonstrated in blood of volunteers who had ingested the plant material. A 50% methanol extract, a water extract, as well

as isolated corilagin and brevifolin carboxylic acid have demonstrated strong β-glucuronidase inhibitory action. However, phyllanthin and hypophyllanthin were ineffective. Fresh plant material and a methanol extract showed strong antioxidant activity in various antioxidant assays. A correlation between the antioxidant activity and total phenolic content was observed. Drying of the plant material caused a significant reduction in antioxidant properties. On the other hand, boiling water extracts exhibited significantly stronger antioxidant potentials even from dried plant material due to greater solubility of compounds, breakdown of cellular constituents as well as hydrolysis of tannins. A whole plant extract showed significant radio-protective activity when given orally to mice, by decreasing the damage to intestinal cells, decreasing the lipid peroxidation levels, decreasing the percentage of chromosomal aberrations and by elevating the antioxidant enzymes in the intestine, blood and liver (Chandewar and Dhongade, 2013). Methanol and aqueous extracts inhibited all the phases of inflammation in standardized tests in rats. A hydroalcoholic extract, given intraperitoneally to rats, exhibited pronounced antinociception. Given orally, the extract was less potent. The hexane extract, the lignan-rich fraction and the lignans phyltetralin, nirtetralin and niranthin, but not hypophyllanthin or phyllanthin, inhibited carrageenan-induced rat paw oedema and neutrophil influx. Furthermore, niranthin exhibited anti-inflammatory and anti-allodynic activities. An ethanol and a hexane extract showed significant anti-inflammatory potential *in vitro* and *in vivo* in mice (Oudhia *et al.,* 2008). Aqueous and methanolic extracts of the aerial parts showed anti-diabetic activity in mice, rats and rabbits. However, a 1-week treatment with the aqueous extract was incapable of lowering blood glucose in untreated non-insulin dependent diabetic patients. Oleanolic acid, ursolic acid and lupeol isolated from this fraction were shown to inhibit α-amylase (Adeneye *et al.,* 2006)

2.5 Antimicrobial Properties of Medicinal Plants

The beneficial medicinal effects of plant materials typically result from the secondary products present in the plant although, it is usually not attributed to a single compound but a combination of the metabolites. The medicinal actions of plants are unique to a particular plant species or group, consistent with the concept that the combination of secondary products in a particular plant is taxonomically distinct (Joshi *et al.*, 2011). The screening of plants usually involves several approach; ethno botanical approach is one of the common methods that are employed in choosing the plant for pharmacological study.

Plant essential oils and extracts have been used for many thousands of years, in food preservation, pharmaceuticals, alternative medicine and natural therapies. It is necessary to investigate those plants scientifically which have been used in traditional medicine to improve the quality of healthcare (Joshi *et al*, 2011). Medicinal plants have some advantages over antibiotics such that there is better patient tolerance, relatively less expensive, acceptance due to long history of use and being renewable in nature (Vermani and Garg, 2002).

2.6 Description of *Staphylococcus aureus*

Staphylococcus aureus are Gram-positive, cocci, catalase positive and coagulase positive belonging to the Micrococcaceae family (Becker *et al.,* 2004). They are approximately 0.5-1.5 μm in diameter, nonmotile, non-spore-forming, facultative anaerobes (with the exception of *S.aureus* anaerobius) that usually form in clusters. Many strains produce staphylococcal enterotoxins, the superantigen toxic shock syndrome toxin (TSST-1), and exfoliative toxins. *Staphylococcus aureus* are part of human flora, and are primarily found in the nose and skin.

S. aureus can cause a range of illnesses, from minor skin infections, such as pimples, impetigo, boils (furuncles), cellulitis, folliculitis, carbuncles, scalded skin syndrome, and abscesses, to life-threatening diseases such as pneumonia, meningitis, osteomyelitis, endocarditis, toxic shock syndrome (TSS), bacteremia, and sepsis. Its incidence ranges from skin, soft tissue, respiratory, bone, joint, endovascular to wound infections. It is still one of the five most common causes of nosocomial infections and is often the cause of post-surgical wound infections (Becker *et al.,* 2004).

2.6.1 Pathogenesis of *Staphylococcus aureus*

S. aureus is an opportunistic pathogen that causes a variety of self-limiting to life threatening diseases in humans. The anterior nares are the main ecological niche for *S. aureus*. Approximately 20% of individuals are persistently nasally colonized with *S. aureus*, and 30% are intermittently colonized. However, numerous other sites may be colonized, including the axillae, groin, and gastrointestinal tract. Colonization provides a reservoir from which bacteria can be introduced when host defenses are breached, whether by shaving, aspiration, insertion of an indwelling catheter, or surgery, it also clearly increases the risk for subsequent infection. Those

with *S. aureus* infections are generally infected with their colonizing strain. Colonization also allows *S. aureus* to be transmitted among individuals in both health care and community settings. (Rachel and franklin, 2008) *Staphylococcus aureus* causes a variety of suppurative (pus-forming) infections and toxinoses in humans. This infection causes superficial skin lesions such as boils, styes and furuncules; more serious infections such as pneumonia, mastitis, phlebitis, meningitis, and urinary tract infections; and deep-seated infections, such as osteomyelitis and endocarditis. *S. aureus* is a major cause of hospital acquired (nosocomial) infection of surgical wounds and infections associated with indwelling medical devices. *S. aureus* causes food poisoning by releasing enterotoxins into food, and toxic shock syndrome by release of superantigens into the blood stream. *S. aureus* expresses many potential virulence factors:

(1) surface proteins that promote colonization of host tissues; (2) invasins that promote bacterial spread in tissues (leukocidin, kinases, hyaluronidase); (3) surface factors that inhibit phagocytic engulfment (capsule, Protein A); (4) biochemical properties that enhance their survival in phagocytes (carotenoids, catalase production); (5) immunological disguises (Protein A, coagulase); (6) membrane-damaging toxins that lyse eucaryotic cell membranes (hemolysins, leukotoxin, leukocidin; (7) exotoxins that damage host tissues or otherwise provoke symptoms of disease (TSST); and (8) acquired resistance to antimicrobial agents. Once *S. aureus* adheres to host tissues or prosthetic materials, it is able to grow and persist in various ways. *S. aureus* can form biofilms (slime) on host and prosthetic surfaces, enabling it to persist by evading host defenses and antimicrobials *S. aureus* can also invade and survive inside epithelial cells, including endothelial cells, which theoretically may also allow it to escape host defenses, particularly in endocarditis During infection, *S. aureus* produces numerous enzymes, such as proteases, lipases, and elastases, that enable it to invade and destroy host tissues and metastasize

to other sites. *S. aureus* is also capable of producing septic shock. It does this by interacting with and activating the host immune system and coagulation pathways. In addition to causing septic shock, some *S. aureus* strains produce superantigens, resulting in various toxinoses, such as food poisoning and toxic shock syndrome (Rachel and Franklin, 2008)

2.6.2 Epidemiology of *Staphylococcus aureus*: The natural reservoir of *Staphylococcus aureus* is the human host but it can survive on domesticated animals such as dogs, cats and horses. Asymptomatic infection is more common and in the colonization of the skin. *S. aureus* also occurs in the nose frequently and the throat less commonly. In developing world, mortality associated with severe *Staphylococcus aureus* infections far exceeds that in developed countries (Ryran and Ray, 2004). *Staphylococcus aureus* is one of the most common causes of skin, soft-tissue, and nosocomial infection. Around 20% of individuals are persistent carriers of *Staphylococcus aureus*, about 60% are intermittent carriers, and approximately 20% rarely carry it (Miller *et al.,* 2005). Children are more likely to be persistent carriers of the bacteria. Young women are at a higher risk for toxic shock syndrome. *S. aureus* is the most frequently occurring bacterial pathogen among clinical isolates from hospital in-patients in the United States and is the second most prevalent bacterial pathogen among clinical isolates from outpatients (Christoph, 2009).

2.6.3 Diagnosis

Depending upon the type of infection present, an appropriate specimen is obtained accordingly and sent to the laboratory for definitive identification by using biochemical or enzyme-based tests. A Gram stain is first performed to guide the way, which should show typical Gram-positive

bacteria, cocci, in clusters. Second, the isolate is cultured on mannitol salt agar, which is a selective medium with 7–9% NaCl that allows *S. aureus* to grow, producing yellow-colored colonies as a result of mannitol fermentation and subsequent drop in the medium's pH.

Furthermore, for differentiation on the species level, catalase (positive for all *Staphylococcus* species), coagulase (fibrin clot formation, positive for *S. aureus*), DNAse (zone of clearance on DNase agar), lipase (a yellow color and rancid odor smell), and phosphatase (a pink color) tests are performed. For staphylococcal food poisoning, phage typing can be performed to determine whether the staphylococci recovered from the food were the source of infection.

2.6.4 Treatment

The treatment of choice for *S. aureus* infection is penicillin. Penicillin, an antibiotic derived from *Penicillum* fungus, inhibits the formation of peptidoglycan cross-linkages that provide the rigidity and strength in a bacterial cell wall. The four-membered β-lactam ring of penicillin is bound to enzyme DD-transpeptidase, an enzyme that when functional, cross-links chains of peptidoglycan that form bacterial cell walls. The binding of β-lactam to DD-transpeptidase inhibits the enzyme's functionality and it can no longer catalyze the formation of the cross-links. As a result, cell wall formation and degradation is imbalanced, thus resulting in cell death. In most countries, however, penicillin resistance is extremely common, and first-line therapy is most commonly a penicillinase-resistant β-lactam antibiotic (for example, oxacillin or flucloxacillin, both of which have the same mechanism of action as penicillin). Combination therapy with gentamicin may be used to treat serious infections, such as endocarditis, but its use is controversial because of the high risk of damage to the kidneys. The duration of treatment depends on the site of infection and on severity (Bayer *et al.*, 2000).

2.6.5 Control

Spread of *S. aureus* (including MRSA) generally is through human-to-human contact, although recently some veterinarians have discovered the infection can be spread through pets (Sing *et al.*, 2008) with environmental contamination thought to play a relatively unimportant part. Emphasis on basic hand washing techniques is, therefore, effective in preventing its transmission. The use of disposable aprons and gloves by medical staff reduces skin-to-skin contact and, therefore, further reduces the risk of transmission. Personal care items such as razor, towels or similar items should not be shares with others. Infected people should be kept in a separated place in the hospital. Transmission of the pathogen is facilitated in medical settings where healthcare worker hygiene is insufficient. Ethanol has proven to be an effective topical sanitizer against MRSA. The prevention of nosocomial infections involves routine and terminal cleaning. An important and previously unrecognized means of community-associated MRSA colonization and transmission is during sexual contact (Sing *et al.*, 2008)

2.7 Antibiotic Resistance

Antibiotic resistance in *S. aureus* was uncommon when penicillin was first introduced in 1943. Indeed, the original petri dish on which Alexander Fleming of Imperial College London observed the antibacterial activity of the *Penicillium* fungus was growing a culture of *S. aureus*. By 1950, 40% of hospital *S. aureus* isolates was penicillin-resistant; and, by 1960, this had risen to 80% (Chambers, 2001). Methicillin-resistant *S. aureus*, abbreviated MRSA is one of a number of greatly feared strains of *S. aureus* which have become resistant to most β-lactam antibiotics. For this reason, vancomycin, a glycopeptide antibiotic, is commonly used to combat MRSA. Vancomycin inhibits the synthesis of peptidoglycan, but unlike β-lactam antibiotics, glycopeptide antibiotics target and bind to amino acids in the cell wall, preventing peptidoglycan cross-linkages from forming. MRSA strains are most often found associated with institutions such as hospitals, but are becoming increasingly prevalent in community-acquired infections. A recent study by the Translational Genomics Research Institute showed that nearly half (47%) of the meat and poultry in U.S. grocery stores were contaminated with *S. aureus*, with more than half (52%) of those bacteria resistant to antibiotics (Waters *et al.*, 2011) This resistance has been caused by the widespread use of antibiotics in the husbandry of livestock, including prevention or treatment of an infection as well as promoting growth according to the study.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Test Organism:

A referenced pure culture of antibiotic resistance *Staphylococcus aureus* (*S. aureus* W241-Ojo) were obtained from the Drug Discovery and Development Research Unit, Department of Microbiology, Federal University Oye Ekiti, Ekiti State.

3.2 Confirmation of Test Organism:

Colonies growing on slants were streaked on top of freshly prepared plates of Nutrient Agar and incubated again at 35°C. Primary characterization of isolates was based on gram stain, morphological and cultural characteristics growth on nutrient agar and fermentation on Mannitol salt agar, Catalase test, Coagulase test and other sugars (Lactose, Sucrose, Maltose, and Fructose) and Citrate utilization test.

3.3 Plant Collection

The whole plant of *Phyllanthus amarus* were collected from their natural habitat at the Federal University Oye-Ekiti, Ekiti State, Nigeria. The whole plant of *Phyllanthus amarus* were thoroughly washed and rinsed in flowing tap water and air-dried for two weeks.

3.4 Processing of Plant Extract

The dried plant was pulverized into powdered form using a household blender (Nakai Magic blender; Model No: SI-889BD). The powdered material was subjected to hot and cold extraction of ethyl-acetate and methanol to obtain crude extracts. The hot extraction was done with a soxhlet apparatus using 75g of the plant in 500ml of methanol and ethyl-acetate respectively for 5-6hrs.The cold extraction was carried out by weighing 75g of the pulverized plant into the beaker and 500ml of respective solvents were added, covered with foil paper and kept for 48hrs.

Whatman No 1 filter paper was used to filter the extract out and were concentrated for 3 hours until the solvent used is extracted out using a rotary evaporator (Senco Technology Co. Ltd; Model No:R205; SN: 13605).at a speed of 39-40rpm.

3.5 Animal Study

Twenty (20) Swiss albino rats of either sex weighing 120-200kg were purchased from the animal house of Afe Babalola University Ado-Ekiti, Ekiti State. The animals were housed in Polycarbonated cages at ambient environmental condition of 23±2°C and 50-60% relative humidity. The animals were allowed to acclamatize to the environment. The animals were subjected to light and dark cycles of 12 and 12 hrs respectively for 7 days before the experiment. Animals were given the rodent commercial diet and water ad libitum as described by (Jena *et al.*, 2012).

3.5.1 Prophylactic Treatment of Animal Subjectsx

For prophylactic studies, 20 Swiss albino rats were used. The rats were sorted into 4 groups of 5 rats each according to their body weights and a control group. Group 1 with average weight of 126.5kg, Group 2 with average weight of 150kg, Group 3 with average weight of 165kg,
Group 4 with average weight of 180kg, and the Control group with average weight of 190kg
The rats were defasted for 4 hour prior to treatment and the extract dosage was given according to the weight of rats each and the solvents of extraction. The dosages were administered orally using an oral canular with 1ml syringe of 0.5ml of sterile extracts. Group 1 received 50mg/kg of 0.5ml of *Phyllanthus amarus* methanol extract. Rats in group 2 received 100mg/kg of 0.5ml of *Phyllanthus amarus* methanol extract. Rats in group 3 received 50mg/kg of 0.5ml of *Phyllanthus amarus* ethyl-acetate extract. Rats in group 4 received 100mg/kg of 0.5ml of *Phyllanthus amarus* ethyl-acetate extract while the control group received 0.5ml of DMSO in distilled water.

They were observed for 1 hour, 2 hours and 4 hours after treatment and intermittently for 24 hours for 7 days for clinical signs such as weakness, aggressiveness, loss of weight, diarrhea, discharge from the eye and ears, inflammation on skin and number of deaths.

3.5.2 Organism Challenge Test

The treated and untreated rats were challenged with 0.5ml of 1×10^8 cfu/ml of the test organisms in Mueller Hington broth standardized to 0.5McFarland standard at the fourth day (4^{th}) of pre-treatment. The protection offered by the extracts was determined by recording the observed signs and mortality rate of rats in each group up to 7 days.

Plate 2: Swiss albino rat in polycarbonated metabolic cage

CHAPTER FOUR

4.0 RESULTS

4.1 **Microscopic and Biochemical Characterization of Reference Strain**

Table 1 showed that the referenced pure culture of antibiotic resistance strain of *Staphylococcus aureus* obtained on confirmation showed Gram positive cocci, Coagulase positive, Catalase positive and fermentation on Mannitol salt agar, citrate was utilized. For Lactose, there was production of Acid but no production of gas. For maltose there was production of acid but no production of gas and then for fructose and sucrose there was production of acid and gas respectively

Table1. Microscopic and Biochemical Characterization of Reference Strain

Test organism	Coagulase	Catalase	MSA	Citrate	Lactose		Maltose		Fructose		Sucrose		Gram staining
					A	G	A	G	A	G	A	G	
S.aureus W241-Ojo	+	+	+	+	+	-	+	-	+	+	+	+	+ cocci

KEYS: A= Acid production G= Gas production + = Positive - = Negative

4.2 **Dosage Administration and Response on Pre-treatment of Swiss albino rats with *Phyllanthus amarus* extract**

The dosage administration of the crude extracts of *Phyllanthus amarus* and the observation of clinical signs is as shown in table 2. The result shows that at the concentration given to the swiss albino rats in this study, the effect of the extract is not toxic to the animal subjects. From Group 1-Group 4 for the four days given, there was no weakness, no fever, no inflammation, no diarrhea, No death, no redness from the eye/skin, no discharge from the eye/ear. From day 1 to day 3, each group was very aggressive. But for group 1 on the fourth day, none of the rat was aggressive. For group 2 two were aggressive, for group 3 and group 4 all were aggressive. For the control group during the 4 days of pre-treatment all were active. It shows that the extract was not toxic to any of the groups.

Table 2.Dosage administration (Pre-treatment) of *Phyllanthus amarus* crude extracts on Swiss albino rats

				DAY 1								DAY 2							
Grp mean weight(kg)	T_x	C (mg)	D_o (ml)	Response to dosage								Response to dosage							
				F (n)	Agg (n)	Inf (n)	W (n)	R (n)	D (n)	Di (n)	De (n)	F (n)	Agg (n)	Inf (n)	W (n)	R (n)	D (n)	Di (n)	De (n)
1 (126.5)	ME_1	50	0.5	0	5	0	0	0	0	0	0	0	5	0	0	0	0	0	0
2 (150)	ME_2	100	0.5	0	4	0	0	0	0	0	0	0	4	0	0	0	0	0	0
3 (165)	EA_1	50	0.5	0	4	0	0	0	0	0	0	0	4	0	0	0	0	0	0
4 (180)	EA_2	100	0.5	0	4	0	0	0	0	0	0	0	4	0	0	0	0	0	0
Control (190)	DMSO + DW		0.5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

KEY:

ME- Methanol extract, EA- Ethylacetate extract, DMSO- Dimethylsulphoxide, T_x- Treatment, C- Concentration, D_O-Dosage, F-fever, Agg-aggressiveness, Inf- inflammation, W- weakness, R-redness of the eye, D-discharge from eyes, Di-diarrhoea, De-death, DW-Distilled water, n-number of rat.

Table 2.Dosage administration (Pre-treatment) of *Phyllanthus amarus* crude extracts on Swiss albino rats (contd)

				DAY 3								DAY 4							
Grp mean weight(kg)	T_x	C (mg)	D_o (ml)	Response to dosage								Response to dosage							
				F (n)	Agg (n)	Inf (n)	W (n)	R (n)	D (n)	Di (n)	De (n)	F (n)	Agg (n)	Inf (n)	W (n)	R (n)	D (n)	Di (n)	De (n)
1 (126.5)	ME_1	50	0.5	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2 (150)	ME_2	100	0.5	0	4	0	0	0	0	0	0	0	2	0	0	0	0	0	0
3 (165)	EA_1	50	0.5	0	4	0	0	0	0	0	0	0	4	0	0	0	0	0	0
4 (180)	EA_2	100	0.5	0	4	0	0	0	0	0	0	0	4	0	0	0	0	0	0
Control (190)	DMSO + DW		0.5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

KEY:

ME- Methanol extract, EA- Ethylacetate extract, DMSO- Dimethylsulphoxide, T_x- Treatment, C- Concentration, D_O-Dosage, F-fever, Agg-aggressiveness, Inf- inflammation, W- weakness, R-redness of the eye, D-discharge from eyes, Di-diarrhoea, De-death, DW-Distilled water, n-number of rat.

4.3 Prophylactic Activity of *Phyllanthus amarus* extracts on Organism Challenged Swiss albino rats

The protection offered by *Phyllanthus amarus* to Swiss albino rats into receiving a challenge dose of *S.aureus* of 10^8 cfu/ml was shown in Table 3. The result shows that after 1 hr both the treated and non-treated group showed signs of weakness, but after 4hrs the treated groups adjusted back to their normal state of health but for the non-treated they were still very weak. At 24hrs, 48hrs, 72hrs also the non-treated group was still very weak. It took a longer period of time before the non-treated groups could be able to adjust to its normal state of health compared to the treated groups.

Table 3. Protection offered by *Phyllanthus amarus* on Swiss albino rats into receiving a challenge dose of *S.aureus*

Type	Group	Extract injected per rat	Response to dosage				
			1hr	4hrs	24hrs	48hrs	72hrs
Non treated	Control (n=4)	0.5ml DMSO+H_2O	W	W	W	W	W
Treated	Grp1 (n=9)	50mg/kg	W	N	N	N	N
	Grp2 (n=8)	100mg/kg	W	N	N	N	N

Key: DMSO- Dimethyl sulphoxide, W- Weak N- normal

5.0 CHAPTER FIVE

DISCUSSION

5.1 Dosage Administration and Response on Pre-treatment of Swiss albino rats with *Phyllanthus amarus* extracts

The use of *Phyllanthus amarus* as herbal medicine has been well reported and been referred to as wonder plant because of its health enhancing properties including antibacterial, antioxidants and anti-inflammatory (Babatunde *et al.*, 2014).

This study shows the solvent of the crude extracts at different concentration rate (50mg/kg of Methanol and ethyl-acetate crude extracts and 100mg/kg of methanol and ethyl-acetate crude extracts). The results after four (4) days revealed that the concentrations used as Pre-treatment on the swiss albino rats had no toxic effect and clinical signs such as weakness, diarrhoea and no inflammation observed. The findings by Chandewar and Dhongade (2013) observed protective activity and anti-inflammatory potential on the methanolic and ethyl-acetate extract of *Phyllanthus amarus* at concentration rate of 150mg/kg and 200mg/kg which corroborate with the report of this study on the non-inflammatory response recorded at concentration rate of 50mg/kg and 100mg/kg.

5.2 Prophylactic activity of *Phyllanthus amarus* extracts on Organism challenged Swiss albino Rats

The protection offered by *Phyllanthus amarus* to swiss albino rat against *Staphylococcus aureus* after 72 hours of observation for clinical signs and symptoms such as weakness and death and which there was no observable negative effect has shown that *Phyllanthus amarus* crude extract

of ethyl-acetate and methanol at different concentrations of 50mg/kg and 100mg/kg respectively has a more significant efficiency with the time of action on the organism at the dosage given with no adverse effect on the treated group of rats. The survival of the animals after four days of Pre-treatment revealed that the ethyl-acetate and methanol extracts was able to inhibit the growth of *Staphylococcus aureus* which was similar to a report by Ushie *et al.* (2013) who reported that the methanol and ethyl-acetate crude extracts of *P. amarus* exhibited significant antibacterial activity against *Staphylococcus aureus* with a diameter range of 25mm. Additionally, Adegoke *et al.* (2010) who worked on the ethanolic extracts of *Phyllanthus amarus* observed that the ethanolic extract of *Phyllanthus amarus* inhibited the growth of *Staphylococcus aureus* at concentration rate of 10mg/ml, 50mg/ml and 100mg/ml. the findings in this study revealed that the extract was able to successfully protect the animal subjects from *Staphylococcus aureus* infection.

6.0 CONCLUSION AND RECOMMENDATION

6.1. CONCLUSION

The results of this study shows that the crude extracts of *Phyllanthus amarus* possess antibacterial and prophylactic activity against *Staphylococcus aureus* on the animal subjects at each of the concentrations used. Since no mortality is observed during the administration period, it can therefore be deduced that this medicinal plant is effective with good potency.

6.2. RECOMMENDATION

1. Further studies are however recommended to identify the specific active compounds and to characterize the potential antibacterial agents in the crude extracts of the plants.
2. Evaluation of the phytochemical properties and toxicological studies of *Phyllanthus amarus* must be carried out to ascertain its relative safety as a possible antimicrobial agent.
3. The extracts of *Phyllanthus amarus* should also be tested on other pathogenic microorganisms that cause serious human infections.

6.3. CONTRIBUTION TO KNOWLEDGE

1. The concentration of ethyl-acetate and methanol (50mg/kg and 100mg/kg) used in pre-treatment of swiss albino rats do not cause any feverish or inflammatory condition.
2. *Phyllanthus amarus* offered a prophylactic activity on the treated groups after being challenged with *Staphylococcus aureus* at various concentrations of 50mg/kg and 100mg/kg.

REFERENCES

Adegoke, A.A., Iberi, P.A., Akinpelu, D.A., Aiyegoro, O.A., and 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(3), 6-12.

Adeneye, A.A., Amole, O.O. and Adeneye, A. K. (2006). The hypoglycemic and hypocholesterolemic activities of the aqueous leaf and seed extracts of *Phyllanthus amarus* in mice. *Fitoterapia* **77**: 511-514.

Aiyegoro, O.A, Akinpelu, D.A. and Okoh, A.I. (2007). *In Vitro* antibacterial potentials of the stem bark of Redwater Tree (*Erythrophleum suaveolena*). *Journal of Biological Sciences* **7**(7): 1233-1238.

Ajayi, A.O. and Akintola, T.A. (2010). Evaluation of antibacterial activity of some medicinal plants on common enteric food-borne pathogens. *African Journal of Microbiology Research* 4(4): 314-316.

Akinyemi, K.O., Smith, S.I., Oyefolu, A.O. and Coker, A.O. (2005). Multidrug resistance in *Salmonella enterica* serovar typhi isolated from patients with typhoid fever complications in Lagos, Nigeria. *Public Health* 119: 321-327.

Becker, K., Harmsen, D., Mellmann, A., Meier, C., Schumann, P., Peters, G., & von Eiff, C. (2004). Development and evaluation of a quality-controlled ribosomal sequence database for 16S ribosomal DNA-based identification of *Staphylococcus* species. *Journal of Clinical Microbiology*, 42(11), 4988-4995.

Bayer, A.S., Bolger, A.F., Taubert, K.A. (2000). "Diagnosis and management of infective endocarditis and its complications". *Circulation* 98 (25): 2936-2948.

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

Chambers, H.F. (2001). The changing epidemiology of *Staphylococcus aureus*? *Emerging Infectious Disease* **7** (2): 178–82.

Chandewar, A.V., and Dhongade, H., (2013). A review on pharmacognostical, phytochemical,pharmacological properties of *Phyllanthus amarus*. *International Journal of Biomedical and Advance Research*. 04(05): 2229-3809

Chaudhury, R.R. (2007). Healing Power of Herbs. Sterling Publishers Pvt Ltd-new Delhi. p. 94.*Science* 1(4): 41-416.

Christoph, K.N. (2009). *Staphylococcus aureus* bacteremia. *Clinical Infectious Disease*. 48:5231-5237.

Etta, H. (2008). Effects of *Phyllanthus amarus* on liter traits in albino rats. *Scientific Research and Essay*. 3(8): 370-372.

Jena, B.K., Ratha, B., Kar, S., Mohanta, S.R., Tripathy, M., Nayak, A.K., (2012). Healing potential of Ziziphus xylopyrus Welld (Rhamnaceae) stem bark ethanol extracts using *In-vitro* and *In-vivo* model. *Journal Drug and Development*. 2(6): 41-46

Joshi, B., Sah, G.P., Basnet, B.B., Bhatt, M.R., Sharma, D., Subedi, K., Pandey, J and Malla, R. (2011). Phytochemical extraction and antimicrobial propertiesof different medicinal plants: *Ocimum sanctum* (Tulsi), *Eugenia caryophyllata* (Clove), *Achyranthes bidentata* (Datiwan) and *Azadirachta indica* (Neem). *Journal of Microbiology and Antimicrobials* 3(1): 1-7.

Miller, L. G., Perdreau-Remington, F., Rieg, G., Mehdi, S., Perlroth, J., Bayer, A. S., Tang, A. W., Phung, T. O., and Spellberg, B. (2005). Necrotizing fasciitis caused by community associated methicillin-resistant *Staphylococcus aureus* in Los Angeles. *The New England Journal of Medicine*; 352(14), 1445.

Obianime A.W. and Uche F.I. (2009). The comparative effects of methanol extract of *Phyllanthus amarus* leaves and Vitamin E on the Sperm parameters of Male guinea pigs. *Journal of Applied Sciences and Environmental Management* 13(1): 37-41.

Ojo, S.K.S., Ejims-Enukwe, O., Esumeh, F.I., (2013). *In Vitro* Antibacterial Time Kill Assay of *Phyllanthus amarus* and *Diodia scandens* Crude extracts on Staphylococci Isolated from wounds and burns of patient. *International Journal of Pharmaceutical Science Investment* (2): 2319-6718.

Oluduro, A. and Omoboye, O. (2010). *In Vitro* Antibacterial Potentials and Synergistic Effect of South-Western Nigerian Plant Parts Used in Folklore Remedy for *Salmonella typhi* infection.*Nature and Science* 8(9): 52-59.

Oluma H.O, Umoh E.U, One kutu, A. and Okolo, J. (2004). Antibacterial potentials of eight medicinal plants from the lower benue valley of Nigeria against *Salmonella typhi. Journal of Botany* 17:1-11.

Oudhia, P., Schmelzer, G.H. and Gurib-Fakim, A (2008). *Phyllanthus amarus* Schumach. & Thonn. In: (Editors). Medicinal plants/Plantes Wageningen, Netherlands. Prota 11(1).

Rachel.J.Gordon and Franklin. D. Lowy (2008). Pathogenesis of Methicillin-Resistant *Staphylococcus aureus* infection. *Journal of Clinical Infectious Disease* 46(5):5350-5359

Ryan, K.J. and Ray, C.G. (2004). *Sherries Medical Microbiology*, fourth edition. McGraw Hill. ISBN. 8385-8529.

Sen, A. and Batra, A. (2013). The study of *in vitro* and *in vivo* antioxidant activity and total phenolic content of *Phyllanthus amarus* Schum. and Thonn.: A Medicinally Important Plant. *International Journal of Pharmacy and Pharmaceutical Sciences,* 5(3), 942-947.

Sing, A., Tuschak, C., Hoermansdorfer, S., Tuschak, Hörmansdorfer. (2008). "Methicillin-Resistant *Staphylococcus aureus* in a Family and Its Pet Cat". *Natural England Journal Medicine;* 358(11):1200–1209.

Sonia, V., Hitender, S., and Munish, G. (2014). *Phyllanthus amarus*: A review. *Journal of Pharmacognosy and Phytochemistry* 3(2): 18-22.

Unander, D.W., Webster, G.L., and Blumberg, B.S. (2005). Usage and bioassays in *Phyllanthus* (Euphorbiaceae) IV. Clustering of antiviral uses and other effects. *Journal Ethnopharmacology;* 45: 1–18.

Ushie, O.A., Aleji,P.A., Ettim, E.E., and Nson, G.E. (2013). Phytochemical screening and antimicrobial activities of *Phyllanthus amarus* stem bark extracts; *International Journal of Modern Biology and Medicine*; 3(3): 101-112.

Vermani, K. and Garg, S. (2002). Herbal medicine for sexually transmitted diseases and AIDS.*Journal of Ethnopharmacology;* 80: 49-66

Waters, A.E., Contente-Cuomo, T., Buchhagen J. (2011). "Multidrug-Resistant *Staphylococcus aureus* in US Meat and Poultry". *Clinical. Infectious. Disease;* 52 (10): 1227–30.

APPENDIX

BIOCHEMICAL TEST

Equipment

Autoclave, incubator, oven, conical flask, electric weighing balance, beaker, measuring cylinder, test tubes, aluminium foil, paper tape, wire loop, McCartney bottles, swab sticks, absorbent cotton wool, Hand gloves, Bunsen burner, microscope, petri dishes, brush, durham tubes.

- **Gram staining**

Materials used include: Crystal violet, iodine ethanol, Bunsen burner, inoculating loop, clean glass slide.

Procedure

Heat fix the glass slide by passing it over blue flame, colonies of the test organism was picked and smeared on the glass slide. The smear was flooded with crystal violet for 60seconds and mildly rinsed, some drops of iodine was added also for 60 sec. the iodine decreases the solubility of the added dye-iodine complexes which was then mildly rinsed with water. The purple dye-iodine complex decolourized with 95% ethanol for 15secomds and rinsed with water. The smear was then counter stained with safranin for 30seconds. The excess stain was then rinsed off with water, slide was then allowed to air-dry and a drop of oil immersion was drpped on the smear and examined under the microscope.

- **Sugar fermentstion**

Sugar fermentation is used to test for the ability of organism to differentiate sugars by breaking them down to alcohol. The product of sugar metabolism is dependent on the type of enzymes produced by organism. Sugar utilization coupled with acid production whuch can be formed during the reaction and it is detected by the use of durham tube in inverted position in a given

tube. Sugars used were lactose, sucrose, fructose and maltose. 1g of each sugar was weighed into different beakers; peptone water was added and made up to 100mls with distilled water. Exactly 0.01g phenol red was added as an indicator. About 2ml each of the 100ml sugar solution was dispensed into different test tubes, cotton wool and aluminium foil paper was used to plug the test tube and sterilized in an autoclave for 10mins at 121°C. Tubes were allowed to cool before inoculation. The sugar solution in test tubes were then inoculated with test organism and incubated at 36°C for 24 hours.

Results

A change in the colour to yellow indicates acid production while bubble in durham tube indicates gas production.

Catalase Test

This test is used to differentiate those bacteria that produce the enzyme catalase such as staphylococci. Catalase acts as catalyst in the breaking down of oxygen and water.

A colony of the test organism was picked and placed in a drop of 3% hydrogen peroxide on a clean glass slide bubbles was checked for immediately and result was recorded.

Results

Effervescence caused by the liberation of oxygen as gas bubbles indicate the production of catalase by the organism while an absence of gas bubbles indicates a negative result

Coagulase Test

Materials used include: Blood plasma, normal saline

The test is used to identify *S.aureus* which produces the enzyme coagulase. Coagulase causes plasma to clot by converting fibrinogen to fibrin. A colony of test organism was emulsified in normal saline on a clean glass slide and an equal volume of plasma was added and mixed

together.

Results

A positive coagulase test was indicated by clumps in the mixture, while a negative coagulase test shows no agglutination.

ANIMAL STUDY

Grouping of the Swiss albino rats according to their weight

Grp I: 118-135g

Grp II: 144-156g

Grp III: 173-188g

Control IV: > 190g

To calculate for the average weight of the rats

For grp I: 118+135=253÷2= 126.5g

For grp II: 144+156g=300÷2= 150g

For grp III: 173+188= 361÷2=180.5g

For grp IV: 190g.

Preparation of Extract Concentration

For 25mg/kg body weight

Average weight = 126.5g= $\frac{25\times126.5}{1000}$ =3.16mg

To give 0.5ml × 5 animals × 7 days = 17.5ml

$\frac{3.16}{1000}$ = 0.00316g/17.5ml

For 50mg/kg body weight

Average weight=150g=$\frac{50\times150}{1000}$=7.5mg/17.5ml

$\frac{7.5}{1000}$ = 0.0075g/17.5ml

For 100mg/kg body weight

Average weight = 180.5g = $\frac{100\times180.5}{1000}$ = 18.1mg/17.5ml

$\frac{18.1}{1000}$ = 0.0181g/17.5ml.