# PROPHYLACTIC ACTIVITY OF *Phyllanthus amarus* and *Diodia scandens* IN SYNERGISM ON SWISS ALBINO RATS

BY

**BALOGUN, DAMILOLA MARY**

**MCB/11/0331**

**DEPARTMENT OF MICROBIOLOGY**

**FACULTY OF SCIENCE**

**FEDERAL UNIVERSITY OYE-EKITI**

**EKITI STATE, NIGERIA**

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A RESEARCH PROJECT SUBMITTED TO THE DEPARTMENT OF MICROBIOLOGY, FACULTY OF SCIENCE, FEDERAL UNIVERSITY OYE-EKITI IN PARTIAL FULFILMENT OF THE REQUIREMENT 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 research work was carried out by Balogun, Damilola Mary of the Department of Microbiology, Faculty of Science, Federal University Oye-Ekiti, Ekiti State under the supervision of Dr S.K. Ojo.

.....  14-12-2015

Dr S.K. Ojo  Date

Supervisor

.....  16/12/2015

Prof. B.O. Ogeneh  Date

Head of Department

## DEDICATION

This project is dedicated to GOD Almighty, invisible and all sufficient for His loving kindness throughout my course of study.

## ACKNOWLEDGEMENT

My sincere appreciation goes to the Most High God for the strength, wisdom and grace sufficient for this academic achievement and above all His guidance throughout my period of academic career in this citadel of learning. My unreserved gratitude goes to my hard working supervisor, Dr S.K. Ojo who properly and meticulously guided me through my project work. My heartfelt thanks goes to my beloved parents Mr Balogun Kehinde and Mrs Balogun Bamidele they are indeed loving and caring parent. My deepest and profound gratitude goes to my siblings Balogun Oluwatobiloba, Balogun Titilayo, Balogun Oluwatimileyin.

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Thanks for making a good microbiologist out of me and I won't forget you all. To Yahweh be the glory because I came, I saw and I conquer.

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## ABSTRACT

This study is aimed at evaluating the prophylactic activity of *Phyllanthus amarus* and *Diodia scandens* in synergism on the Swiss albino rats. The two plants were obtained from Federal University Oye-Ekiti and its environs, thoroughly washed with distilled water and air dried at room temperature for two weeks. The air dried plant materials were pulverized into powdery form using house-hold electric blender. Ethyl-acetate extracts of the two plants combined was administered orally in the mouth of the Swiss albino rat as pre-treatment before challenging them with test organism (Coagulase Negative *Staphylococcus*). Results showed that the concentration of combined extracts of the two plants at 25mg/kg and 50mg/kg were effective as a prophylactic measures on the Swiss albino rats with no symptoms of diarrhoea, redness of the eyes and skin, discharge from the eyes or death of the infected rat group. Conclusively, the synergistic effect of the two plants combined had good antibacterial activities on Coagulase negative *Staphylococci* therefore the plants can be administered in the treating related symptoms and other infectious disease caused by Coagulase negative *Staphylococcus*.

# CHAPTER ONE

## 1.0 INTRODUCTION

Coagulase negative *Staphylococcus* is a Gram positive organism which does not produce the enzyme coagulase; it usually appears under the microscope as spherical (coccus) organisms appearing in pairs, short chains, or bunched, grape-like clusters. Coagulase negative *Staphylococcus* has been implicated as a causative agent in acute food poisoning episodes, toxic shock syndrome, impetigo, scalded skin syndrome, cellulitis, folliculitis and furuncles. It is also a common cause of systemic infections such as infective endocarditis, osteomyelitis, epiglottitis, and sinus infections among others. (Parsonnet *et al*., 2005).

There is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action for new and re-emerging infectious diseases (Rojas *et al.,* 2008). Therefore, researchers are increasingly turning their attention to folk medicine, looking for new leads to develop better drugs against microbial infections. The increasing failure of chemotherapeutics and antibiotic resistance exhibited by pathogenic microbial infectious agents has led to the screening of several medicinal plants for their potential antimicrobial activity (Kapil, 2005). A medicinal plant, as defined by World Health Organization (W.H.O), is a plant in which some or all of its parts can be used directly in the management of a disease (Acharya and Shrivastava 2008). (Acharya and Shirivastava 2008) reported that the active constituent of drugs from plants is usually more concentrated in storage organs like roots, stem, bark and leaves. Flowers are not commonly used while woods and woody plants are usually inert. Report by W.H.O has estimated conservatively that, between 60 and 90 per cent of the population of the non-industrialized countries rely on medicinal plants to meet their health care needs, either totally or partially. Antimicrobials of

plant origin have enormous therapeutic potentials to heal many infectious diseases and are mostly not associated with serious side effects. Hence, the potential for developing antimicrobials from higher plants appears rewarding as it will lead to the development of phyto-medicines to act against pathogenic microbes. Medicinal plants are finding their way into pharmaceuticals, cosmetics, and neutraceuticals. In pharmaceutical field, medicinal plants are mostly used for the wide range of substances present in plants which have been used to treat chronic as well as infectious diseases (Bassam, 2012).

The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the human body and these chemical substances are called phytochemicals. These phytochemicals were used to cure the disease in herbal and homeopathic medicines (Chitravadivu *et al.,* 2009). According to Ahmed and Urooj (2009), these phytochemical substances are non-nutritive substances, and have protective or disease preventive property. With advances in phytochemical techniques, several active constituents of many medicinal plants have been isolated and introduced as valuable drug in modern systems of medicine. The most important of these bioactive compounds are alkaloids, flavonoids, tannins and phenolic compounds, which are the important raw materials for drug production (Bassam, 2012).

Several health-related benefits including antibacterial, anticarcinogenic, antimutagenic, antithrombotic and vasodilatory activities have been obtained from these chemical constituents. Most plants contain several compounds with antimicrobial properties for protection against aggressor agents, especially microorganisms (Chitravadivu *et al.,* 2009). The abundance of medicinal plants in nature and the traditional knowledge increases the understanding of the medicinal plants properties, safety and efficacy. This concern has been expressed because of the resistance of clinically pathogenic microorganisms to antibiotics that has been produced in the last decades. The rising prevalence of these drug-resistant

pathogenic microorganisms raises the demand for finding new alternative antimicrobial agents. The drugs already in use to treat infectious disease are of concern because drug safety remains an enormous global issue. Most of the synthetic drugs cause side effects and also most of the microbes develop resistance against the synthetic drugs (Chanda and Rakholiya, 2011).

*Diodia scandens (Rubiaceae)* is an evergreen perennial herb, which has an alternate leaf arrangement, petiole is present. It has compound leaves, ovate to lanceolate in shape, reticulate venation, and entire in margin. *Diodia scandens* has a dark green coloration which has been use in the Western Africa system of medicine. It has enormous usefulness and importance; whole parts are useful in curing various ailments, treatment of venereal diseases and cutaneous and subcutaneous fungal infections. (Essitt *et al.,* 2010).

*Phyllanthus amarus* is a small erect, annual monoecious herb that grows to 30-40 cm in height. (Sonia *et al.,* 2014). It belongs to the family *Euphorbiaceae* with leaves that alternate distichous and crowded along lateral branchlets. About 150 species have been identified in tropical Africa. It was reported to have originated from tropical America and has spread as weed throughout the tropics and subtropics. *Phyllanthus amarus* is a plant with reported medicinal properties and broad spectrum of pharmacological activities including antiviral, antimicrobial, anti-plasmodial, anti-inflammatory, anticancer, antidiabetics, antioxidant and diuretics properties among others. Extracts from the roots and leaves of *Phyllanthus amarus* has been reported to have antimicrobial action against drug resistant pathogenic bacterial strains such as *Enterococcus faecalis (*Sonia *et al.,* 2014*)*.

**Research problem**

The scepticism often encountered in the use of herbal medicine for treatment of various pathological and physiology diseases owing to the undetermined toxic level of such herbal medicines have plaque both the primary and tertiary health care system in Nigeria.

**Aim of study**

To evaluate the prophylactic activity of *Phyllanthus amarus* and *Diodia scandens* on Swiss albino rat challenged with Coagulase negative *Staphylococcus*

**Objectives of Study**

1. To carry out the viability of the reference strains using culturing and biochemical methods
2. To carryout crude extraction ,of bioactive ingredients using hot (soxhlet) and cold extraction method on ethyl-acetate solvent
3. To determine the prophylactic activities of *Phyllanthus amarus* and *Diodia scandens* on Swiss albino rat using ethyl-acetate crude extract concentration for pre-treatment
4. To challenge the animal subjects with Coagulase negative *Staphylococcus* strains

# CHAPTER TWO

## 2.0 LITERATURE REVIEW

### 2.1 Medicinal Plants as potential antimicrobial agents

Medicinal plants include a various types of plants used in herbalism and some of these plants have medicinal activities, which are considered as a rich resources of ingredients that can be used in drug development and synthesis (Bassam, 2012). Besides that these medicinal plants play a critical role in the development of human cultures around the whole world, studies have shown that about half million of medicinal plants have not been explored and their medical activities could not be decisive in the treatment of present or future studies (Bassam, 2012).

The World Health Organization (W.H.O) has estimated that approximately 80% of the global population relies on traditional herbal medicines as part of standard healthcare (Foster *et al.*, 2005). Public, academic and governmental interest in traditional medicines is growing exponentially due to increased incidence of the adverse drug reactions and economic burden of the modern synthetic drugs. Further to this, consumers concern about the safety of food containing synthetic chemicals as preservatives and the increasing antibiotic resistance of food and water borne pathogens has also necessitated a growing interest in the use of natural antibacterial compounds such as those from medicinal plants because they have little side effects and possess great antimicrobial activity. Many drugs presently prescribed by physicians are either directly isolated from plants or are artificially modified versions of natural products. In Western countries, approximately 25% of the drugs used are of natural plant origin (Foster *et al.*, 2005).

Plants produce a whole series of different compounds which are not of particular significance for primary metabolism, but represent an adaptive ability of a plant to adverse abiotic and biotic environmental conditions. They can have a remarkable effect to other plants,

microorganisms and animals from their immediate or wider environment. All these organic compounds are defined as biologically active substances, and generally represent secondary metabolites, given the fact that they occur as an intermediate or end products of secondary plant metabolism. These secondary metabolites, apart from determining unique plant traits, such as: colour and scent of flowers and fruit, characteristic flavour of spices, vegetables, they also complete the functioning of plant origin, showing both biological and pharmacological activity of a plant. Therefore, medicinal properties of plants can be attributed to secondary metabolites (Taylor *et al.,* 2001).

Plants contain constituents that are bioactive and thus are used in the treatment of many human diseases. Diverse array of phytochemicals such as flavonoids, phenolic acids, tannins, lignins and other compounds are contained in products derived from plants. Several health-related benefits including antibacterial, antiplasmodial, antioxidant, anticarcinogenic, antimutagenic, antithrombotic and vasodilatory activities have been obtained from these chemical constituents (Chitravadivu *et al.,* 2009). These compounds either act on different systems or biochemical pathways of animals including man, and/or act through interfering in the metabolism of microbes. Each compound in a medicinal plant extract, exhibits different biological activity, which include: direct antimicrobial activity showing effects on growth and metabolism of microorganisms and indirect activity as antibiotic resistance modifying compounds which, combined with antibiotics, increase their effectiveness. It has been proposed that the degree of antimicrobial activity of plant extracts against microbes is influenced by conditions such as the inhibition of various cellular processes, followed by an increase in plasma membrane permeability and finally ion leakage from the cells (Chitravadivu *et al.,* 2009).

### 2.1.1 The use of medicinal plant (herbal drugs)

Many of the pharmaceuticals currently available to physicians have a long history of use as herbal remedies, including opium, aspirin, digitalis, and quinine. The World Health Organization (W.H.O) estimates that 80% of the population of some Asian and African countries presently use herbal medicine for some aspect of primary health care (Bassam, 2012). Pharmaceuticals are prohibitively expensive for most of the world's population, half of which lives on less than $2 U.S. per day. In comparison, herbal medicines can be grown from seed or gathered from nature for little or no cost. The use of, and search for, drugs and dietary supplements derived from plants have accelerated in recent years.

Pharmacologists, microbiologists, botanists, and natural-products chemists are combing the Earth for phytochemicals and leads that could be developed for treatment of various diseases. In fact, according to the World Health Organisation, approximately 25% of modern drugs used in the United States have been derived from plants. (Bassam, 2012). Among the 120 active compounds currently isolated from the higher plants and widely used in modern medicine today, 80 percent show a positive correlation between their modern therapeutic use and the traditional use of the plants from which they are derived (Bassam, 2012). More than two thirds of the world's plant species - at least 35,000 of which are estimated to have medicinal value - come from the developing countries. At least 7,000 medical compounds in the modern pharmacopoeia are derived from plants. In many medicinal and aromatic plants (MAPs), significant variations of plants characteristics have been ascertained with varying soil traits, and the selective recovery and subsequent release in food of certain elements have been demonstrated. Great attention must be paid to choice soil and cropping strategies, to obtain satisfactory yields of high quality and best-priced products, respecting their safety and nutritional value. (Bassam, 2012)

### 2.1.2 Extinction of Medicinal Plant species

It has been reported that over 50% of prescription drugs derived from chemicals first are identified in plants. A 2008 report from the Botanic Gardens Conservation International (representing botanic gardens in 120 countries) warned that cures for ailments such as cancer and HIV may become extinct before they are ever found. They identified 400 medicinal plants at risk of extinction from over-collection and deforestation, threatening the discovery of future cures for disease. These included yew trees (the bark is used for the cancer drug paclitaxel); Hoodia (from Namibia, a potential source of weight loss drugs); half of Magnolias (used as Chinese medicine for 5,000 years to fight cancer, dementia and heart disease); and autumn crocus (for gout). Their report said that five billion people still rely on traditional plant-based medicine as their primary form of health care (Bassam, 2012). The results of monitoring by traffic of selected species at high risk show few signs of recovery (Bassam, 2012).

### 2.1.3 Synergistic effects of chemosynthetic antimicrobials and herbal drugs.

The long standing successful use of herbal drug combination in traditional medicine makes it necessary to find a rationale for pharmacological and therapeutic superiority of many of them in comparison to isolated single constituents. Synergistic effect can be produced if the constituent of an extract affect different target or interact with one or several substances of an extract. A special synergistic effect can occur when antibiotics are combined with an agent that antagonizes bacterial resistance mechanisms. The verification of a real synergy effect can be achieved through detailed pharmacological investigations and by means of controlled chemical studies performed in comparison with synthetic drugs. The new generation of phytopharmaceuticals could lend phytotherapy a new legitimacy and enable their use to treat diseases which have hitherto been treated using synthetic drugs alone (Wagner and Ulrich-Merzenich, 2009)

## 2.2 Description of *Phyllanthus amarus*

*Phyllanthus amarus* belongs to the family Euphorbiaceae. It grows up to 10-60cm high and it 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 10 or 11 subgenera: *Botryanthus*, *Cicca*, *Conani*, *Emblica*, *Ericocus*, *Gomphidium*, *Isocladus*, *Kirganelia Phyllanthodendron*, *Phyllanthus*, and *Xylophyll* (Unander *et al.,* 2005). It has an erect stem, naked below and slender spreading leaf branches and it is also reported as a troublesome weed in pulses, soyabean, groundnut, cereals, sugar cane, cassava, taro, sesame, sunflower and cotton. (Oudhia *et al.,* 2008).

### 2.2.1 Origin and Geographical Distribution of *Phyllanthus amarus*

The widespread throughout the tropics and subtropics in sandy regions as a weed in cultivated and wastelands, make the plant to be indigenous to tropical America, the Philippines and India (Oudhia *et al.,* 2008). Plants in the genus *Phyllanthus* are found around all tropical regions of the world from Africa to Asia, South America and the West Indies. Some related species with medicinal importance include *Epiphyllanthus*, *P. niruri, P. urinaria*, *P. acuminatus* and *P. emblica.* The plant can be found along roads, valleys, on riverbanks and near lakes. *P. amarus* is sometimes mistaken and wrongly identified with the closely related *P. niruri*. In appearance, phytochemical structure and history of use, *P. niruri* also reaches a height of 60 cm with larger fruits and dark brown and warty seeds than that of *P. amarus* (Oudhia *et al.,* 2008).

Plate 1: *Phyllanthus amarus* (Source: Federal University Oye-Ekiti and environs)

### 2.2.2 Traditional use of *Phyllanthus amarus*

*Phyllanthus amarus* is a better and astringent that is used in folk remedies in many countries. It has a long history of use in the treatment of liver, kidney and bladder problems, diabetes and intestinal parasites. *P. amarus*, *P. niruri* and *P. urinaria* have been used in the treatment of kidney related problems, gallstones, appendix inflammation and prostate problems has also shown to work as an antifungal, antibacterial and antiviral agent (Oudhia *et al.,* 2008). 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.

The plant is a popular medicinal herb used as a remedy around the world for influenza, dropsy, diabetes and jaundice (Ushie *et al.,* 2013).Whole plant is employed in some genitourinary infections. The young tender shoots are used in chronic dysentery and the juice of the stem is mixed with oil in ophthalmia for eye treatments. According to Ushie *et al.,* (2013), the aerial part of *Phyllanthus amarus* is highly valued in traditional medicine for its healing properties. Fresh leaf juice of the plant can be applied externally for the treatment of cuts and bruises. It is also good for treating Arthritis and Asthma in patients (Ushie *et al.,* 2013). The plant is also used in traditional medicine as herbal decoction in treating bladder and kidney disorders, cramps and uterus complaints (with other herbs) and also works as an appetizer. Sonia *et al.* (2014), reported that plant extracts of *P. amarus* can be used as blood purifiers, for light malaria fevers, anaemia, colic and also helps the release of phlegm.

*Phyllanthus amarus* which is otherwise called Eyin olobe in South-Western Nigeria has healing effects on hypertensive patients (Etta, 2008). 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

### 2.2.3 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. It constantly addresses a challenge because of the large number of compounds present as mixture in the extract in trace amounts. *Phyllanthus amarus* has been reported to possess two lignin namely phyllanthin and hypophyllanthin obtained from the leaves of the plant that has been noted to enhance the cytotoxic responses with cultured multidrug-resistant cells. Niranthin, nirtetralin, phyltetralin and lintetralin the four flavanone glycoside has been reported to be obtained from the leaves of *Phyllanthus amarus*. Alpha-lrhamno pyranoside, a flavanol could be obtained from the stem of the plant (Sonia *et al*., 2014).

Moreover, the structure of three new lignans namely -desmethoxy seco-isolintetralin, 2, 3-desmethoxy seco-isolintetralin diacetate and demethy-lenedioxyniranthin were determined from leaves of *Phyllanthus niruri*. An unusual ellagitannin, Phyllanthusiin D, was found to be isolated from the biological active polar fraction of aerial parts of *Phyllanthus amarus* whose structure was established as 1-galloyl-2,4-(acetonyl dehydrohexahydroxydiphenoyl)-3,6-hexahydroxy di phenoyl-glucopyranoside by chemical and spectroscopic methods (Sonia *et al*., 2014).

### 2.2.4 Pharmacological Properties

*Phyllanthus amarus* have been found to have pharmacological activity against viral, bacterial, fungal, infection and also cancer and antifertility

**1. Antiviral**: *Phyllanthus amarus* target different steps of the HIV life cycle, thereby presenting multiple antiviral activities. The water/alcohol extracts blocks HIV-1 attachment and the HIV-1 enzymes integrase, reverse transcriptase and protease to different degrees. A gallotannin containing fraction and the isolated ellagitannins geraniin and corilagin were

shown to be the most potent mediators of these antiviral activities. The *P. amarus* derived preparations blocked the interaction of HIV-1 gp120 with its primary cellular receptor CD4 at 50% inhibitory concentrations of 2.65 (water/alcohol extract) to 0.48 μg/ml (geraniin).

**2. Antimicrobial**: *P. amarus* showed the most promising antibacterial properties, inhibiting all of the strains tested with minimum inhibitory concentrations (MICs) ranging from 0.25 to 16 mg/ml. The strains isolated from both HIV seropositive patients were susceptible to various concentrations of the P. amarus extracts (5, 10, 20, 40 and 80 mg ml-1) which were assessed against extend spectrum β-lactamase producing Escherichia coli isolated from the stool samples of HIV seropositive patients with or without diarrhoea.

**3. Anticancer**: Aqueous extract of *P. amarus* treatment exhibited potent anticarcinogenic activity against 20- methylcholanthrene (20-MC) induced sarcoma development and increased the survival of tumour harbouring mice. The extract administration (p.o) was also found to prolong the life span of Dalton's Lymphoma Ascites (DLA) and Ehrlich Ascites Carcinoma (EAC) bearing mice and reduced the volume of transplanted solid tumours.

**4. Antifertility**: Antifertility effects of an alcohol extract of the whole plant, *P. amarus* a dose of 100 mg/kg body weight for 30 days orally was investigated in cyclic adult female mice. The results revealed no significant change in absolute body and organ weights in extract-fed animals, indicating no alteration in general metabolic status. Further, feeding had no effect on haematological and clinical biochemical tests reflecting its non-toxicity. Similarly, uterine and ovarian biochemical tests showed no change except in 3β and 17β hydroxy steroid dehydrgenase (HSDs) levels, probably affecting hormonal conversions in the latter. Cohabited females with normal male mice were unable to become pregnant as their cyclicity was affected. Upon withdrawal of feeding for days, these effects were reversible.

## 2.3 Description of *Diodia scandens*

*Diodia scandens* belongs to the family Rubiacea, it has a dark green colouration, it is tasteless, odourless and has solitary inflorescence. It is a straggling herb with slender angular stems of 1m to 3m long. It is used in the Western Africa system of medicine in curing various ailments and also used as antidotes (venomous stings, bites etc.), antibacterial treatment of veneral diseases and cutaneous and for subcutaneous fungal infection. The common names include: Abure naosi (Ivory Coast), Sierra leone - Kissi yendeyendo, Yoruba - ewe idasha, Ibo – onaedi and Delta – okpo. The most important plant parts to be used are roots and leaves. Roots are bitter in taste with external colour greyish yellow to brown and internal colour is whitish grey, surface is slightly wrinkled and rough with coarse longitudinal markings. In India, many indigenous plants are used in herbal medicine to cure diseases and heal injuries. Some important chemical substances found in plants are alkaloids, carbon compounds, hydrogen, nitrogen, glycosides, essential oils, fatty oils, resins, mucilage, tennin, gums and others (Essiett *et al.*, 2010).

Most of the chemical substances are potent bioactive compounds found in medicinal plant parts that can be used for therapeutic purpose or which are precursors for the synthesis of useful drugs. The active principles differ from plant to plant due to their biodiversity and they produce a definite physiological action on the human body (Essiett *et al.*, 2010). The importance of these medicinal plants and their importance in the pharmaceutical industry has been elucidated (Edeoga *et al.*, 2005).

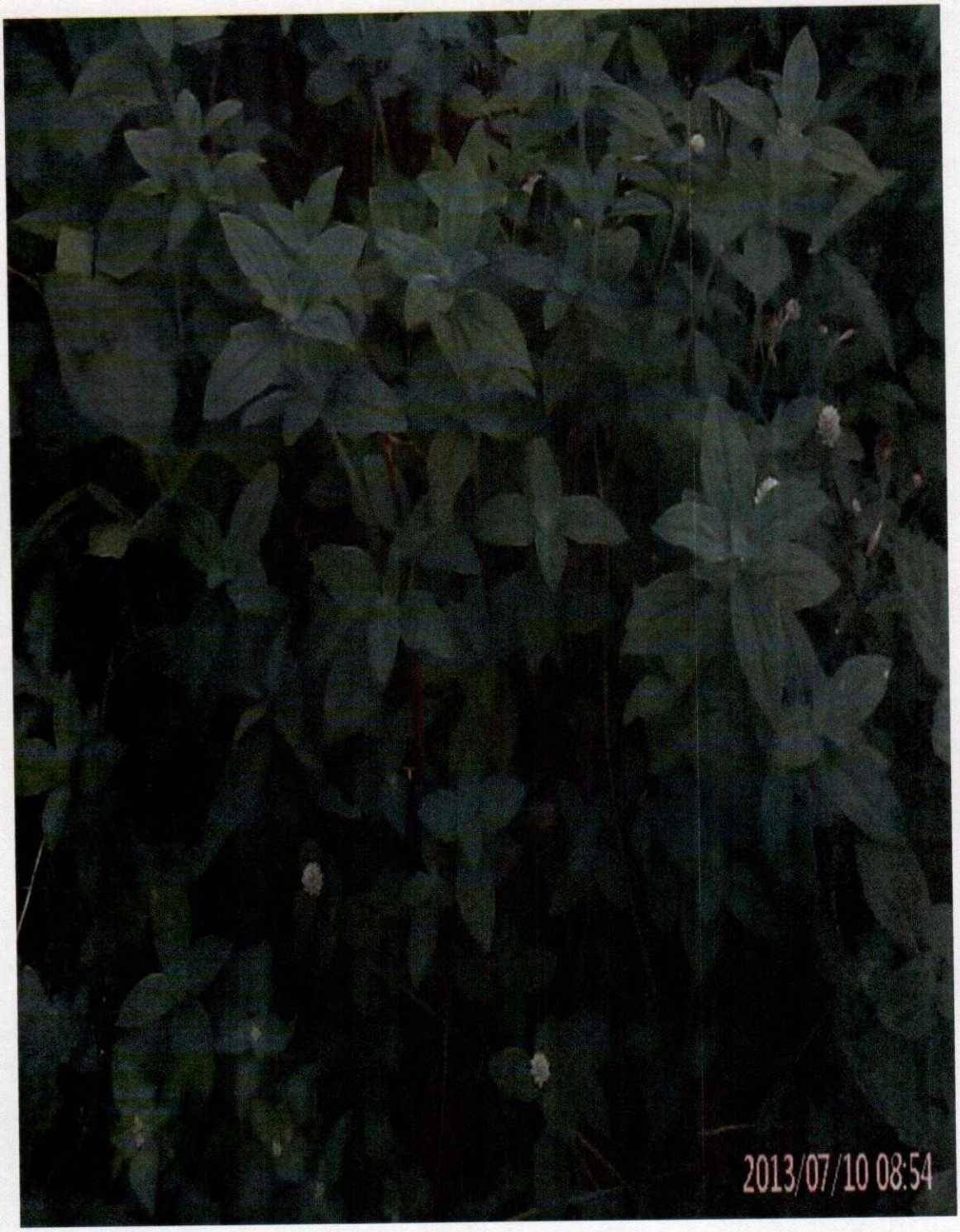


Plate 2: *Diodia scandens* (Source: Federal University Oye-Ekiti and environs)

## 2.4 Description of Coagulase negative *Staphylococcus*

Coagulase negative *Staphylococci* is a Gram positive bacterium that usually appears under the microscope as spherical (coccus) organisms appearing in pairs, short chains, or bunched, grape-like clusters. Coagulase negative *Staphylococcus* has been implicated as a causative agent in acute food poisoning episodes, toxic shock syndrome, impetigo, scalded skin syndrome, cellulitis, folliculitis and furuncles. It is also a common cause of systemic infections such as infective endocarditis, osteomyelitis, epiglottitis, and sinus infections among others. (Parsonnet *et al.*, 2005).

### 2.4.1 Epidemiology

Coagulase negative *Staphylococcus* is one of the most common causes of skin, soft-tissue, and nosocomial infection. Rates of infection in community settings are increasing. Coagulae negative *Staphylococcus* can cause illness by preformed toxin production as well as by infecting both local tissues and the systemic circulation. According to W.H.O 2010 disease transmission can occur in the following settings:

a) Gastrointestinal: Coagulase negative *Staphylococcus* causes acute episodes of food poisoning via preformed enterotoxins. Food items likely to be infected by staphylococcal food poisoning include meat and meat products; poultry and egg products; salads such as egg, tuna, chicken, potato, and macaroni; bakery products such as cream-filled pastries, cream pies, and chocolate éclairs; sandwich fillings; and milk and dairy products.

b) Skin and hair infections: Coagulase negative *Staphylococcus* commonly colonizes many skin surfaces on the nasopharynx, and perineum; but can cause infection of these surfaces particularly if the cutaneous barrier has been disrupted or damaged.

c) Systemic infections: Coagulase negative *Staphylococcus* commonly causes infective endocarditis in IV drug abusers; osteomyelitis, sinus infections in the general population; and epiglottitis in young children.

d) Nosocomial infections: Methicillin resistant Coagulase negative *Staphylococcus* is a strain of the bacteria that is commonly implicated in nosocomial infection. Risk factors for MRCoNS colonization or infection in the hospital settings include prior antibiotic exposure, admission to an intensive care unit, surgical incisions, and exposure to infected patients.

### 2.4.2 Incubation Period

Onset of symptoms after consuming contaminated food is usually 30 minutes to 8 hours, colonies of Coagulase negative *Staphylococcus* can be carried for an undetermined amount of time; some individuals may carry it chronically, and some may carry it intermittently, Incubation Period for foodborne Coagulase negative *Staphylococcus d*isease is 1-6 hours (Parsonnet *et al*., 2005).

### 2.4.3 Diagnosis of Coagulase negative *Staphylococcus*

Gastrointestinal illness can be diagnosed by isolating the preformed toxins from the contaminated food item. Systemic infections are best diagnosed by blood cultures (Parsonnet *et al*., 2005).

### 2.4.4 Treatment

Coagulase negative *Staphylococcus* is susceptible to beta-lactamase resistant penicillins such as ticarcillin and pipercillin. Vancomycin is the drug of choice for MRCoN infections.

### 2.4.5 Mode of transmission

Coagulase negative *Staphylococcus* is transmitted via ingestion of food containing enterotoxins, vertical transmission during vaginal delivery is uncommon. Person-to-person transmission occurs through contact with a purulent lesion or with a carrier. Unsanitary

conditions and crowded community settings increase exposure to Coagulase negative *Staphylococcus.* Infection may be spread from person-to-person through health care workers or patients (Murray *et al*., 2003).

### 2.4.6 Reservoir

Coagulase negative *Staphylococcus* is found in humans in the nose, groin, axillae, perineal area (males), mucous membranes, the mouth, mammary glands, hair, and the intestinal, genitourinary and upper respiratory tracts. Many animals act as reservoirs, particularly cows with infected udders (Murray *et al*., 2003).

# CHAPTER THREE

## 3.0 MATERIALS AND METHODS

### 3.1 Collection of Plants

The whole plants of *Phyllanthus amarus* and *Diodia scandens* were collected from vegetation in Federal University, Oye-Ekiti, Ekiti State and its environs.

### 3.2 Confirmation of test organism (Coagulase negative *Staphylococcus*)

A referenced pure culture Coagulase negative *Staphylococcus* (CONS 10b: OJO) was obtained from the Drug Discovery and Development Research unit of the Department of Microbiology, Federal University Oye Ekiti. The colonies of Coagulase negative *Staphylococcus* growing on slants were streaked on top of freshly prepared plate of mannitol salt agar and incubated for 35°C for 24hours. The primary characterization of the isolate was based on Gram staining, morphology and cultural characteristics. Identification of this isolate includes growth on different media including nutrient agar, fermentation on mannitol salt agar, catalase, coagulase and citrate utilization and sugar test were performed for the biochemical characterization.

### 3.3 Preparation of Extract

The fresh whole plants of *Phyllanthus amarus* and *Diodia scandens* washed thoroughly with sterile distilled water and were air dried at room temperature for exactly 2weeks to ensure the sample loose most of its moisture content. The dried plant materials were then pulverized into fine powdered form using an electric-blender (Nakai Magic blender, Model No: SI-889BD). The Powdered material of the plant was subjected to successive soxhlet hot and cold extraction with ethyl-acetate to obtain the respective extract. The hot extraction was done with soxhlet extractor using 75g of the plant material in 500ml of ethyl acetate under water bath regulated at 65$^0$C for 6hours while the cold extraction was done using 75g of the plant

material in 500ml of ethyl acetate in an enclosed container for 48hours. The extract component of the cold extraction method was filtered with Whatman No. 1 filter paper. The filtrate was concentrated using rotary evaporator (Senco Technology Co. Ltd; Model No: R205; SN: 13605) at a speed of 39-40rpm. The extracts obtained were transferred into clean containers and stored until ready for use. The extracts were subjected to evaporation using mild temperature of a regulated water bath at $50^0$C until molten semi-solid extracts were obtained and the rotary evaporator was used to recover the solvent (Mbata and Saika, 2008).

**3.4 Animal Study**

Twelve (12) Male and female Swiss albino rats, free from contaminating organisms, weighing 150-165g were used in the study. They were obtained from the animal house, Afe Babalola University Ado Ekiti, Ekiti State. The rats were grouped into three with four rats per group and a control group. The rats were kept in a polycarbonated metabolic cage at ambient environmental condition of about 25°C at 50-60% relative humidity. The rats were fed on a standard rodents diet (growers' mash) consisted of crude protein, fat, calcium, available phosphate, vitamins, crude fibre and constant supply of water. The animals were subjected to light and dark cycles of 12hours respectively and they were allowed to acclimatize for seven days. (Jena *et al.,* 2012).

Plate 3. Swiss albino rat in a polycarbonated metabolic cage

### 3.5 Prophylactic treatment

Each mouse in group 1 to group 3 were intubated orally for 4 days after acclimatization, the mouse in group 1 were administered with 0.5ml of sterile extract of 25mg/kg dose of ethyl-acetate extract of *Phyllanthus amarus* and *Diodia scandens* and also the mouse in group 2 were administered with 0.5ml sterile extract of 50mg/kg dose of ethyl-acetate extract of *Phyllanthus amarus* and *Diodia scandens* was administered orally into the mouth of the rats while the group 3 which serves as the control were administered with the mixture of 2.5ml DMSO ( Dimethyl sulphoxide) in addition with 15ml of distilled water for seven days before incubating with the test organism. Their weights were taken every other day for the 4 days of the experiment.

**Group A**: Infected rats treated with ethyl-acetate extract of *Phyllanthus amarus* and *Diodia scandens* at the concentration of 25mg/kg

**Group B**: Infected rats treated with ethyl-acetate extract of *Phyllanthus amarus* and *Diodia scandens* at the concentration of 50mg/kg

**Group C**: Not infected but treated with DMSO and Distilled water (Control).

**3.6 Organism Challenge Test:** On the fourth day of the pre-treatment with the administration of the sterile extract, each group were challenged with 0.5ml of $1x10^8$cfu in 0.5 McFarland Standard of the Coagulase negative *Staphylococcus* in Mueller Hinton broth. The control group was challenged with the test organism after injection with 0.5ml of the mixture of sterile distilled water and DMSO. The protection offered by the compound was determined by recording the mortality rate of rats in different groups up to 3 days.

# CHAPTER FOUR

## 4.0 RESULTS

### 4.1 Microscopic identification and Biochemical characterization of Coagulase negative *Staphylococcus*

The reference organism on microscopic examination showed Gram positive cocci in clusters. There is the production of bubbles indicating catalase positive, no clump on serum, indicating coagulase negative. The organism ferment maltose, sucrose, lactose and fructose with production of acid and gas with citrate utilization changing colour from red to yellow (Table 1).

**Table 1: Microscopic identification and Biochemical characterization of Coagulase negative *Staphylococcus.***

| Test organism | Coagulase | Catalase | MSA | Citrate | Lactose | | Maltose | | Fructose | | Sucrose | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| CoNS 10b: | - | + | - | + | A | G | A | G | A | G | A | G |
| OJO | | | | | + | + | + | - | + | + | + | + |

Key: += Positive, -= Negative, A= Acid production, G= Gas production

### 4.2 Combined dosage administration and response of *Phyllanthus amarus* and *Diodia scandens* pre-treatment of Swiss albino rats.

The prophylactic pre-treatment of the two plant *Phyllanthus amarus* and *Diodia scandens* combined are represented in table 2. The result shows that the synergism of the concentration of 25mg/kg and 50mg/kg of the two plants were very effective on the rats. The extracts were not toxic to the rats for the 4days of pre-treatment with no indication of diarrhoea, no redness of the eyes and skin, no discharge from the eyes, or death. Infected mice in group A treated with 0.5ml sterile extract of 25mg/kg ethyl-acetate extract of *phyllanthus amarus* and *Diodia scandens* were aggressive and active from the 1st to the 4th day, while infected rats in group 2 treated with 0.5ml sterile ethyl-acetate extract of 50mg/kg of combined *Phyllanthus amarus* and *Diodia scandens*, were very aggressive and hyperactive from the 1st to the 4th day. The control group treated with DMSO and distilled water appeared normal.

**Table 2: Combined dosage administration and response of *Phyllanthus amarus* and *Diodia scandens* Pre-treatment of Swiss albino rats.**

| | | | | DAY1 | | | | | | | | DAY 2 | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Groups Mean weight(kg) | $T_x$ | C (mg/kg) | $D_o$ (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 (155) | EA1 | 25 | 0.5 | 0 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2 (165) | EA2 | 100 | 0.5 | 0 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 0 | 0 | 0 | 0 | 0 | 0 |
| Control (190) | DMSO+ Distilled $H_2O$ | | 0.5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

Key: Tx- Treatment, $D_0$- Dosage, C- Concentration, R- Redness of the eye and skin, RD- Response to dosage, Agg- Aggressiveness, INF- Inflammation, D-Discharge in eyes, Di- Diarrhoea, D- Death, RD- Response to dosage, EA 1 and EA2- Ethyl acetate 1 and 2, W-Weakness, DMSO+ds $H_2O$= distilled water

**Table 2.1: Combined dosage administration and response of *Phyllanthus amarus* and *Diodia scandens* Pre-treatment of Swiss albino rats**

| | | | | DAY3 | | | | | | | | DAY 4 | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Groups - Mean weight(kg) | $T_x$ | C (mg/kg) | $D_o$ (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 (155) | EA1 | 25 | 0.5 | 0 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2 (165) | EA2 | 100 | 0.5 | 0 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 0 | 0 | 0 | 0 | 0 | |
| Control (190) | DMSO+ Distilled $H_2O$ | | 0.5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |

Key: Tx- Treatment, $D_0$- Dosage, C- Concentration, R- Redness of the eye and skin, RD- Response to dosage, Agg- Aggressiveness, INF- Inflammation, D-Discharge in eyes, Di- Diarrhoea, D- Death, RD- Response to dosage, EA 1 and EA2- Ethyl acetate 1 and 2, W- Weakness, DMSO+ ds. $H_2O$= DMSO + distilled water

### 4.3 Protection offered by the combined extract of *Phyllantus amarus* and *Diodia scandens* on Swiss albino rats.

Table 3 shows protection offered by the combined extract of *Phyllanthus amarus* and *Diodia scandens* to the Swiss albino rats being challenge with Coagulase negative *Staphylococus*. After 1 hour of the challenged test, rats in group A and B remains hyperactive and normal (healthy) without any observable signs or symptoms and no death recorded, whereas the control group appeared dull (not active).

**TABLE 3: Protection offered by the combined extract of *Phyllantus amarus* and *Diodia scandens* on Swiss albino rats.**

| 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 | Gp1 (n=4) | 25mg/kg | W | N | N | N | N |
| Treated | Gp2 (n=4) | 50mg/kg | W | N | N | N | N |

**Key**: W= weak N= normal DMSO= Dimethyl sulphoxide

# CHAPTER FIVE

## 5.0 DISCUSSION

### 5.1 Combined dosage administration and response of *Phyllanthus amarus* and *Diodia scandens* pre-treatment of Swiss albino rats.

The use of combined ethanol and n-hexane extracts of *Phyllanthus amarus* and *Diodia scandens* as herbal medicine has been well reported by Ojo *et al.* (2013). And also the antimicrobial and phytochemical properties of the combined plant extract of *Phyllanthus amarus* and *Diodia scandens* has revealed the potency of the plants.

This study showed the efficacy of ethyl-acetate crude extracts of different concentration of 25mg/kg and 50mg/kg on the Swiss albino rats which was in corroborated by Ojo *et al.* (2013) who used 128ug/ml and 256ug/ml of the two plant extracts combined on *Staphylococcal* isolates at an in vitro study.

This results after four (4) days revealed that the concentration used as pre-treatment on the Swiss albino rats had no toxic effects on the rats, with no indication of diarrhoea, redness of the eyes and skin, no discharge from the eyes or death of the animals. This result is in agreement with Mb Abudul *et* al. (2015) who reported on the efficacy of the plant extracts of *Phyllanthus amarus* and *Leucas aspera* at the concentration of 150mg/kg and 300mg/kg

### 5.2 Prophylactic activity of *Phyllanthus amarus* and *Diodia scandens* ethyl-acetate extract on organism challenged Swiss albino rat.

The protection offered by the combined ethyl-acetate extracts of *Phyllanthus amarus* and *Diodia scandens* on Swiss albino rat against Coagulase negative *Staphylococcus* after

72hours of observation for clinical signs and symptoms indicating weakness but no death at the different concentration used. This was synonymous with an earlier report by Mb Abudul *et* al. (2015), who reported on the *Phyllanthus amarus* and *Leucas aspera* at the concentration of 150mg/kg and 300mg/kg on Swiss albino rats, showing weakness and no death of the animal subjects.

It was also noted that the longer the time of exposure of the animals on different dosage or concentration, gave a good and lasting prophylaxis on the animal subjects with no adverse effect. The survival of the rats after four days of pre-treatment revealed that the ethyl-acetate extracts was able to inhibit or kill the growth of Coagulase negative *Staphylococcus* which was similar to a report by Ojo *et al.* (2013) who reported a synergistic activity against Coagulase negative *Staphylococci* in vitro at 128u/ml and 256u/ml.

# 6.0 CONCLUSION AND RECOMMENDATION

## 6.1 Conclusion

The whole plant of *Phyllanthus amarus and Diodia scandens* combined has broad spectrum activities which have great potential as antibacterial compounds against Coagulase negative *Staphylococcus.* Thus, they can be used in the treatment of antibacterial related symptoms and other infectious diseases caused by these selected bacteria. The demonstration of their synergistic effect against the test organisms is an indication that there is possibility of sourcing for an alternative antibacterial compounds in this plant for the development of newer antibacterial agents.

## 6.2 Recommendation

The synergistic effect of *Phyllanthus amarus and Diodia scandens* has proven its antimicrobial activity on Coagulase negative *Staphylococcus*. It is therefore recommended that the synergistic effect of these plants be evaluated on other causative organisms of bacterial infections.

## 6.3 Contribution to Knowledge

1. The ethyl-acetate of 25mg/kg and 50mg/kg pre-treated on the Swiss albino rats did not cause any feverish condition, inflammation, diarrhoea, redness of the eyes, weakness nor death.

2. *Phyllanthus amarus* and *Diodia scandens* combined offered a prophylactic activity on the treated groups after been challenged with Coagulase negative *Staphylococcus* at different concentration of 25mg/kg and 50mg/kg

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## 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 dropped on the smear and examined under the microscope.

- **Sugar fermentation**

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 which 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 sugar1 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 and coagulase negative *Staphylococcus* which does not produce 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

Group I: 155-160g

Group II: 165-170g

Control III: > 190g

To calculate for the average weight of the rats

For group I: 155+160=315÷2= 157.5g

For group II: 165+170g=335÷2= 167.5g

For group III: 190g.

**Preparation of Extract Concentration**

For 25mg/kg body weight

Average weight = 157.5g= $\frac{25\times157.5}{1000}$ =3.94mg

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

$\frac{3.94}{1000}$ = 0.00394g/17.5ml

For 50mg/kg body weight

Average weight=167g=$\frac{50\times167}{1000}$=8.4mg/17.5ml

$\frac{8.4}{1000}$ = 0.0084g/17.5ml