ANTIBACTERIAL ACTIVITIES AND PHYTOCHEMICAL ANALYSIS OF SELECTED MEDICINAL PLANTS ON SELECTED PATHOGENIC BACTERIA FROM PATIENTS ATTENDING GENERAL HOSPITAL MINNA, NIGERIA.

By

IBRAHIM, Dawud
MTech/SLS/2018/8093

JULY, 2021

ANTIBACTERIAL ACTIVITIES AND PHYTOCHEMICAL ANALYSIS OF SELECTED MEDICINAL PLANTS ON SELECTED PATHOGENIC BACTERIA FROM PATIENTS ATTENDING GENERAL HOSPITAL MINNA, NIGERIA.

By

IBRAHIM, Dawud
MTech/SLS/2018/8093

A THESIS SUBMITTED TO THE POSTGRADUATE SCHOOL, FEDERAL UNIVERSITY OF TECHNOLOGY, MINNA, NIGERIA IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF MASTER OF TECHNOLOGY IN MICROBIOLOGY (PHARMACEUTICAL)

JULY, 2021

ABSTRACT

Medicinal plants are effective source of both traditional and modern medicines. This study was aimed to determine the antibacterial activities and phytochemical analysis of selected medicinal plants on selected pathogenic bacteria from patients attending General Hospital Minna, Nigeria. The pathogenic organisms (*Escherichia coli, Pseudomonas aeruginosa, Salmonella bongori* and *Klebsiella pneumonia)* and the plant materials (*Calotropis procera* leaf, *Leptadenia hastata* stem and *Securidaca longipedunculata* stem) were collected from Minna metropolis. The isolates were identified, characterized and confirmed using cultural, morphological and molecular techniques while the plant materials were extracted using cold maceration techniques and further partitioned into n-Hexane, ethylacetate and residual aqueous. The extracts were subjected to phytochemical screening, antimicrobial susceptibility testing, minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), gas chromatography-mass spectrometry (GC-MS) analysis. The qualitative and quantitative phytochemical screening of plants revealed the presence of flavonoids, phenols, tannins, alkaloids and saponings from all the three plants but tannins was found to be absent in *Leptadenia hastata* stem. The highest activity of *Calotropis procera* leaf extract was recorded against *Pseudomonas aeruginosa* (19.60±0.2 mm) whereas with *Leptadenia hastata* stem and *Securidaca longipedunculata* the activities of 21.55±0.65 mm and 13.15±0.15 mm were observed against *Klebsiella pneumonia* and *Escherichia coli* all at the concentration of 300 mg/mL respectively. The least MIC and MBC values of 37.5 mg/mL and 75 mg/mL were observed against *Escherichia coli* and *Pseudomonas*, 18.75mg/ mL and 37mg/ mL against *Klebsella pneumonia,* 75mg/ mL and 150mg/ mL *Escherichia coli* from *Calotropis procera* leaf, *Leptadenia hastata* stem and *Securidaca longipedunculata* stem respectively. Some of the fractions of the partitioned extract showed some increased level of activity against some of the organisms whereas some showed decreased level of activity. The most common compound presents in the extracts when analyzed using GC-MS was 9,12-Octadecadienoic acid (Z,Z)-($C_{18}H_{32}O_2$). The study revealed that the plants material could be a good candidate for use in the management of bacteria and other related illness as used in the research, although further evaluation need to be carried out in other to fully validate its usage.

TABLE OF CONTENTS

LIST OF TABLES

Table

Page

LIST OF FIGURES

Figure

Page

LIST OF PLATES

Plate

Page

LIST OF APPENDIX

ABBREVIATIONS

MIC Minimum inhibitory concentration

MBC Minimum bactericidal concentration

GC-MS Gas chromatograph interfaced to a mass spectrometer

BP Base pair

PCR Polymerase chain reaction

DMSO Dimethyl sulfoxide

MM Millimeter

CLSI Clinical and Laboratory Standards Institute

DNA Deoxyribonucleic acid

GC&M Growth characteristics on media,

MCM Microscopic morphological characteristics

IDZU7 *Pseudomonas aeruginosa* strain MK572634.1

IDZU8 *Salmonella bongori* strain MN623691.1

IDZU9 *Escherichia coli* strain CP074120.1

IDZU10 *Klebsiella pneumoniae* strain AB642255.1

WDP Weight of dry powder

WE Weight of extract

PY Percentage yield

G Gram

SLS Stem extract of *Securidaca longipedunculata*

LHS Stem of *Leptidenia hastata*

CP *Calotropis procera*

ND Not Detected

mg/mL Milligram per mil

WDE Weight of dry Extract

A Residual aqueous fraction

E Ethyl acetate fraction

H n-Hexane fraction

P-K Peak Number,

R-T Retention Time

M.F Molecular formula

M-W Molecular Weight,

% Conc. Percentage Concentration,

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background to the Study

Medicinal plants are effective source of both traditional and modern medicines which are genuinely useful for primary health care (Mohammadhosseini, 2017) and a safe remedy for ailment of both microbial and non-microbial origin **(**Chang *et al.,* 2019). Medicinal plants are plants that are used in treatment of diseases and this may be a whole or part of a tree, shrub or grass. Plants have an old record of medical use since the history of mankind. They play a very crucial role in the development of human cultural heritage around the Globe (Ahn, 2017). In western world, the use of plants in treating diseases is common and is known as "alternative medicine". These plants are frequently used as raw materials for extraction of active ingredient which is used in the synthesis of drugs (James *et al.,* 2018). Based on current global medical trend, medicinal plants have a bright future because there are over million species of plant around the world which their medical activity have not been investigated, thus their medical activity could be decisive in the treatment based on present or future studies (Atanasov *et al.,* 2015). More so, plants with Medicinal properties plays a central role not only as traditional medicine but also as commercial commodities which improves a Nation's economy (Ahn, 2017)

In Africa and other continents of the world, phyto-medicines have been used since time immemorial to treat various ailments long before the introduction of modern medicine (Mahomoodally, 2013). Herbal medicines are still widely used in many

parts of the world especially in areas where people do not have access to modern medicines (Mamza *et al.,* 2015). Moreover, in most African countries where herbal medicines are still heavily relied upon because of the high cost of chemotherapeutic drugs, there is need for scientific research to determine the biological activities of medicinal plants (Mahomoodally, 2013). The use of plant extracts with antimicrobial properties may be of importance in therapeutic treatment, whereas in the past few years, a number of studies have been conducted in different countries to prove such efficiencies (Smith *et al.,* 2012). Before a herb is used for any medicinal purpose, it has to be prepared, the use of herbs for medical purpose is referred to as phyto-medicine or phyto-therapy (Mamza *et al.,* 2015). Presently, there is a rising trend of including phyto-therapy in the curriculum of medical schools. Over 70 % of German physicians prescribe herbs to their patients than any other synthetic drug (Mahmoud, 2012). Herb's preparation can be in various forms, which include; extract, capsule, tablets powder and cream (Abubakar and Haque, 2020). Phyto-medicine is known to many and considered relatively safe because of their easy availability, cost effectiveness and also contains multiple chemicals with a specific composition that act as effective antimicrobials and side effect neutralizers (Mahmoud, 2012).

Calotropis procera, Leptidenia hastata and *Sacuridaca longipedunculata* are flowering plants natives to Tropical and North Africa (Moustafa *et al.,* 2010; Ibrahim *et al.,* 2012; Abubakar *et al.,* 2018). These plants have been used traditionally for the treatment of many diseases such as diarrhoea, stomatic, sinus fistula, skin disease and

the leaf are used to treat jaundice, fevers, rheumatism, indigestion, cold, eczema (Ibrahim *et al.,* 2012; Tabuti *et al.,* 2012; Mafulul *et al.,* 2018).

1.2 Statement of the Research Problems

Emergence of new and re-emergence of old diseases caused by microorganisms and continuous evolvement of drug resistance strains, high cost and side effect of many available drugs due to their toxicity and undesirable side effects in some patients are some of the problems that require quick and rapid attention (Atanasov *et al.,* 2015). However, this issue become more difficult for clinicians to empirically select an appropriate antimicrobial agent. High prevalence of morbidity and mortality mostly associated with infectious diseases are heavily recorded among people in developing countries, where as in developed countries small number of cases are recorded among the vulnerable minorities (GianLuca *et al.,* 2012). Combating these problems requires new effective drugs with high therapeutic index, hence there is an immense need for the discovery and development of new bioactive compounds (Atanasov *et al.,* 2021).

Urinary tract infection is among the most common bacterial infection that affect both gender (male and female) in community and hospital settings and it contribute greatly to the global health problem with millions of cases recorded annually with a significant mortality rate around the globe (Emiru *et al.,* 2013). Bacteria are the common etiological agents of urinary tract infections accounting for about 95 % of all cases, the most common prevalent bacteria in urinary tract infection are the members of *Enterobacteriaceae* (Abbo and Hooton, 2014; Ganesh *et al.,* 2019). *Escherichia coli* account for about 80 % of the cases followed by other organisms such as

Staphylococcus aureus Klebsiella pneumoniae, Proteus mirbilis and *Candida albicans* (Chongs *et al.*, 2018). Infection of wound injuries delays the healing process of the wound and increases pain and treatment cost to the patients. Five years surveillance carried out by Oladeinde *et al.* (2013) on wound infection at some rural tertiary hospitals in Nigeria stood to be 70 % with a significant morbidity and mortality rate in which *staphylococcus aureus* and *Pseudomonas aeruginosa* account for 24 % of the total infection rate. Furthermore, a fatal multi-systemic illness called enteric fever and dysentery caused by *Salmonella species* of bacteria contribute adversely to the Global health security challenges. Without a proper treatment, these bacteria can spread to various part of the body and a high mortality rate are recorded annually from infections caused by various species of this organism (Andino and Hanning, 2015). Furthermore, emerging and re-emerging infectious diseases are expected to remain a noticeable important challenge for the nearest future.

1.3 Aim and Objectives

The aim of this study was to evaluate antibacterial activities and phytochemical analysis on selected medicinal plants on some selected pathogenic bacteria from patients attending General Hospital Minna, Nigeria while the objectives of this study were to:

i. Identify and characterize the selected bacterial isolates.

ii. Determine the molecular characterization of the bacterial isolates

iii. Extract the plant materials; *Calotropis procera* (leaves), *Leptidenia hastata* (Stem) and *Securidaca longipedunculata* (stem) using cold water maceration techniques

iv. Determine the antibacterial activities of methanol extracts of the plants.

v. Determine the minimum inhibitory concentrations (MIC) and minimum bacteriocidal concentrations (MBC) of the active plants extracts.

vi. Fractionate the crude from (iv) above.

vii. Determine the compounds presents in the purified fraction using GC-MS.

viii. Determine the phytochemical constituents of the plants.

1.4 Justification for the Study

According to Harvey *et al.* (2015), among all the natural product derived from plant, microorganisms and animals, plant remain the highest source of therapeutic substances since the extinct civilization. This terrain is still maintained as about 60 % of major portion of new drugs are obtained from natural products and their derivatives. About 75-80 % of the world population relies primarily on traditional medicines most of which are prepared from plants, also many people believed that plant base remedies have minimal or no side effects (Alves and Humberton, 2011).

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 *Calotropis procera*

Calotropis procera is a wild growing plant belonging to the family Apocynaceae. It is popularly known as “Tunfafia” in Hausa language and “Bomubomu” in Yoruba in northern and western part of Nigeria respectively, other common name includes Rubber Bush, Apple of Sodom in India & Pakistan (Mali *et al.,* 2019). It is well recognized for its diverse medicinal properties as such several researchers have reported it diverse medicinal activity such as anti-inflammatory, analgesic, anti-tumor, anti-helmintic, hepatoprotective, anti-diarrhoeal, anticonvulsant, anti-microbial, oestrogenic, anti-nociceptive, antimalarial and antioxidant properties (Preeti, *et al.*, 2017). These medicinal activities of *Calotropis procera* is possibly achieved as a result of the phyto-constituents present in the plant which are responsible for different pharmacological activities such as alkaloids, flavonoids, saponins, sterol, cardenolide, triterpinoids, resins, tannins, anthocyanins, proteolytic enzymes in latex and cardiac glycosides. (Preeti *et al.* 2017; Vishnukant and Vidyut, 2017). Traditionally, the plant is used in treatments of various diseases such as: diarrhoea, stomatic, sinus fistula, eczema rheumatism, indigestion, fever, jaundice, and skin disease (Preeti *et al.,* 2017; Maflul *et al.,* 2018).

More so, it is used as a purgative, digestive, emetic, expectorant, sedative, blood purifier and an antidote for snake poisoning (Preeti *et al.,* 2017). Therefore, evaluating

the potentials of *Calotropis procera* in pathogenic bacteria of medical importance is of outermost importance.

2.1.1 Botanical classification and description of *Calotropis procera*

Taxonomically *Calotropis procera* is classified into:

Kingdom: Plantae (Plants)

Subkingdom: Tracheobionta (Vascular plants)

Superdivision: Spermatophyta (Seed plants)

Division: Magnoliophyta (Flowering plants)

Class: Magnoliopsida (Dicotyledons)

Subclass: Asteridae

Order: Gentianales

Family: Asclepiadaceae/Apocynaceae

Genus: *Calotropis*

Species: *C. procera.*

The plant grows as a small tree, evergreen, soft-wooded and perennial shrub that attains a minimum height of 2.5 m and maximum 6m. Furthermore, the plant has a corky, furrowed, and light gray bark and whenever any part of the plant is cut, a copious amount of white sap is generated (Ismaiel and AbdAlrheam 2015). The plant root is simple, branched, and woody at the base covered with a corky bark. The leaves of the plant are simple, subsessile, slightly leathery, opposite-decussate and estipulate; blade oblong-obovate to broadly-obovate, measures about 2.5-15cm, apex abruptly and shortly acuminate to apiculate, base cordate, succulent with entire margins. They

appear tomentose white when young and later becomes glabrescent and glaucous as shown as describrd in Plate I. The Flowers of *Calotropis procera* are bell-shaped shallow, bracteate, hypogynous, complete, actinomorphic, bisexual, pentamerous, multi-flowered, pedicellate, umbellate, peduncled cymes with axillary or terminal inflorescence, it has a five lobed shortly united sepals that ranges between 4-5 mm long and Five-lobed petals (Corolla), twisted aestivation, and gamopetalous are observed in the flower. It Androecium has stamens namely: gynandrous, anther and dithecous. Gynoecium is apocarpus, bicarpellary, and in apex united styles, peltate stigma with 5 lateral stigmatic surfaces. Anthers are joined to the stigma forming a gynostegium (Verma and Yadav 2017). Fruit is simple, inflated, fleshy, and subglobose to obliquely ovoid follicle. Seeds are present in large amount, flat, small, obovate, compressed with silky white pappusat at one end with a length of about 3 or more centimeter (Parihar and Balekar, 2016).

2.1.2 Distribution and habitat of Calotropis procera

Calotropis procera is native to North Africa, Tropical Africa, Western Asia, South Asia, and Indochina. *C. procera* favors open habitat with little competition (Neha, *et al.,* 2018**)**. It grows in dry habitat with limited rainfall of about 150 to 1000 mm and also inhabit areas of excessive drained soil as much as 2000 mm of annual precipitation; its common habitat includes: road-side, beachfront dunes, and widely disturbed in the urban areas (Parihar and Balekar, 2016). More so, it can be found at the elevated areas of 1,000m.

2.1.3 Medicinal uses of *Calotropis procera*

Calotropis procera have been reported widely in several folklore preparations and ethno-medicines. It was reported to have an Expectorant, Resolvent, Detergent, Purgative, Corrosive and Analgesic properties which are used in treating various ailments. A review on the pharmacological Study conducted by Shamim and Lubna (2019), revealed that the plant has a therapeutic potential in treating leprosy, ascites, arthritis, asthma and inflammatory conditions. It also has anticancer, antimicrobial, anti-diabetic and antioxidant properties. (Hayat *et al.*, 2020). *Calotropis procera* is strongly recommended in leprosy, hepatic and splenic enlargement, dropsy and worms. The latex and the fresh leaves can be applied to painful joints and swelling, the oil extracts from the leaves can be applied as a remedy in a paralyzed part of the body (Abhay *et al.,* 2019). The root bark is used to promote secretion and useful in treating skin disease, enlargement of viscera, intestinal worms, abdominal, ascites and ancesraca. in India, the milky juice is used as purgative, and the flowers are used to aid digestive, tonic, stomachic and useful in cough, thrush, mycotic enteritis asthma, catarish and loss of appetite (Goyal *et al.*, 2013; Abhay *et al.*, 2019).

The leaves of *Calotropis procera* is practically useful in treating backache and Joint pains and if warmed and tied around any body organ it relief pain (Abhay *et al.*, 2019). It is also use in treating Piles, dysentery, dropsy, ring worm and thorn removal from body (Verma and Yadav 2017), scabies, sores and malaria (Yaniv and koltai, 2018). The bark and leaves are also known to exhibit wound healing ability, anti-hyperglycemic effect, Analgesic, Anti pyretic, anti-cancer, and neuromuscular

blocking activities (Mali *et al.,* 2019), Acaricidal, schizonticidal,antimicrobial, anthelmintic,insecticidal,antidiarrheal,anticancerous, and larvicidal activities (Parihar and Balekar, 2016). Leaf part is used to treat jaundice, it is also used as sedative, blood purifier and an antidote for snake poisoning (Preeti *et al.,* 2017).

2.1.4 Antibacterial activity *of Calotropis procera*

The leaves of *Calotropis procera* have been reported to have activity against various pathogenic bacteria as indicated by many scientists. Thus, the scientist findings indicate the possibilities of the plant extracts to be used in treating susceptible bacterial infections. Hayat *et al.* (2020), reported that methanolic extract of *Calotropis procera* leaf has antibacterial activity against *Proteus mirabilis, Pseudomonas aeruginosa* and *Bacillus cereus, Escherichia coli, Klebsiella pneumoniae, Salmonella typhi* and *Enterococcus faecalis.*

The antibacterial activity carried out by Akin-Osanaiye and Okhomina (2018), using ethanol as a solvent shows the ethanolic leaves extracts to be potent against *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa and Salmonella typhi* more than the aqueous and methonolic solvents. So also studies by Akindele *et al.* (2017) show significant inhibitory zone from ethanolic extracted leaves of *Calotropis procera* against *Staphylococcus aureus* and *Escherichia coli.* In another investigation by Asoso *et al.* (2018), in their effort to find the possible leakages of sodium and potassium ions from microbial cells of some selected pathogens caused by *Calotropis procera* extract of various solvent (aqueous, acetone and methanol), a promising result was obtained from aqueous extract against *Escherichia coli.* When

compared with other commercial antibiotics (amoxicillin and tetracycline), the amount of sodium and potassium ions released by the extract shows to be higher than the commercial antibiotics. Whilst acetone extracted leaves shows high activity against *Pseudomonas aeruginosa, Proteus mirabilis* and *Citrobacter youngae* in the report of Oyesola *et al.* (2016). Furthermore, in investigating the activity of aqueous, petroleum ether and ethyl acetate leaves extract of *Calotropis procera* against *Escherichia coli* and *Proteus vulgaris*, the extracts from all the three solvents have antibacterial activity but the maximum activity is recorded from the aqueous extract against *Proteus vulgaris (*Sharma *et al.,* 2018). In a research conducted by Al-Ghanayem *et al*. (2017), moderate antibacterial activity of the methanolic leaves extract was observed against *Pseudomonas aeruginosa, Enterobacter cloacae, Enterococcus faecalis, Escherichia coli, Klebsiella pneumoniae*, *Proteus vulgaris* and *Salmonella typhi* isolates. Even the fungal isolates gotten from the root of *Calotropis procera* shows significant antibacterial activity as described by Verma and Yadav (2017), they isolated a total number of ten endo-phytic fungi (*Aspergillus* species) from the *Calotropis procera*, and they all show a great antibacterial activity against 24 human pathogenic bacteria (*E. coli*, *Streptococcus pneumoniae, B. subtilis, Staphylococcus hyicus, Bacillus sphaericus, S. aureus,* and *Pseudomonas aeruginosa*).

All these studies are pointing fingers to the plants to be a potential source of bioactive compounds to be used in managing bacterial infections. However, several studies as shown above laid emphasis on the inhibitory capability of *Calotropis procera* on

pathogenic bacteria using ethanolic solvent, but is important to note that ethanol itself have an antibacterial activity and its more toxic than methanol either by adopting agar disc or agar well diffusion techniques, therefore need to determine the exact antimicrobial effect of the plant with less toxic solvent, and there is need to properly investigate toxicological activity of the plant on laboratory animal for further clinical trials.

2.1.5 Antifungal activity of *Calotropis procera*

Apart from the antibacterial activity of *Calotropis procera* against pathogenic bacteria, several research pin point the plant to have a vast antifungal activity against many pathogenic fungi. *Calotropis procera* potency with methanol as solvents for extraction, was tested by Waheed *et al*. (2016) for their antifungal potential against *M. phaseolina* and the result obtained was promising. The in-vitro antifungal activity of *Calotropis procera* leaves extract evaluated by Goyal *et al*. (2013) against three different genera of dermatoplytes namely: *Microsporium, Trichophyton* and Epidermophyton by agar dilution method concludes with the statement "ethanolic extract of *Calotropis procera* is active against dermatophytes causative fungus of many superficial infections". However, using various solvent (petroleum ether, chloroform, ethyl acetate and ethyl alcohol) for extraction in testing the efficacy of *C. procera* on dermatophytic fungi, Verma *et al.* (2011) used the paper disc diffusion method to demonstrate anti-dermatophytic efficacy of foliar extract of Calotropis procera against three target fungi: *Microsporum canis, Microsporum fulvum, and Trichophyton mentagrophytes.* Furthermore, the bark extract of C. *procera* may be

used in developing phyto-medicines with antifungal properties based on the result obtained from aqueous bark extract that shows good zone of inhibition against *Tinea capitis, Candida albicans,* whereas it revealed moderate activity against *Microsporum canis* and *Epidermophyton floccosum* (Bajpayee, 2015).

Fungi toxic potentials of *Calotropis procera* leaves of four different solvents namely; aqueous, methanol, acetone and ethanol against the fungus *Alternaria alternate* determined by Deepika and Singh (2015), shows that the Ethanolic extract exhibit 100 % inhibition against the organism while methanol inhibited 76 % , acetone 86 % and aqueous gave least inhibition of less than 3 %. This give an insight on the ability of *Calotropis procera* ethanolic extract to serve as bio-fungicide in controlling disease caused by *Alternaria alternata*. In another study, conducted by Keta *et al.* (2018), using the latex of *Calotropis procera* against*Epidermophyton floccosum*, *Trichophyton rubrum* and *Microsporum canis,* a broad spectrum of activity was observed from the assay ranges from 13.28 mm to 16.10 mm inhibition zone. This signifies the effect of the latex against fungi. More so, ethanolic solvent was found to be the best solvent to be used in extracting the leaf extract of *Calotropis procera* than the aqueous as described by Manoorkar *et al.* (2015) in a research conducted to determine the antifungal activity of leaf and latex extracts of *Calotropis procera* against dominant seed-borne storage fungi of some oil seeds. The seed-borne dominant fungi includes; *Cuvularia lunata, Alternaria alternata, Rhizoctonia solani, Fusarium solani, Penicillium chrysogenum, Aspergillus niger, A.* fl*avus, A. terrus A. fumigatus, and Rhizopus sp.* using agar well diffusion methods, the ethanolic leaf and

latex extract shows a reasonable activity against the fungal isolates followed by aqueous.

All the above mentioned works point fingers on the ability and scientific proof on *Calotropis procera* to possess those bioactive compounds of anti-fungal efficacy.

2.1.6 Antiviral activity of *Calotropis procera*

Due to the multiple medicinal important and verse bioactive compound of content of the leaves and roots of *Calotropis procera*, their activity was tested against HIV type 1 virus by Mohanraj *et al.* (2010). Hot water extraction and p24 antigen's expression inhibition are used in evaluating the efficacy against the growth of HIV. And the extract proofs to be efficient against HIV-1 virus. The cytotoxic effect of different extract of various medicinal plants are evaluated by Latif *et al.* (2014) in which *Calotropis procera* was among, the cytotoxicity obtained leads to the suggestion of utilizing and testing of the *Calotropis procera* extract as antiviral using cell culture model. Though many studies give more attention to the antibacterial and antifungal activity of *Calotropis procera*, Antiviral activity of *Calotropis procera* has limited published data.

2.1.7 Antiprotozoal and antihelminthes activity of *Calotropis procera*

Several studies have suggested *Calotropis procera* to be an anti-helminthic and anti-protozoal plant. Murti *et al*. (2015), reported the activity of ethanolic, water, and *n*-butanol fractions of *Calotropis procera* leaf extract against helminthic, there findings shows that some of the phyto-components of the leaf shows anti-helminth activity as they tend to act by interfering with energy generation in the helminthe parasites as a

result of uncoupling of oxidative phosphorylation. However, a research conducted by Upadhyay (2015), reported that the leaves of *Calotropis procera* show no activity anti-trypanosomal activity. But a review carried out by Al-Snafi (2016), hinted the activity of various part of *Calotropis procera* against many parasitic infection which includes; the activity of crude aqueous and methanolic extracts of flowers against *Haemonchus contortus,* ethanolic extracts of the root and bud against *Plasmodium falciparum,* saponins-rich fraction phytocompnent against *Trypanosoma evansi,`* ethanolic extract of the leaves against *Plasmodium falciparum and* crude aqueous and methanolic extracts of flowers against *Haemonchus contortus.* Furthermore, the extract of *Calotropis procera* is used widely in treating filariasis infection in sub-saharan Africa (Mwangi, 2017).

Protozaons and helminthic infections are cardinal of the Neglected Tropical Disease particularly in developing countries, further research needs to be conducted so as to strategize on how to reduce the plant toxicity in other to validate it traditional usage so as to curve the menace of Neglected Tropical Disease worldwide

2.2 *Laptadenia hastata*

Laptadenia hastata also known as yadiya in Hausa and iran-aji in Yoruba language. The plants are mostly shrubs and herbs with white sap that comprises of about two hundred and fifty genera, and two thousand species many of which have a reduced leaves and succulent body like cactus plants (Thomas, 2013). The plant is non domesticated wild plant that play an important role in food security during seasonal changes or famine particularly among the poor. Furthermore, the plant is edible and

the leaves are cooked and eaten as vegetable (Hassan *et al.,* 2017). The plant is important fodder for goat, cows, camel and other herbivorous animals. The plant plays an important role in ethnomedicine for many centauries due to it phyto-constituents that are responsible for different pharmacological activities (Umaru *et al.*, 2017).

2.2.1 Botanical classification and description of *Laptadenia hastata*

Laptadenia hastata is classified into:

Kingdom: Plantae (Plants)

Phylum; tracheophyta (Vascular plants)

Division: Magnoliophyta (Flowering plants)

Class: Magnoliopsida (Dicotyledons)

Order: Gentianales

Family: Asclepiadaceae/Apocynaceae

Genus: leptadenia

Species: *L. hastata*

It is an evergreen perennial climber plants that attains a height of about 1.5m. At young stage, the plant is latex containing herb but as it grows older it becomes woody thereby developing strong branched climbing stems that becomes corky with age. The leaves are tapper and alternate upwards towards the tips, with a simple opposite petiole and variable ovated limb shape at the base. It has an acuminate apex limb with

entire margin at both sides. The stems are light green and shoot curve upward. Furthermore, it has umbellate inflorescences with several flowers arranged of about 1.5 cm long peduncle. The flowers of *Laptadenia hastata* are bisexual, scented and yellowish in colour with five pubescent corolla. The fruits come in a pair of follicles with a conical length of about 1 °Cm each and a tuft of hairs at the apex of the seeds (Bello *et al.,* 2011; Umaru *et al.,* 2018). All these are shown in plate II

2.2.2 Distribution and habitat *Laptadenia hastata*

Laptadenia hastata is widely distributed across tropical African countries such as Senegal, Kenya, Ethiopia and Uganda, Nigeria, Gambia, Ghana and Togo. In Nigeria, it grows mostly in the northern part of the country, the plant is tolerant to draught, insects and grows very well in dry, harsh and nutritionally poor soil conditions.

2.2.3 Medicinal uses of *Leptadenia hastata*

Several studies reveal the medicinal uses of various part of *Leptadenia hastata*. In northern Nigeria, the plant leaves and stem are grinded as spice and use in sauces (Ibrahim *et al.,* 2012). Moreso, traditional healers use this plant in treating catarrh, skin diseases and hypertention (Dambatta and Aliyu, 2011). The leaves can be decocted and taking orally or medicinal bath so as to treat onchocercosis and ophthalmia, scabies can be treated using the roots of *Leptadenia hastata*, (Anwar *et al.*, 2014). Repots by Maina *et al.* (2018) suggest the plant usage in improving sexual potency, treating trypanosomiasis, wound healing and skin diseases. Moreso, the leaves are reported to be used for lactation and purgative and treating rheumatism and prostate complaints (Ibrahim *et al.,* 2012). The plants is also used in stimulating the

flow of urine, anuria, stomach ache urethral discharge tertiary syphilis and gonorrhea infection can all be treated with *Leptadenia hastata* (Bello *et al.,* 2011).

2.2.4 Antimicrobial activity of *Leptadenia hastata*

2.2.5 Antibacterial activity of *Leptadenia hastata*

Leptadenia hastata exhibit certain antibacterial activity against some pathogenic bacteria as reported in some findings. Report by Imam *et al.* (2019) proof the effectiveness of *Leptadenia hastata* leaves against some gastro intestinal isolates which includes *Proteus mirabilis, Escherichia coli, Klebsiella pneumonia, Salmonella typhi , Staphylococcus aureus* and *shigella dysentariae,* thus the study showed that ethanol leaf extract have better activity than the aqueous extract. The studies of Umaru *et al.* (2017) also repots the activity of this plants leaf extracts against *Escherichia coli, Klebsiella pneumonia, Salmonella typhi ,* and *Staphylococcus aureu.* Furthermore, studies by Umaru *et al.* (2018) shows the potential activity of the stem-bark extract against *Klebsiella pneumonia, Escherichia coli, Staphylococcus aureu* and *Salmonella typhi.* The methanol extract of *Leptadenia hastata* has no activity against *Escherichia coli, Staphylococcus aureus* and *Pseudomonas aeruginosa* rather the ether extract have a very good activity against the organisms (Anwar *et al.,* 2014). Although much research has not been done in justifying the antibacterial activity of this plant, but the few available have shown it great potentials in treating bacterial infections.

2.2.6 Antifungal activity of *Leptadenia hastata*

Leptadenia hastata extract shows promising antifungal activity against *Aspergillus niger*, *Aspergillus flavin*, *Candida tropicalis* and *Fusarium oxysporium* (Umaru *et al.*, 2017). Another study by Aboh *et al.* (2019) shows the potential activity of the plant methanolic leaves extract against *Aspergillus niger* and *Fusarium oxysporium.* Furthermore, the activity of this plant extract is recorded against *Cryptococcus neoformans* and *Candida albicans* by Bello *et al.*, (2019). Though the study of Anwar *et al.* (2014) shows that the methanol–leaf extract of *Leptadenia hastata* have no activity against *Candida albicans* but the ether extract of the plant have activity against the fungal. These findings also hint on the potentiality of *Leptadenia hastata* in treating fungal infections.

2.2.7 Antiprotozoal and antiviral activity of *Leptadenia hastata*

Some studies shows the extract of *Leptadenia hastata* to have activity against trypanosomal parasite (Umar *et al.*, 2018). A moderate activity was recorded from the findings of Bello *et al.* (2017) during the primary screening of *Leptadenia hastata* methanol and chloroform root extract against *Tripanosoma brucei* and *plasmodium falciparum. Leptadenia hastata* contains a compound called lupeol which is suggested top have antiprotozoal activity (Mailafiya *et al.*, 2020). There is limited or no studies on the antiviral activity of *Leptadenia hastata.*

The antimicrobial potential of *Leptadenia hastata* needs to studied more sue to the increase in microbial infection and continuous emergence of microbial resistance strains worldwide.

2.3 *Securidaca longipedunculata*

Securidaca longipedunculata is also known as Sanya or Uwar magunguna in Hausa and Ipeta in Yoruba language. Its common English names includes the Fiber tree, Violet tree, and Rhodesian violet. It is commonly collected from the wild for medicinal use and also as a food and source of material (Abubakar *et al.,* 2018). The plant is widely distributed around Africa but due to overexploitation, the plant become much rarer in the wild and which makes it to be considered under threat in its native range. Thus makes various Governments to impose a legislative law that protects the plant (Tabuti *et al.,* 2012). It is a beautiful flowering tree that has ornamental potentials in garden and parks.

2.3.1 Botanical classification and description *Securidaca longipedunculata*

Securidaca longipedunculata Belongs to the kingdom Plantae phylum Tracheophyta (vascular plant), division; Angiosperm (flowering plant); super division; Spermatophyta (seed plant); Order (fabales); family (Polygalaceae); Genus (Securidaca); specie longipedunculata).

The tree is semi-deciduous spiny shrub with many open branches, and sweet scent which grows in small bunches on a peduncle. It grows between 4-12 meters long with a slightly flattened or fluted bole. The Leaves are somehow smaller, often elliptical and crowded towards the stem tips. The fruits are round, heavily veined, smooth, oblong, purplish green when at young stage and attached to a wing which becomes up to 40 mm long. The flowers are small pinkish or purple in color sweet scented that are usually produced in early summer. The plant woods are light yellow with

markedly dark growth rings which is soft, spongy, and durable that resists the attacks of termites. Although it is liable to split upon drying (Borokini *et al.,* 2013; Eziah *et al.,* 2013; Keshebo *et al.,* 2014). All this are shown in plate III.

2.3.2 Habitat and distribution of *Securidaca longipedunculata*

Securidaca longipedunculata is native to Africa, but it also be found in tropical and subtropical region of Africa which includes, Ethiopia, Gambia, Ghana, Kenya, Malawi, Namibia, Angola, Benin, Botswana, Cameroon, Chad, Eritrea, Niger and Nigeria (Tshisikhawe *et al.,* 2012). It habitat varies depending climatic conditions of the area. It can be found in wood lands and arid Savannah which includes various bush habitat and forest gallery at elevation level up to 1,600 meters. Furthermore, it can also grow in rocky or acidic soil environment. It can also withstand bush fire (Oni *et al.,* 2014)

2.3.3 Medicinal uses of *Securidaca longipedunculata*

The plant has a lot of medicinal uses traditionally this includes using the root to treat wide range of ailments such as toothaches, gout, fevers, constipation, diabetes, coughs, chest complaints, and microbial infections (Bruschi *et al.,* 2011). Snake bites can also be treated using this plant and Some part of the world uses the plant in treating mental disorders and immune booster during breastfeeding the bark and the root powder can be mixed and infused to treat inflammation, abortion, ritual suicide, tuberculosis, infertility problems and some venereal diseases (Kadiri *et al.,* 2013). Furthermore, the root can also be mixed with custard apple to treat gonorrhea. The leaves are locally used in treating epilepsy, headaches, stomach ache, infertility,

snakebite, toothache, cancer, skin infections, dislocated jaw, contraceptive purposes and to expel the placenta (Mustapha, 2013), whilst it stems bark is used in treating dysentery, malaria, typhoid, inflammation, chest complaints, abortion, constipation, viral infections, snake bites and infertility problems (Oladunmoye and Kehinde, 2011).

2.3.4 Antibacterial activity of *Securidaca longipedunculata*

Securidaca longipedunculata have a good antibacterial activity as reported by many researchers. Antibacterial activity of the stem bark aqueous extract reported by Abubakar *et al.* (2018) show a good result against *Escherichia coli, Salmonella typhi, Staphylococcus aureus, Shigella dysenteriae, Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. But a study by Ndamitso *et al.* (2013) Shows that the aqueous, chloroform and methanolic root and leaves extract of the plant have activity against *Escherichia coli* and *Salmonella typhi* but devoid of activity against *Pseudomonas aeruginosa*. Greater antibacterial activity was reported against *Escherichia coli, Pseudomonas aeruginosa* and *Salmonella typhi*, in both aqueous and ethanolic extract of the leaves and stem bark (Sanusi *et al.*, 2015). All these activities reported are due to the bio active ingredient poses by the plant.

2.3.5 Antifungal activity of *Securidaca longipedunculata*

Securidaca longepedunculata has shown to have antifungal effect against *fusarium solani* and *Fusarium moniliform* and complete inhibition of their mycelia was also observed by Nitiema *et al.,* (2020.). Also Adejuwon *et al.* (2019) reported the activity of ethanolic extract of the root bark against *Candida albicans*. The essential oil

extracted from root bark of *sacuridaca longipedunculata* was found to be active against *Aspergillus ochraceus* (Alitonou *et al.,* 2012). The leave was also reported to have activity against *Candida albicans* (Ngonda *et al.,* 2012).

2.3.6 Antiprotozoan activity *Securidaca longipedunculata*

Tauheed *et al.* (2016) reported the activity of the methanolic crude, ethyl acetate fraction and aqueous fraction of the stem bark extract against *Trypanosoma brucei*. The plant root extract was also said to have activity against Malaria parasite as reported by Nguta (2019). Lavicidal effect of the root extract was also seen by Adiele *et al.* (2013), when trying to determine the anti-helmintic effect of the extract.

2.4 Antibacterial Agents (Antibiotics)

Antibacterial agents are various group of compounds or materials that fight against pathogenic bacteria either by destroying the bacteria or reducing it metabolic activities. The threat caused by pathogenic bacteria to human kind leads to the revolutionary discovery of the antibacterial agents, although the first antibacterial agents was discovered accidentally by Sir Alexander Fleming in 1928 and is called penicillin from the fungus *Penicillium nonatum.* Alexander Fleming was quoted saying "I did not discover penicillin, nature did but I only discovered it by accident" (Tan and Tatsumura 2015). This also unfold the mystery behind the use of natural microbial product to antagonize pathogenic bacteria. Antibacterial drugs are among the most commonly prescribed and misused antibiotics worldwide by both the patients and physicians. As a result of this, the emergence of antibiotic resistant species of the pathogenic bacteria has been in acceleration and threatening to the

global public health continuously. Therefore, a renewed effort is required researching and developing new drugs that will be effective against the pathogenic bacteria species that are resistant to the currently prescribed and consumed antibacterial drugs (Moellering, 2011; Belete, 2019).

2.4.1 Classification of antibacterial agent

Antibacterial agents can be classified mainly into the following categories which are on the basis of their source, functions and mode of action and spectrum of activity.

2.4.2 Classification base on their source

On the basis of their source antibacterial agent can be classified into natural, semi-synthetics and synthetics. Natural antibacterial agents are obtained mostly from plants and fungi, examples of natural antibacterial agents includes the penicillin, gentamycin, cephalosporins and cefamycin (Ullah and Ali Khan 2016; Wiese and Imhoff, 2019**)**. Semi-synthetic antibacterial agents are derived chemical alteration of naturally sourced antibacterial agents and examples includes amikacin and ampicillin (Mathur and Hoskins 2017). Synthetics antibacterial agents are produced from synthesizing various chemical compounds so as to design the drug that will target and antagonized are particular components of the bacterial cell and examples includes sulfonamides, imidazole derivatives and diaminopyrimidine derivatives (Azmi *et al.*, 2017). Though it is said that drugs from natural sources have higher toxicity than that of the semi-synthetic and also the toxicity level of synthetic based sourced antibacterial agent is lower than the semi-synthetic and natural sources.

2.4.3 Classification based on their functions and mode of action

Based on the antibacterial mode of action, it can be grouped in to bactericidal or bacteriostatic. Bactericidal agents destroy the bacteria completely. For example the cell wall synthesis interfering antibacterial agents such as Beta- drugs which includes the penicillin and it derivatives, penicillin and it derivatives; cell wall synthesis inhibiting antibacterial agent for example vancomycin; protein synthesis inhibiting antibacterial agents such as aminoglycosides , amikacin, gentamycin and tobramycin; DNA replication inhibiting antibacterial agent this includes the quinolones and floroquinolones; cell membrane disrupting antibacterial agents such as polymixin B and colistin. Whilst bacteriostatic inactivate or slows the bacterial growth, this antibacterial agents comprises of the various drugs within certain classes such as the sulphonamides class that inhibit the initial stage of folic acid synthesis; the amphenicols class that interrupt protein synthesis by binding to 30S sub-unit; the tigecycline class that block amino acid incorporation to a developing peptide chain; the macrolides class, oxazolidinone class and tetracyclines class all work by act on protein synthesis inhibition (Adzitey, 2015; Kang and Park 2015; Etebu and Arikekpar 2016).

2.4.4 Classification based on the spectrum of activity

In this regard, antibacterial are classified on the basis of their target specification as either narrow or broad spectrum of activity. The narrow spectrum of activity antibacterial agents are those that work on a narrow range of microorganisms, they exact their effect on one group of bacteria, either Gram positive or Gram negative but

not both at a time. Examples includes the first generation Beta-lactam drugs such as procaine penicillin, penicillin G and penicillin V; methicillin, Cloxacillin, oxacillin, nafcillin and temocillin; first and second generation Cephalosporins are also considered to be relatively narrow spectrum antibiotics. On the other hand, broad spectrum activity antibacterial agents are those that work effectively against both Gram positive and Gram negative bacteria. Examples includes Ampicillin and its derivative amoxicillin, the Quinolones drugs, Chloramphenicols, Aminoglycosides, Macrolides and Tetracycline (Coates *et al.,* 2011; Josef 2018; O'Rourke *et al.,* 2020). Thus the narrow spectrum antibacterial agents are considered to be safer as they did not destroy the microbial flora in the body and also do not cause super infection and has less ability to cause resistance.

2.5. Plants as Antibacterial Agents

Talking about the medicinal usage of plants, large proportion of the plants species are of great medicinal important. Going back to the first century, Dioscorides wrote the medicinal plant catalog called *De Materia Medica* that becomes the prototypes of today's pharmacopoeias, furthermore, the Bible also makes a description of about 30 healing plants (Staub *et al.,* 2016). Presently an estimate number of about 250,000-500,000 plant species are known worldwide and only a small percent (1-10 %) are relatively used as food for human consumption (Pattanayak 2018). Plants and plants products have been used by traditional healers in treating various diseases and ailments for many years. Although there has always been a lingering questions in scientific circles about the therapeutic efficacy index, dosage index, and toxicity index

of various medicinal plants. In other to find the answers to this questions, scientist make relentless efforts in studying the pharmacological activity of many medicinal plants. Even with that, there are still vast majority of medicinal plants whose metabolites are waiting to be studied for their pharmacological effects (Panda *et al.,* 2018).

2.5.1 Plant metabolites as potential therapeutic agent

Plants serves as source of food to human and animals through the provision of sufficient nutrients to meet the metabolic requirement for their growth, productivity and wellbeing (Pisoschi *et al.,* 2018). Medicinally, plants make a major contribution to in achieving an optimal health and development and also play an essential role in delaying or reducing the disease infestation and disorder. Medicinal plants exhibit their therapeutic potentials due to their ability of biosynthesizing various complex metabolites also known as phytochemicals and are grouped in to the flavonoids, phenolics and terpenoids (Anand *et al.,* 2019). Most at times the overall activity of a medicinal plant may be as result of synergistic interaction between two or more metabolites present in that particular plant. Clinical and pharmacological research of the metabolites present in plants justified their therapeutics and medicinal potentials also serves as the backbone of traditional medicines. Medicinal plants have long been used to supplement the health care needs of both rural and urban residents. Plant extract, formulations and other pure or natural compounds from living beings can be used in controlling and treating arrays of diseases ranging from inflammations,

coughs, diarrhea, bacterial, fungi, viral infections both in human and animal medicine (Mushtaq *et al.,* 2018).

2.6 Phytochemicals Properties

Phytochemical or phyto-components of plants are secondary metabolites synthesized by tool in defending itself against microbial pathogens, predators or competitors. They can also be produced as a waste product within the various parts of the plant. Example of these metabolites includes; cardiac glycosides, phenols, tannins, alkaloids, anthraquinones, saponins, terpens, striods, and terpens. These biological active compounds present in the plants have a lot of medicinal uses and sometimes are used as source of nutrients (Koche *et al.,* 2016; Grynkiewicz, 2020). A lot of interest has been geared towards the isolation identification, characterization and biological activity determination of phytochemical compounds over the years (Rao, 2003; koche *et al.,* 2010). Due to the recognition and encouragement of folk medicines, phytochemicals become keen interest of pharmaceutics and pharmaceutical industries as a result of their therapeutic index potentials (Grynkiewicz, 2020).

2.6.1 Classes of phytochemical compounds

Phytochemical components of plants are still research subject worldwide due to their complex nature. The general classifications of such compounds is still unavailable, though few researchers were able to classify them in to the following; phenols, terpenoids and steroids and also alkaloids.

2.6.2 Phenols Component

Phenols are also called phenolics and are group of phytochemicals which are largely found naturally within kingdom Plantae. These chemical compounds consist of one or more hydroxyl group attached directly to an aromatic hydrocarbon group (Ozcan *et al.*, 2018). Due to the fact that phenolics compounds are synthesized as secondary metabolites in plants, they possess many beneficial effects to human which includes antioxidant properties (Balasundram *et al.*, 2006; Dai and Mumper, 2010). The three most common group of dietary phenolics compounds are polyphenols, flavonoids and phenolic acids.

2.6.3 Terpenoids and steroids

They are the largest group of naturally occurring compounds because they can be found in both plant and animals. Most of these compounds are attract industrial interest because of their commercial values of flavours in food industries and fragrance in cosmetic industries. Terpenoids are class of secondary metabolites which are grouped on the basis of isoprene unit or number of carbon in their structure (Yadav *et al.*, 2014). In plants, they are the main constituent of essential oils. Steroids are also known therapeutically as cardiac drugs and are also observed to promote nitrogen retention in oesteoporosis and animals with wasting illness (Sultan, 2015). Example of terpenoids and steroids includes α-cubebene, terpenolen, β-caryophyllene, diosgenin and cevadine.

2.6.4 Alkaloids Component

The name alkaloid was derived from alkaline which describe a nitrogen based containing compounds. Alkaloids are group of compounds whose basic characteristics is heterocyclic nitrogen atoms. Most alkaloids are derived from amino acids precursors and have a biter test (Bribi, 2018). Alkaloids have a good pharmacological reputation due to their bactericidal ability and are classified based on their structure as pyridine, tropane or pyrolizidine alkaloids (Bribi *et al.,* 2013; Hussain *et al.,* 2018).

2.7 Medicinal functions and activity of phytochemical compounds

2.7.1 Anti-diabetic activity

Diabetes is a serious public health issue that is characterized by chronic hyperglycemia and can result in a variety of macro and microvascular problems. Moreso, due to the hyperlipidemia hyperglycemic infection, diabetes mostly surfaced with the increase of oxidative stress or production of free radicals (Zhang and Liu 2011). Phytochemicals extracted from many plants such as *Chrysobalanus icaco, Lactuca sativa, Aloe greatheadii, amaranthus, raphanus and cinnamon* and their various parts (leaves, roots, stems, flower and juice) have shown to have anti-oxidants, lowering of blood glucose and increases the amount of thrombotic thrombocytopaenic purpura (Rahigude *et al.,* 2012; Xie *et al.,* 2013; Cheong *et al.,* 2014).

2.7.2 Antimicrobial activity

Generally, phytochemicals are synthesized and employ by plants to protect itself from pathogenic organisms such as fungi, insect, bacteria, fungi and protozoa, which is the

reason for their application in human medicines (Nascimento *et al.,* 2000). Some phytochemicals such as phenolic acids play role in reducing the adherence of the organism from the site of infection such as the cells lining the bladder, and the teeth (Jakhetia *et al.,* 2010). Furthermore, plants have the ability to exert bacteriostatic or bactericidal effect on the organisms, there by inhibiting their growth and metabolism or destroying the organisms completely.

2.7.3 Anti-Cancer Activity

Due to their independent potential to create DNA mutation, free radicals or oxidants are recognized to be actively implicated in multistage carcinogenic processes. Phytochemicals containing phenolic compounds have proof their potentials in carcinogenic process inhibition. Phenolic acids can significantly minimize the formation of specific cancer-promoting nitrosamines from the dietary nitrates and nitrites (Sreelatha *et al.,* 2012). Presently cancer have few or no specific treatment and preventive dug except for the chemotherapy options that many opt to. Thus the chemotherapeutic option is capital extensive and attached with several post treatment complications. Several studies show that vegetables and fruits consumption can inversely treat and prevent varieties of cancer. For example, cabbage, broccoli and cauliflower are good source of glucosinolates which provide a substantial protective support against colon cancer (Liu, 2004). Active intake of Brussel sprouts can cause a rapid appreciable enhancement of glutathione-Stransferase, and also a noticeable reduction in the urinary concentration of a specific purine metabolites which serves as a marker of DNA-degradation in cancer (Liu *et al.,* 2009). Furthermore, polyphenols

from phytochemicals showed a promising anticancer activity in various research. For example, epicatechin gallate and ellagitannins polyphenols showed anti-carcinogenic properties. Grape seeds proanthocyanidins phytochemicals, polyphenols from green tea and silymarine from milk thistlehad the ability toprotect the skin from theadverse effects of Ultra Violate radiation (Barrajon-Catalan *et al.,* 2010; Cordero *et al.,* 2013). Thus this minimize the risk of having skin cancers.

2.7.4 Anti-ulcer activity

Some phytochemicals have been reported to inhibit the growth of ulcer causative organism and it enzyme (helicobacter pylori). Some plants phyto-constituent very efficiecient of in a liquid medium at an optimum pH which help in enhancing their in-vivo potency in the human stomach. Moreso, the inhibitory effect in the kidney and intestinal electrolytes (K^+/Na^+ ATPase) (Jakhetia *et al.,* 2010; Orsi *et al.,* 2012).

2.7.5 Antioxidants activity

Antioxidants help in protecting the cells from damaging effect due to activity of free radicals or oxidative stress such as. This free radicals are sourced from reactive oxygen species and examples includes; super oxide, single oxygen, hydroxyl radicals, peroxyl radicals, and peroxynite which all induced oxidative stress thereby leading to cellular damage (Mattson and Cheng, 2006; Deng *et al.,* 2012). Natural antioxidants obtained from phytochemical substances aid in the prevention of chronic and degenerative diseases such rheumatoid arthritis, heart and cerebral ischemia, atherosclerosis, and neurological disorders. (Uddin *et al.,* 2008; Jayasri *et al.,* 2009). Antioxidants play their role by exerting their activity so as to scavenge the free

radicals and give rise to stable radicals. The free radicals are unstable chemical entities that draw electrons from their immediate environment if they are ignored, they tend to damage crucial biomolecules such as proteins and lipids present across all the membranes, mitochondria and also DNA. Wide array of free radicals scavenging molecules also known as antioxidants are present in vegetables, fruits, cereal grains, edible macro fungi, microalgae, and many other medicinal plants (Deng *et al.,* 2012; Guo *et al.,* 2012), this phyto-compounds includes; the phenols, vitamins, terpenoids and flavonoids. Example of some plants that have the antioxidants properties are grape, berries, Chinese date, persimmon, guava, pomegranate, sweetsop and Chinese wampee.

2.7.6 Anti-inflammatory activity

Ulcerative colitis and crohn's disease are inflammatory bowel diseases, which is a chronic inflammatory disorder caused by deregulated immune responses in a genetically predisposed individual. Phytochemicals with antioxidant properties are found to have anti-inflammatory activities (Talero *et al.,* 2012; Sung *et al.,* 2013). Essential oil from *C. osmophloeum* twigs has good anti- inflammatory activities and cytotoxicity against Human Hepatocellular Liver Carcinoma Cell Lines. They also have the ability to suppress nitric oxide production by Lipopolysaccharide stimulated macrophages (Jakhetia *et al.,* 2010).

2.7.7 Anti-Aging Activity activity

Phyto-components of plants are suggested to have anti-aging activity because aging is said to be a progressive deleterious alteration and functional decline of an organism,

this leads to an increase in disease susceptibility and death due to the advancing age and presenting cognitive and motor deficits (Shukitt-Hale *et al.,* 2013). Oxidative stress or free radicals are associated with biological aging couple with many aging associated diseases. (Kolosova *et al.,* 2012). Therefore, the phyto-components of plants containing antioxidants have the ability to fight against the aging and it associated diseases. Reductionof both motor and cognitivedeficits in agedrats was observed using coffee because it contains is a high levels of antioxidant phytochemicals (Sultan, 2015).

2.8 Plant Materials and Extraction of Plant Materials

2.8.1 Plant materials

Plant medicine is the oldest form of medicinal material known to mankind. As much as 80 % of the world's population depends on herbal medicine, as most of the commonly prescribed medicines worldwide contain at least one active ingredient derived from plant material (Elumalai and Eswarial 2012). Medicines derived from plant are used in all civilizations and cultures and, hence, plants have always played a key role in health care systems worldwide. Herbal treatment remedies are considerably effective, socially acceptable and economically viable (Ezenyi *et al.,* 2016).

Plants are powerful biochemists that create a wide range of bioactive substances that have been used in phytomedicine from the beginning of time. Approximately 20 % of all known plants have been employed in pharmacological studies, resulting in positive effects on the healthcare system, such as the treatment of harmful diseases. (Jonathan

and Micheal 2014). These natural Plant based bioactive constituents are derived from various plant material such as the grasses, rushes, barks, woods, gourds, stems, roots, seeds, and leaves. Bark, stems, leaves, flowers, roots, fruits, and seeds. The analysis of these plant components is carried out scientifically using a logical pathway (Serino *et al.,* 2019). Plants can be collected at random or based on information provided by local healers in the locations where the plants are located. Plant materials, whether fresh or dried, can be used to extract secondary plant components. Many researchers have reported on the reasoning for extracting plant extracts from fresh plant tissues, and this logic stems from traditional and tribal peoples' ethnomedical utilization of fresh plant materials (Halliwell 2007). Due to variances in water content within different plant tissues and the fact that many plants are utilized in the dry state (or as an aqueous extract) by traditional healers, plants are normally air dried to a constant weight before extraction (Dwivedi and shridhar 2007). Otherresearchers dries the plants in the ovenat about 40 °C for 72 h (Karabegovic *et al.,* 2014). Many research shows that the underground parts of plant (roots, tuber, rhizome and bulb) have more water content than other parts and were used more frequently and extensively compared with other part (above the ground) in search for bioactive compounds possessing antimicrobial properties (Turner *et al.,* 2007).

More so, the basic principle consist of grinding the plant material (dry or wet) finer, so as to increase the surface area for extraction thereby increasing the rate of extraction.

2.8.2 Improving the quality, safety and efficacy of herbal medicine

Popularity of Herbal medicines has risen and continuously rising globally, therefore improving the quality, safety and efficacy becomes paramount important prior to consumption. In past decades, upsurge consciences in quality of herbal medicine was observed, through which herbal medicines were used as food initially with few or no quality requirement. Presently they their usage is beyond food and are classified into various categories such as drugs, health products, neutraceuticals (nutritional substances), cosmetics and many others depending on the laws governing a country (Zhang *et al.,* 2011). Furthermore, the face and faith of herbal product is also changing rapidly as a result of its incorporation into modern science. A lot of efforts have been put in place by various countries around the world so as to maintain and improve the quality, safety and efficacy of herbal medicines and other herbal product (Thakur *et al.,* 2011). This leads the developed nations in enacting certain rules and regulations governing the usage of herbal and herbal products also known as pharmacopeias which provides a specific monograph to be used in their prescription. These countries include the United States, Indian, United Kingdom and other European countries. Thus several other developing countries such as Brazil are still in the process of providing their own standard monograph, and also some countries are using other country's pharmacopeias to standardize their product. This monographs are subjected to review on timely basis therefore it varies from one edition to another (Mandal and Mandal, 2011).

2.8.3 Encouraging mediculture

The act of producing therapeutic plants on a scientific basis is known as mediculture. Cultivation of such plants (medicinal plants) should be encouraged, with a greater focus on genetic stability and homogeneity of the plant population to assure repeatable outcomes. Realization the existing connection between plants and health form the basic backbone for a new generation of botanical therapeutics which includes multicomponent botanical drugs, plant derived pharmaceuticals, dietary supplement, recombinant proteins, functional foods and plant supplements. This concept is gradually changing plant Biotechnology and medicine in which some crops are grown for medicinal purpose rather than food or fiber (Sen *et al.,* 2011; Cragg and Newman, 2013).

2.8.4 Correct identification of plant material

The importance of carefully identification and classification of medicinal plant materials is of paramount important prior to it cultivation and development of a qualitative herbal or medicinal products. Classical methods of plant taxonomy for identification of plant material provide an authentic and viable methodology. But in the situation when the whole plant is not available to the taxonomist, a genetic method will be more reliable in the identification and authentication methods. Because genetic materials such as Deoxyribonucleic acid (DNA) are more reliable markers than chemical based on caryotyping and proteins due to the uniqueness of genetic composition of each individual. This DNA can be extracted from various part of the

plant such as the stem, leaves, fruit and roots (Estrada *et al.,* 2018; Seeland *et al.,* 2019).

2.8.5 Minimizing contamination of the herbal medicine or herbal product

Most herbal medicines contained mixture of crude or raw herbs as contaminants which may occur directly from wild or cultivated fields. Also herbal medicines can be contaminated by microorganisms, or toxic chemicals which can occur from the processing agencies, environmental factors, packaging and storage facilities. These are vital areas aspects that are required to be check mate and ensure all that the products are devoid of such contaminants prior to production and consumption (Kosalec *et al.,* 2010; Vuuren *et al.,* 2014).

Extraction is a process use in isolating substances or compounds from the matrix (mixture). Extraction methods used pharmaceutically involves the separation of medicinally active portions of plant tissues from the inactive/inert components by using selective solvents through standard procedures (Azwanida, 2015). During extraction, solvents diffuse into the solid plant material and solubilize compounds with similar polarity. The main aim of all standardized extraction procedure is to separate the soluble plant metabolites and leaving behind the insoluble cellular residues (Newman and Cragg, 2016). The use of selective solvent also known as menstruum can be employed in treatment of crude drugs (medicinal parts of plant) so as to attain desired portion with therapeutic potentials and eliminate the unwanted material. The end products from plants extraction may be relatively impure obtained in either of the following forms, liquids, semisolids or solid form (Atanasov *et al.,*

2015). Thus, the standardized form of the obtained extract can be used as medicinal agent in form of tinctures or fluid extracts and can be processed further to be incorporated in any dosage form such as tablets and capsules. The standardized obtained product contains several mixture of complex plant metabolites, such as flavonoids, alkaloids, terpenoids, glycosides and lignin.

2.9 Some General Plant Extraction Techniques and Procedures

2.9.1 Maceration method

This technique is used in making wine and is been adopted and widely in medicinal plants research. Maceration techniques is a simple extraction technique by which the whole or coarsely powdered plant material is soaked with the solvent and kept in a stoppered container for a specified period of time with a frequent vigorous agitation until the soluble matter is dissolved. The processed tend to breakdown and soften the plant's cell wall in other to release the soluble phyto-constituent of the plant. The disadvantage of this techniques incudes energy and time consuming with low extraction efficiency, but the advantage of this technique is that, it is most suitable method in extracting thermo labile (Pandey and Tripathi 2014; Zhang *et al.,* 2018).

2.9.2 Decoction method

This is a method used for the extraction of water soluble and heat stable compounds, hard plants materials such as roots and barks, and also oil soluble compounds from the plant material. The plant material is subjected to boiling in a specified volume of water for a defined period of time which is then cooled and filtered. This method is suitable for extracting water soluble, heat stable constituents of the plant material, though the extract from decoction contains a large amount of water-soluble impurities

and is not a suitable method in extracting volatile components of the plants material (Li *et al.,* 2010; Daswani *et al.,* 2011).

2.9.3 Percolation method

This technique is more efficient than maceration as result of it being a continuous process by which the saturated solvent is constantly replaced by a fresh solvent. This procedure is frequently used to extract active ingredients in the preparation of tinctures and fluid extracts. A unique narrow, cone-shaped vessel open at both ends which is called a percolator is generally used. A specified amount of the menstruum can be use to moisten the dried powdered samples packed in to the percolator and allowed to stand for 4 h in an airtight container, after which the mass is packed and the top of the percolator is closed. The menstruum is added to until a shallow layer is formed above the mass, and the mixture is allowed to macerate in the closed percolator for 24 h. After which the outlet of the percolator is opened and the liquid contained therein is allowed to drip slowly. The menstruum is added continuously as required, until the percolate measures is about three-quarters of the required volume of the finished product. The mixed liquid is clarified through filtration or decantation (Azwanida, 2015; Benitez *et al.,* 2016; Ravanfar *et al.,* 2018; Xiong *et al.,* 2018).

2.9.4 Soxhlet extraction (Hot continuous extraction)

This is an automatic continuous extraction method that has a high extraction efficiency. This method consumes less time, energy and solvent when compared to maceration or percolation. In this procedure, the finely grounded plant material sample should be placed in the thimble that is made from a cellulose or strong filter

paper. The thimble is then transferred in to the thimble chamber as provided in the soxhlet apparatus. The solvent is heated in the bottom flask and vaporizes in to the thimble and condenses in the condenser and drip back continuously until a siphon level is reached. The recycling process will continue until a clear drop of solvent from the siphon tube does not leave residue when evaporated. The advantage of this technique is that large amounts of drug can be extracted with small amount of solvent, and it has tremendous effects economically in terms of time, energy and financial inputs. Though the disadvantage of this technique is that, prolong heating increases the possibilities of thermal degradation. (Amid *et al.,* 2010; Lau *et al.,* 2010; Pandey and Tripathi 2014; Azwanida, 2015).

2.9.5 Plant tissue homogenization

Plant tissue homogenization is one of the old methods widely used by researchers. A wet or dried plant material should be grinded into fine particles and soaked in certain amount of solvent with vigorous shaking for five to ten minutes or allowed for 24 h, after which the extract is then filtered and the filtrate may be centrifuged or dried under reduced pressure and re-dissolved in the solvent to determine the concentration. (Xing *et al.,* 2019).

2.9.6 Sonication (Ultrasound-assisted extraction)

In this extraction, an ultrasound frequency ranging from 20 kHz to 2000 kHz is involved and it mechanical effect helps to produce cavitation and due to the increase in the cell wall permeability. Because Physical and chemical properties of the subjected plant materials are altered and the plant cell wall are disrupted, so as to

facilitate the release of compounds and enhance mass transport of the solvents into the plant cells. The advantage of this technique is that it is relatively simple and low cost technologically which can be used in both small and larger scale of phytochemical extraction. Whilst the disadvantage is that the procedure may lead to an occasional deleterious effect active constituents of the plant material through formation of free radicals and undesirable changes in the drug molecules consequently as a result of ultrasound (above 20 kHz) (Dhanani, *et al.,* 2013; Ebrahim *et al.*, 2014; Yingngam *et al.*, 2014).

2.9.7 Basic parameters influencing the quality of a plant extract

Some of the basic parameters that influence the quality of an extract are; (1). The Plant part used as starting material, this includes the various part of the plants such as the leaf, stem, bark or roots. (2) The solvent used for the extraction this includes the pH, boiling point, polarity and toxicity of the solvent to be used for the extraction. (3) The extraction procedure which depends on the type and nature of the compounds need to be extracted, for examples are the compounds thermo-stable, oily or not (Odetoye *et al.*, 2016; Tambunan *et al.*, 2017). And the variation in the choice of extraction methods usually depends upon the Temperature required, Particle size of the plant tissues, Length of the extraction period, Solvent used, pH of the solvent and the ratio of the solvent-to-sample.

2.10 Solvents for Plant extraction

Solvents selected to be used for extraction play a crucial role in determining the biologically active compounds to be extracted from the plant material. Properties of a

good solvent in plant extractions includes, ease of evaporation at low heat, low toxicity, preservative action, promotion of rapid physiologic absorption of the extract and inability to cause the extract to form complex or dissociate (Xu *et al.,* 2016; Thouri, *et al.,* 2017). The factors affecting the choice of solvent includes the quantity of phytochemicals to be extracted, toxicity of the solvent in the bioassay process, the rate of extraction, diversity of inhibitory compounds extracted, ease of handling of the extracts, potential health hazard of the extractant and diversity of different compounds extracted, (Do *et al.,* 2014; Zia-Ul-Haq *et al.,* 2014; Zhao *et al.,* 2018). The intended use of the extract greatly affects the choice of solvent as traces of residual solvents remains with the final product. Therefore, solvent needs to be non- toxic and should not interfere with the bioassay (Moreno-Quir´os *et al.,* 2017; Złotek *et al.,* 2018). The targets compounds to be isolated also influence the choice of solvents

2.10.1 Ethanol as solvent

Ethanol is a simple alcoholic solvent with a near neutral pH of 7.33, boiling point of 78.21 ℃, melting point of -114.14 ℃. It is volatile, flammable and colorless liquid characterized with a slight odor like smell. It also have a psychoactive drug that is the principal ingredient in drinks such as beer, wine and hard liquor (Menezes *et al.,* 2013; Stogner *et al.,* 2014). In most cases, high antimicrobial activity is recorded in ethanolic when compared to aqueous. Because they are more efficient in cell walls and seeds degradation of the plant material which have unipolar character and causes high content of polyphenols to be released from cells. More so, ethanol was found easier to penetrate the cellular membrane to extract the intracellular ingredients from

the plant material. Some of the phytoextract of ethanol includes Polyacetylenes, Flavonols, Sterols, Alkaloids, Tannins, Polyphenols and Terpenoids. Furthermore, more bioactive flavonoid compounds can be extracted with 70 % (30 % water) ethanol solution than 100 % ethanolic solution (Hasan *et al.,* 2017; Tulashie *et al.,* 2017; Alara *et al.,* 2020;).

2.10.2 Methanol as solvent

This is also an alcoholic polar solvent used for extraction. It is a weak acid solvent with the pH of 6.0, boiling point of 64.7 ℃, and melting point of -97.6 ℃. Also it is light, volatile, colorless, flammable liquid with a distinct odor like smell. It is more polar than ethanol due to it cytotoxic nature (Mincer and Aicher 2016). Some of the phyto-components extracted best using methanol includes; Tannins, Xanthoxyllines, Phenols, Polyphenols, Anthocyanins, Terpenoids, Saponins, Totarol, Quassinoids, Lactones and Flavones. The aromatic or saturated compound from plants that have antimicrobial activity are often more obtained through methanolic extraction (Azwanida, 2015; Huq *et al.,* 2016; Truong *et al.,* 2019).

2.10.3 Acetone as solvent

This is a colorless, flammable organic compound with a fruity odor that dissolves several lipophilic and hydrophilic components of plants. Acetone is miscible with water and have low toxicity to the bioassay used. It has a pH value of 7.0, boiling point of 56.63 ℃ and melting point of -95.4 ℃. Acetone is an important extractant for antimicrobial studies where more phenolic compounds are required to be extracted. More so, Flavonols and saponins from acetone extracts proves to have a reputable

antimicrobial activity (Ghezelbash *et al..,* 2015; Mizielinska *et al.,* 2017; Famuyide *et al.,* 2019).

2.10.4 Water as solvent

Water is universal solvent with some basic universal characteristics such as pH near neutral (7.0), boiling point 100 ℃ (212 ℉) and melting point of 0 ℃ (32 ℉) usedto extractplant products withantimicrobial activity. It is good in phytochemical extraction of polar compounds. It is not volatile and has no toxicity effect on the extract. Some of the phyto-components that are extracted by water are Tannins, Starches, Anthocyanins, Saponins, Terpenoids, Polypeptides and Lectins. Extracts from water solvent (aqueous extracts) have a decreased antimicrobial activity when compared with other solvents, this can be ascribed as a result of the presence of the enzyme polyphenol oxidase in water which degrades the polyphenols compound of the plant materials (Pandey and Tripathi 2014; Umapathy *et al.,* 2015; Tchabo *et al.,* 2018; Yen *et al.,* 2018).

2.11 Research Organisms

2.11.1 *Salmonella bongori*

Salmonella genus is made up of two species which includes *Salmonella enterica* and *Salmonella bongori*. The genus *Salmonella bongori* s an intracellular pathogen of cold blooded animals and it is Gram negative, non-spore forming peritrichously flagellated, predominantly motile, facultative anaerobic straight rods with simple nutritional requirements (Fookes *et al.,* 2011). This organism is transmitted through ingestion of infected animal product, water or direct contact with feces of an infected animal. On ingestion, the organism stays and multiply in the intestine for a period of

one to three (1-3) weeks after which it makes way through the intestinal walls and migrate to the bloodstream which furthermore pave way and spread to other tissues and organs thereby secreting endotoxins (Okoro *et al.,* 2012*).* Due to the ability of the organism to live within the host cells, the host immune system can only do little in fighting off the pathogen (Jantsch *et al.,* 2011). Symptoms of this infection includes headache, nausea, prolong fever, vomiting, abdominal pain, constipation and diarrhea. The complication case of this infection may leads to septic shock and hypovolemic shock (Fabrega and Vila 2013).

2.10.2 *Klebsiella pneumoniae*

This is an opportunistic pathogen that mostly causes bacterial pneumonia also known as lungs infection, it is more severe to immunocompromised individuals. The organism is encapsulated, Gram negative, rod shaped, non-motile, facultative anaerobic and lactose fermenter (Rashid and Ebringer 2007). *Klebsiella pneumoniae* is one of the notorious drug resisting organisms that poses an increase threat to the Global health problem (Sanchez *et al.,* 2013). Infection by this organism occur as a result of direct entry of the bacteria into the respiratory tract of the host and it ability to survive the host defense system (Li *et al.,* 2014*).* The organism can be found naturally in the soil and as normal flora in the intestine, skin and mouth but become infectious when it disseminates to other part of the body. The infection caused by this organism includes upper respiratory tract infection, urinary tract infection, wound infection, thrombophlebitis, cholystitis, diarrhea, meningitis, sepsis and osteomyelitis

(Hudson *et al.,* 2014). *Klebsiella pneumoniae* can be diagnosed via sputum culture, chest radiography and CT scan.

2.10.3 *Escherichia coli*

It is the most commonly found bacterium in the gut of warm blooded animals. This bacterium is motile, non-spore forming, Gram negative, rod shaped and facultative anaerobic organism that is found as a normal flora in the intestinal tract of human and other mammals (Lim *et al.,* 2010*).* Also it can be found generally in water, soil, various foods much especially animal products and on plants. Though most species of *E. coli* are har mLess but few are cause various infections which includes pneumonia, urinary tract infections, gastroenteritis, neonatal meningitis, hemorrhagic colitis and Crohn's disease. And in some rarer cases the pathogenic strains of this organism also causes tissue death known as bowel necrosis and unprogressive perforation (Croxen *et al.,* 2013). Furthermore, some strains of *Escherichia coli* produces shiga toxin which is also classified as bioterrorism agent (Bourgeois *et al.,* 2016). This organism can be diagnosed using microscopy culture and sensitivity method.

2.10.4 *Pseudomonads aeruginosa*

Pseudomonads aeruginosa is Gram negative, rod shaped encapsulated pathogenic bacteria of both plant and animals, the organism can be found in water, soil, skin flora and many habitats throughout the world (Klockgether *et al.,* 2011). This organism is of medical important due to it multidrug resistance ability and intrinsically ubiquity in their advance antibiotic resistance mechanisms (De Smet *et al.,* 2017). It is an opportunistic pathogen which causes infection of already established diseases

conditions and treatment of pseudomonads infections may be difficult due to its natural mechanism of drug resistance, therefore when more advanced drugs are needed and used may result to more adverse effect (Hoiby *et al.*, 2010).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

The study was conducted in Minna which is the capital city of Niger State with an altitude of 9°35'0.8"N and a longitude of 6°32'46.74"E North central Nigeria. The state was created in 1976 and named after River Niger in 1976. Both the isolates and plants materials were collected from the city metropolis

3.2 Ethical Consideration

An ethical clearance that permits the collection of test organisms for this research was obtained from the Research, Ethics and Publication Committee (REPC) the General Hospital, Minna Niger State.

3.3 Media Used

The media used for this research includes Nutrient agar (NA), Eosine Methylene Blue (EMB), *Salmonella Shigella* agar (SSA), Muller Hilton agar (MHA), Nutrient broth (NB), Peptone water, MR-VP broth and Simon citrate agar.

All the media used were prepared according to the manufacturer's instruction and were sterilized by autoclaving at 121 °C for 15 minutes (Cheesbrough, 2006).

3.4 Sample Collection and Transportation

3.4.1 Collection of test organisms

The bacterial isolates used in this research were obtained from three microbiological samples which includes the urine, stool and wound swab from General hospital

Minna. Colonies of the isolates were sub-cultured into nutrient broth in a sterile container, the broths were transported in a maintained cold temperature (4 ℃) to microbiological laboratory of Federal University of Technology Minna (step B) for further identification and characterization.

3.4.2 Collection of plants materials

The plant materials, *Calotropis procera* (leaf), *Leptidenia hastata* (stem) and *Securidaca longipedunculata* (stem) were collected aseptically in a clean polythen bag from their natural habitat around moris area of Barikin sale under Chanchaga Local Government Area of minna metropolis, and transported to the drug discovery unit laboratory of Federal University of Technology Minna (step B) Niger state, Nigeria.

3.5 Culturing of Test Isolates

The broth containing the isolates were incubate at 37 °C for 24 h and a loopful from the broth was further sub-cultured onto the agar media which includes the EMB,NA and SSA and incubated at 37 °C for 24 h. Colonies from the agar plates were sub-cultured further on to nutrient agar with the aid of a wire loop and incubated for another 24 h at 37 °C so as to obtain pure isolates. The pure isolates were then identified and characterized by Gram's staining and other biochemical test as follows: (Cheesbrough, 2006).

3.6 Identification of the Isolates

3.6.1 Gram staining

A drop of normal saline was dropped on a clean glass slide, a loopful of colonies were picked from nutrients agar and were emulsified with the normal saline to form a smear. The smears ware dried by using heat fixing method. Crystal violet reagent was added to the smear and was allowed for one minute and after the minute, the crystal violet was rinsed with water. A logos iodine solution was added to the smear for a minute and rinsed off with water. An alcohol was added in drops for 25 seconds which serves as decolourizer and was rinsed with water also. Safranin solution was added to the smear which serves as counter stain and was allowed for one minute, the safranin solution was rinsed and the smear was allowed to dry (Cheesbrough, 2006).

3.6.2 Microscopy

After drying of the stained glass, an oil immersion was added to the smear and covered with cover slip. The smear was viewed under microscope using 100x oil immersion objective lens (Cheesbrough, 2006).

3.6.3 Indole test

A loopful of the colonies from the Nutrient Agar plate were picked and inoculated in to a pepton water broth. The broths were incubated for five days at 37 °C. After the incubation period, three drops of kovacs reagent was added into the broths and was shook gently and presence or absent of of top layer formation was observed (Cheesbrough, 2006).

3.6.4 Methyl red (MR) test

A loopful of the colonies from the Nutrient Agar plate were picked and inoculated in to the MR-VP broths. The broths were incubated for five days at 37 °C. After the incubation periods a methyl-red reagent was added to the tube and it was observed for change in colour (Cheesbrough, 2006).

3.6.5 Voges Proskaur (VP) test

A loopful of the colonies from the Nutrient Agar plate were picked and inoculated in to the MR-VP broth. The broths were incubated for five days at 37 °C. After the incubation periods a Barritt reagent was added to the tubes and were observed for change in colour (Cheesbrough, 2006).

3.6.6 Citrate test

A loopful of the colonies from the Nutrient Agar plate were picked and inoculated in to the Simon citrate agar slanted in a tube. The media were incubated for 24 h at 37 °C. After the incubation period, the media were observed for change in colour (Cheesbrough, 2006).

3.6.7 Catalase test

A drop of hydrogen peroxide was dropped in a clean glass slide a colony was picked from Nutrient agar and was emulsify on the slide, it was observed for bubble formation within 5seconds (Cheesbrough, 2006).

3.6.8 Oxidase test

A piece of filter paper was placed on a clean a clean petri dish and a 2-3 drops of oxidase reagent (tetramethyl-p- phenylenediamine dihydrochloride). The colonies

were picked with a sterile stick and emulsified on the surface of the filter paper. The filter paper was observed for cloure development within the first 10-30 seconds (Cheesbrough, 2006).

3.6.8 Motility test

Semi agar medium was produced using nutrient broth and agar in a test tube. The organism were stab at the center of the media in the test tube using a sterile wire loop. The tube was incubated at 37 °C for 7 days within routine observation (Cheesbrough, 2006).

3.6.9 Urease test

Urea agar slant was prepared in a bijou bottle and the test organisms were inoculated heavily on the slant surface. The caps of the bottles were left partly loose and the slant were incubated for 7 days at 37 °C.the media was observed for present or absent of color change (Cheesbrough, 2006).

3.7 Molecular Characterization of Bacterial Isolates

3.7.1 DNA extraction

Single colonies grown on medium were transferred to 1.5 mL of liquid medium and cultures were grown on a shaker for 48 h at 28 ºC. After this period, cultures were centrifuged at 4600 g for 5 min. The resulting pellets were re-suspended in 520 µL of TE buffer (10 mMTris-HCl, 1 mM EDTA, pH 8.0). Fifteen microliters of 20 % SDS and 3 µL of Proteinase K (20 mg/ mL) were then added. The mixture was incubated for 1 h at 37 ºC, then 100 µL of 5 M NaCl and 80 µL of a 10 % CTAB solution in 0.7 M NaCl were added and votexed. The suspension was incubated for 10 min at 65 ºC

and kept on ice for 15 min. An equal volume of chloroform: isoamyl alcohol (24:1) was added, followed by incubation on ice for 5 min and centrifugation at 7200g for 20 min. The aqueous phase was then transferred to a new tube and isopropanol (1: 0.6) was added and DNA precipitated at –20ºC for 16h. DNA was collected by centrifugation at 13000g for 10 min, washed with 500 µL of 70 % ethanol, air-dried at room temperature for 3 h and finally dissolved in 50 µL of TE buffer.

3.7.2 Polymerase chain reaction

PCR sequencing preparation cocktail consisted of 10 µL of 5x GoTaq colourless reaction, 3 µL of 25 mM $MgCl_2$, 1 µL of 10 mM of dNTPs mix, 1 µL of 10 pmol each 27F 5'- AGA GTT TGA TCM TGG CTC AG-3' and - 1525R, 5′-AAGGAGGTGATCCAGCC-3′ primers and 0.3units of Taq DNA polymerase (Promega, USA) made up to 42 µL with sterile distilled water 8 µL DNA template. PCR was carried out in a GeneAmp 9700 PCR System Thermalcycler (Applied Biosystem Inc., USA) with a Pcr profile consisting of an initial denaturation at 94 °C for 5 min; followed by a 30 cycles consisting of 94 °C for 30 s, 50 °C for 60s and 72 °C for 1 minute 30 seconds ; and a final termination at 72 °C for 10 mins. And chill at 4 ºC.GEL (2,3)

3.7.3 Integrity

The integrity of the amplified about 1.5Mb gene fragment was checked on a 1 % Agarose gel ran to confirm amplification. The buffer (1XTAE buffer) was prepared and subsequently used to prepare 1.5 % agarose gel. The suspension was boiled in a microwave for 5 minutes. The molten agarose was allowed to cool to 60 °C and

stained with 3µl of 0.5 g/ mL ethidium bromide (which absorbs invisible UV light and transmits the energy as visible orange light). A comb was inserted into the slots of the casting tray and the molten agarose was poured into the tray. The gel was allowed to solidify for 20 minutes to form the wells. The 1XTAE buffer was poured into the gel tank to barely submerge the gel. Two microliter (2 L) of 10X blue gel loading dye (which gives colour and density to the samples to make it easy to load into the wells and monitor the progress of the gel) was added to 4 µL of each PCR product and loaded into the wells after the 100bp DNA ladder was loaded into well 1. The gel was electrophoresed at 120V for 45 minutes visualized by ultraviolet trans-illumination and photographed. The sizes of the PCR products were estimated by comparison with the mobility of a 100bp molecular weight ladder that was ran alongside experimental samples in the gel.

3.7.4 Purification of amplified product

After gel integrity, the amplified fragments were ethanol purified in order to remove the PCR reagents. Briefly, 7.6 µL of Na acetate 3 M and 240 µL of 95 % ethanol were added to each about 40µl PCR amplified product in a new sterile 1.5 µL tube eppendorf, mix thoroughly by vortexing and keep at -20°C for at least 30 min. Centrifugation for 10 min at 13000 g and 4°C followed by removal of supernatant (invert tube on trash once) after which the pellet were washed by adding 150 µL of 70 % ethanol and mix then centrifuge for 15 min at 7500 g and 4 °C. Again remove all supernatant (invert tube on trash) and invert tube on paper tissue and let it dry in the fume hood at room temperature for 15 min. then resuspend with 20 µL of sterile

distilled water and kept in -20 °C prior to sequencing. The purified fragment was checked on a 1.5 % Agarose gel ran on a voltage of 110 V for about 1hr as previous, to confirm the presence of the purified product and quantified using a nano-drop of model 2000 from thermo scientific.

3.7.5 Sequencing

The amplified fragments were sequenced using a Genetic Analyzer 3130xl sequencer from Applied Biosystems using manufacturers' manual while the sequencing kit used was that of BigDye terminator v3.1 cycle sequencing kit. Bio- Edit software and MEGA 6 were used for all genetic analysis

3.8 Processing of Plants Materials

The three plants material used in this study are *Calotropis procera* leaf (Tumfafia), *Leptidenia hastata* stem (Yadiya) and *Securidaca longipedunculata* stem (Sanya) were all selected based on their local consumption and ethno-medicinal uses in treating urinary tract, wound, enteric fever and other bacterial infection.

The plant materials were washed under running tap water, air- dried at room temperature and then pulverized and homogenized to fine powder and stored in airtight glass containers at room temperature (28±2°C) in dark until use as described by Shobowale (2013).

3.8.1 Extraction of plant materials

The plant materials were extracted using cold maceration technique as described by Nenaah and Ahamad (2011). For each plant material, one hundred Gram (100 g) of the dried powder was soaked into the methanol in a clean sterile airtight container for

three days (72 h) at room temperature (28±2°C), while undergoing vigorous shaking at regular interval. The mixture was then filtered through muslin cloth and re-filtered through Whattman`s filter paper No. 1. The filtrate was concentrated by complete evaporation of the solvent at room temperature. The concentrated extract was subsequently transferred into clean sterile airtight glass container and stored in the refrigerator at 4 °C until use. The weight of the plants materials were recorded and the percentage yield were calculated using the formula below;

$$\text{Percentage (\%) yield} = \frac{\text{weight of the extract}}{weight\ of\ dry\ sample}\ x\ 100$$

3.8.2 Preparation of the plant extract concentration

The dried extracts were prepared to the concentration 15 mg/ mL, 25 mg/ mL, 50 mg/ mL, 100 mg/ mL, 150 mg/ mL, 200 mg/ mL, 250 mg/ mL and 300 mg/ mL respectively by dissolving in 2 mL of dimethyl sulfoxide (DMSO) and 10 mL of sterile distilled water (CLSI, 2012).

3.9 Standardization of Inoculum

Zero-point one (0.1 mL) of bacteria suspension from 18 h overnight cultures was suspended into 10 mL of nutrient broth and the suspension was incubated for 2 h at 37 °C. The culture was standardized to 0.5 McFarland turbidity standard. A loopful of the standardized inoculum was used for the antibacterial susceptibility test.

3.10 Antimicrobial Susceptibility Testing of the Crude Extracts.

The antimicrobial activity of the extracts was determined using Agar well diffusion method adopted by Innalegwu *et al.* (2021) with slight modification. Müeller Hinton

agar plates were prepared for all the organisms. A sterile cork borer of diameter 6 mm was used to bore wells onto the agar plates and a sterile cotton swab sticks were submerged into the standardized microbial suspensions. Blotting out the excess fluids, the swabs were used to streak the inoculum on the entire surface of the agar plates and the plates were allowed to dry for few minutes. 0.2 mL of each concentration prepared was transferred into the labelled bored well on the agar plate and ciprofloxacin, amoxicillin was used as positive control whereas distilled water and DMSO were used as negative control.

Thirty minutes' pre-diffusion time was allowed for each plate after which they were incubated at 37 °C for 24 h. After the 24 h incubation period, the diameter zone of inhibition (mm) were measured using a measuring ruler and the measurement were obtained by measuring the Centre of well and subtracting it with the diameter of the cork borer. Test isolate with 10mm zone of inhibition is considered sensitive to that extract as described by Clinical and Laboratory Standards Institute (CLSI, 2012).

3.11 Determination of Minimum Inhibitory Concentration (MIC) of the Extract

The minimum inhibitory concentration (MIC) was determined using the method of Mogana *et al.* (2020) with little modification. A series of two fold dilutions of each extract ranging from 300 mg/ mL to and 4.6875 mg/ mL were prepared in Nutrient broth and 0.1 mL of each standardized culture of the test organisms (0.5 McFarland turbidity standard) was transferred into each labeled dilution tubes. Negative and positive controls were maintained respectively in which the negative control contain

the inoculum in the growth medium organisms and positive controls contained the diluted extracts in the growth medium without the inoculum .

The tubes were incubated at 37 °C for 24 h and at the end of the incubation period, the optical density of the cultures in the test tubes were read using a spectrophotometer at a wavelength of 600 nm. The test tubes with the lowest significant reduction in absorbance was taken as the MIC.

3.12 Determination of Minimum Bactericidal Concentration (MBC) of the Extracts

The minimum bactericidal concentration (MBC) was determined by sub-culturing the tubes with the lowest optical density from the minimum inhibitory concentration into a freshly prepared agar plates. The plates were incubated at 37 °C for 24 h. Thus the plates with minimum or no bacterial growth were recorded as the MBC (Ewansiha, 2020).

3.13 Partitioning of the Crude Extracts

The plants methanolic extract were partitioned as described by Innalegwu *et al.* (2021). Fifteen grams (15 g) of the crude methanol extracts were dissolved into 100 mL of distilled water and were partitioned exhaustively in order of increasing solvent polarity (n-hexane < ethyl acetate < distilled water) using separating funnel. The fractions obtained were evaporated to dryness at a control temperature of 40 °C in water bath. The weight of each fraction was recorded after the dryness. Furthermore, the fractions were subjected to antibacterial screening, also the MIC and MBC of the fractions were also determined using the method previously described.

3.14 Gas Chromatography (GC)–Mass Spectrometer (MS) Analysis

The GC-MS analysis of the purified fractions from methanol sub-fraction of *Calotropis procera* leaf, *Leptidenia hastata* stem and *Securidaca longipedunculata* stem were carried out on a GC-MS-QP 2010 Plus Shimadzu system (SHIMADZU, JAPAN). Gas chromatograph interfaced with a mass spectrometer (GC-MS) instrument was used; Column elite-1 fused silica capillary column (30m x0.25mm 1D x μL df, composed of 100 % dimethyl polysiloxane). In detecting the GC-MS an electron ionization system with ionization energy of 70 eV was used. Helium gas (99.999 %) was used as the carrier gas at constant flow rate1 mL/min and an injection volume of 2 μL was employed (split ratio of 10:1) injector temperature 250 °C; ion source temperature 280 °C. The oven temperature was programmed from 110 °C (Isothermal for 2 min.) with an increase of 10 °C per minute to 200 °C then 5 °C per minute to 280 °C /min, ending with a 9 min isothermal at 280 °C. Mass spectra were taken at 70 eV with a scanning interval of 0.5 s and fragments from 40 to 550 Da. The total GC running time was 27 minutes. The relative percentage amount of each component was calculated by comparing the average peak area to the total areas. Software adapted to handle the mass spectra and chromatogram was a turbo mass and the detection of compounds employed the database of the National Institute of Science and Technology (NIST) NIST Ver. 2.0 year 2009 library.

3.15 Qualitative and Quantitative Determination of Phytochemicals

3.15.1 Total flavonoids determination

Praveen and Rajesh (2019) method was used to determine total flavonoid content of the extracts. 0.5 mL of each extract was measured and added to a test tube containing 1.5 mL of absolute methanol, 0.1 mL of 10 % aluminum chloride, 0.1 mL of 1 % sodium acetate and 2.8 mL of distilled water. The tubes were incubated at ambient temperature for 30 minutes. The absorbance was read at 415 nm with double beam shimadzu UV spectrophotometer, UV-1800. Standard quercetin was used to prepare the calibration curve.

3.15.2 Total phenol determination

Total phenol content of the extracts was determined using the method of (Ajiboye *et al.,* 2013) 0.01 g of each extract was dissolved in 10 mL of distilled water, and 0.5 mL was oxidized by 2.5 mL of 10 % Folin-Ciocalteu's reagent which was then neutralized by 2 mL of 7.5 % sodium carbonate. Followed by vigorous shaking, the mixture was allowed to for 2 h. Finally, the absorbance was read at 765 nm using double beam Shimadzu UV spectrophotometer, UV-1800. Standard garlic acid was used to prepare the calibration curve.

3.15.3 Total saponins determination

Saponins content of the extracts was determined using the method of (Mir *et al.,* 2016). 0.5 g of each extract was weighed and dissolved in 20 mL of 1N HCl and boiled in water bath at 80 $^{\circ}$C for 4 h. The reaction mixture was cooled and filtered. 50 mL of petroleum ether was added and the ether layer was collected and evaporated to

dryness. Thereafter, 5 mL of acetone-ethanol (1:1), 6 mL of ferrous sulphate and 2 mL of concentrated sulphuric acid were added and allowed to stand for 10minutes. The absorbance was taken at 490 nm. Standard saponins was used to prepare the calibration curve.

3.15.4 Total alkaloid determination

Total alkaloid of the extracts was determined using method of Lofty *et al*. (2015). Using this, 0.5 g of each extract was weighed and dissolved in 5 mL of mixture of 96 % ethanol:20 % H_2SO_4 (1:1) and then filtered. 1 mL of the filtrate was then added to a test tube containing 5 mL of 60 % H_2SO_4 and allowed to stand for 5 minutes. Thereafter, 5 mL of 0.5 % formaldehyde was added and allowed to stand at room temperature for 3 h. The absorbance was read at wavelength of 565 nm. Vincristine extinction coefficient (E_{296}, ethanol {ETOH} = 15136$M^{-1}cm^{-1}$) was used as reference alkaloid.

3.15.5 Total tanins determination

Tannin content of the extracts was determined using the method of (Suleiman *et al.*, 2007). 0.2g of each extract was weighed into a 50 mL beaker and 20 mL of 50 % methanol was added to it and covered with para film and heated in water bath at 80 ˚C for 1 hour. The reaction mixture was shaken thoroughly to ensure uniformity. The extract was then filtered into a 100 mL volumetric flask, and 20 mL of distilled water, 2.5 mL of Folin-Denis' reagent, and 10 mL of sodium carbonate were added and mixed properly. The reaction mixture was then allowed to stand for 20 minutes at room temperature for the development of bluish-green coloration. The absorbance

was taken at 760 nm using double beam shimadzu UV-spectrophotometer, UV-1800. Standard tannic acid was used to prepare the calibration curve.

3.16 Data Analysis

The results were analyzed using the SPSS statistical package for WINDOWS (version 21.0; SPSS Inc., Chicago) and Data were expressed as the Mean±Standard Error of Mean of three determinations. Results were subjected to one-way ANOVA followed by the Duncan Multiple Range Test (DMRT) also the Values were considered statistically significant at $p < 0.05$

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Results

4.1.1 Identity and characteristics of the isolates

The isolates collected and identified were *Escherichia coli, Pseudomonas aeruginosa, Salmonella bongori* and *Klebsella pneumonia.* The isolates were confirmed based on their growth and cultural characteristics on the culture media, Grams reaction and microscopical appearance and their reaction to certain biochemical reagents as shown in Table 4.1 and Table 4.2.

4.1.2 Molecular characterization of bacterial isolate

Polymerase chain reaction amplifications of total genomic DNA of the bacteria isolates using primer pair 27F 5'- AGA GTT TGA TCM TGG CTC AG-3' and -1525R, 5′-AAGGAGGTGATCCAGCC-3′ primers produced a PCR product of about 1500 base pair (bp) (Plate I). The PCR products were sequenced (GenBank Accession Number: MK572634.1, MN623691.1, CP074120.1 and AB642255.1 for *Pseudomonas aeruginosa, Salmonella bongori, Escherichia coli* and *Klebsiella pneumoniae* respectively) (Table 4.3). The phylogenetic tree of the test isolates (Figure 4.1) showed the relations among the organisms and their origin using the NCBI database while the result of the sequence anlysis are presented in Appendix A.

Table 4.1: Cultural and morphological characteristics of bacteria

Organism	GC & M	MCM	Gram staining
Escherichia coli	Greenish metallic sheen colonies on EMB	Rod shaped bacilli	Negative
Pseudomonas aeruginosa	Large opaque flat colonies with green pigmentation on NA	Rod shaped bacilli	Negative
Salmonella bongori	Colorless colonies with black center on SSA	Rod shaped bacilli	Negative
Klebsella pneumoniae	Large mucoid purple to pink colonies without metallic sheen on EMB	Rod shaped bacilli	Negative

Key: GC&M = growth characteristics on media, MCM= microscopic morphological characteristics

Table 4.2: Biochemical characteristics of bacteria

Organism	Indole	M-R	V-P	Citrate	Catalase	Motility	Oxidase	Urease
Escherichia coli	+	+	-	-	+	+	-	-
Pseudomonas aeruginos	-	-	-	+	+	+	+	-
Salmonella bongori	-	+	-	-	+	+	-	-
Klebsella pneumonia	-	-	+	+	+	-	-	+

Key: + = Positive and - = Negative

Table 4.3: Molecular characterization of bacteria

Sample ID	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
IDZU7	*Pseudomonas aeruginosa*	287	2623	99 %	0	99.93 %	1473	MK572634.1
IDZU8	*Salmonella bongori*	54736	2647	99 %	0	99.52 %	1526	MN623691.1
IDZU9	*Escherichia coli*	562	2630	99 %	0	99.58 %	4844032	CP074120.1
IDZU10	*Klebsiella pneumoniae*	573	2636	99 %	0	99.79 %	1533	AB642255.1

4.1.3 Percentage yield of extract

The yield of the 500 g weight of the pulverized plant material extracted exhaustively with methanol was measured and recorded. The highest yield was obtained from *Calotropis procera* leaf with 35 g (7 %) follow by *Securidaca longipedunculata* stem with 30 g (6 %) and the lowest yield was recorded from *Leptidenia hastata* stem with 27 g (5.6 %) (Table 4.4).

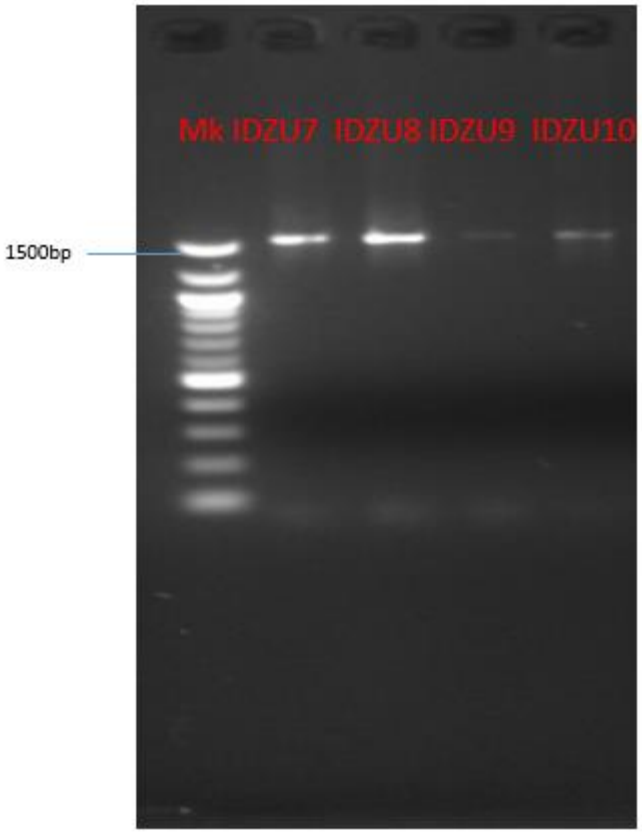

Plate I: PCR amplification of the DNA region of the bacteria isolates using the 27F and 1525R primer.

Keys: MK = 1500bp DNA ladder, IDZU7= *Pseudomonas aerugnosa*, IDZU8 = *Salmonella bongori*, IDZU9 = *Escherichia coli*, IDZU10 = *Klebsiella pnuemoniae*, PCR reaction mixture with DNA of the samples to be identified.

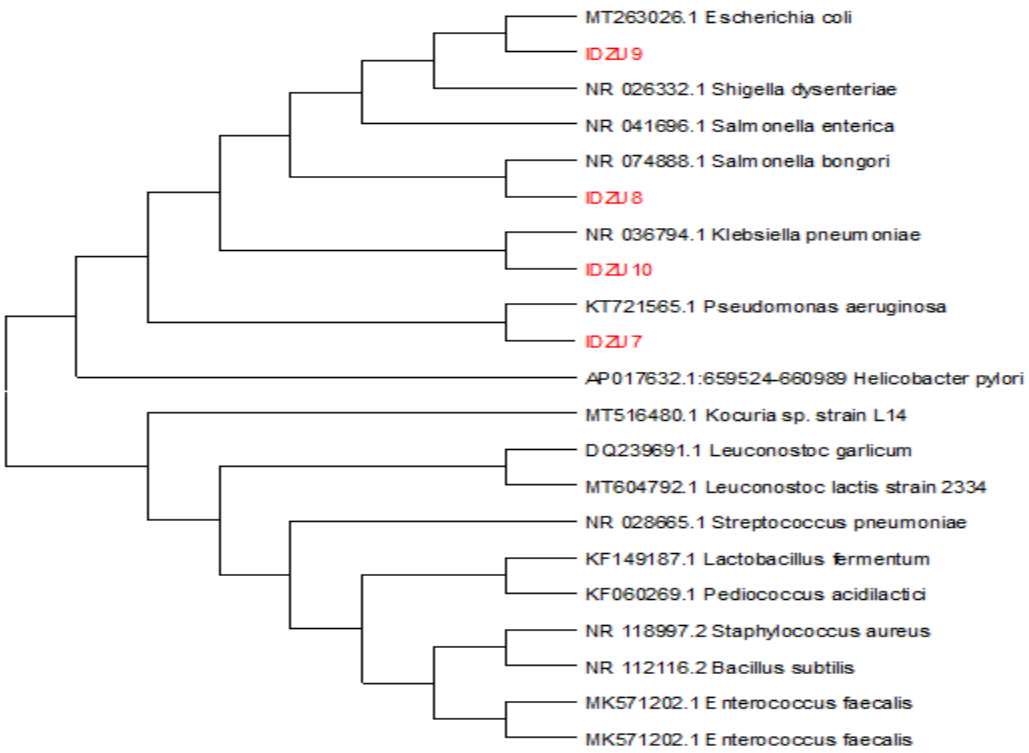

Figure 4.1: Phylogentic tree showing the relations among the bacteria isolates

Key: IDZU7 = *Pseudomonas aeruginosa*, IDZU8 = *Salmonella typhi*, IDZU9 = *Eschrichia coli* and IDZU10 = *Klebsiella pneumoanie*

Table 4.4: Percentage yield of plant extracts

Samples	WDP	WE (g)	PY (%)
Calotrpis procera	500	35	7
Leptedenia hastata	500	27	5.4
Securidaca longipedunculata	500	30	6

Key: WDP= weight of dry powder, WE= weight of extract, PY= percentage yield and g= gram

4.1.4 Antibacterial activity of the crude extract of *Calotropis procera* leaf, *Leptidenia hastata* stem and *Securidaca longipedunculata* stem

Antibacterial activity of the *Calotropis procera* leaf extract against the tested organisms as shows that *Escherichia coli* and *Pseudomonas aeruginosa* are more susceptible to the extract with a zone of inhibition of ranging between 8.00±0.30-17.80±0.20 mm and 14.00±0.50-19.60±0.20 mm respectively but less active against *Salmonella bongori* and *Klebsella pneumoniae* (Table 4.5)

4.1.5 Antibacterial activity of the *Leptidenia hastata* stem crude extract

Crude extratct of *Leptidenia hastata* was active against *Escherichia coli, Klebsella pneumoniae* and *Pseudomonas aeruginosa* with zones of inhibition ranging between 6.70±0.30-14.85±0.5 mm, 12.10±0.10-21.55±0.65 mm and 7.10±1.10-17.65±0.15 mm respectively (Table 4.6).

4.1.6 Antibacterial activity of the *Securidaca longipedunculata* stem crude extract

Securidaca longipedunculata stem extract shows a significant zone of inhibition against *Escherichia coli* ranging between 8.05±2.05-13.15±0.15 mm while *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Salmonella bongori* were resistant against the extract (Table 4.7).

Table 4.5: Mean zones of inhibition of methanol extract of *Calotropis procera* leaf against bacterial isolates

Organisms	Concentration (mg/ mL)									
	15	25	50	100	150	200	250	300	Cipro 50mg/ mL	Amox 50mg/ mL
E. coli	-	-	8.00±0.30^{b}	10.00±0.50^{b}	11.00±1.0^{c}	11.00±0.0^{c}	14.50±0.50^{d}	17.80±0.20^{e}	18.55±1.12^{f}	3.10±0.23^{a}
P. aeruginosa	-	-	14.00±0.50^{a}	14.00±1.00^{a}	15.00±1.00^{ab}	15.15±0.15^{ab}	17.10±0.60^{b}	19.60±0.2^{c}	24.35±1.50^{d}	12.75±0.22^{a}
S. bongori	-	-	-	3.60±0.20^{a}	7.75±0.15^{b}	8.00±1.00^{b}	11.10±0.1^{c}	13.50±0.50^{d}	23.01±0.20^{e}	7.30±1.11^{b}
K. pneumoniae	-	-	-	-	-	4.50±0.50^{a}	9.05±0.05^{b}	10.10±0.10^{b}	24.90±0.13^{d}	12.33±0.6^{c}

Values are expressed in mean ± standard error of mean, values with the same superscript on the same row have no significance difference ($p > 0.05$), n= 3, - = No activity

Table 4.6: Mean zones of inhibition of methanol extract of *Leptidenia hastata* stem against bacterial isolates

Organisms	Concentration (mg/ mL)									
	15	25	50	100	150	200	250	300	Cipro 5mg/ mL	Amox 5mg/ mL
E. coli	-	-	6.70±0.30^{b}	9.25±0.25^{c}	11.85±0.45^{d}	12.40±0.0^{e}	13.15±0.5^{e}	14.85±0.5^{f}	18.55±1.12^{g}	3.10±0.23^{a}
P. aeruginosa	-	-	7.10±1.10^{a}	10.90±0.10^{b}	14.00±1.00^{d}	14.15±0.15^{d}	15.25±0.25^{d}	17.65±0.15^{e}	24.35±1.50^{f}	12.75±0.22^{c}
S. bongori	-	-	-	5.55±0.25^{a}	6.20±0.20^{ab}	6.90±0.10^{ab}	7.30±0.30^{b}	9.10±0.2^{c}	23.01±0.20^{d}	7.30±1.11^{b}
K. pneumoniae	-	4.35±0.25^{a}	12.10±0.10^{b}	12.65±0.15^{b}	14.80±0.8^{c}	17.40±0.10^{d}	17.35±0.85^{d}	21.55±0.65^{e}	24.90±0.132^{e}	12.33±0.60^{b}

Values are expressed in mean ± standard error of mean, values with the same superscript on the same row have no significance difference ($p > 0.05$), n= 3, - = No activity

Table 4.7: Mean zones of inhibition of methanol extract of *Securidaca longipedunculata* stem against bacterial isolates

Organisms	Concentration (mg/ mL)									
	15	25	50	100	150	200	250	300	Cipro 5mg/ mL	Amox 5mg/ mL
E. coli	-	-	-	8.05 ± 2.05^{b}	10.40 ± 0.1^{c}	10.70 ± 0.3^{c}	11.75 ± 0.05^{c}	13.15 ± 0.15^{d}	18.55 ± 1.12^{d}	3.10 ± 0.23^{a}
P. aeruginosa	-	-	-	-	-	-	-	-	24.35 ± 1.50^{b}	12.75 ± 0.22^{a}
S.bongori	-	-	-	-	-	-	-	-	23.01 ± 0.20^{b}	7.30 ± 1.11^{a}
K. pneumoniae	-	-	-	2.70 ± 0.70^{a}	3.40 ± 0.40^{a}	5.20 ± 0.10^{b}	5.85 ± 0.35^{b}	6.35 ± 0.35^{b}	24.90 ± 0.13^{d}	12.33 ± 0.6^{c}

Values are expressed in mean ± standard error of mean, values with the same superscript on the same row have no significance difference ($p > 0.05$), n= 3, - = No activity

4.1.7 Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of *Calotropis procera* leaf extract

MIC and MBC of the crude extracts of *Calotropis procera* leaf was observed against the organisms whereas the least values were recorded against *Escherichia coli* and *Pseudomonas aeruginosa,* (MIC {37.5 mg/mL}, MBC {75mg/ mL}) was observed against *Escherichia coli* and *Pseudomonas* and the highest value was recorded against *Klebsella pneumonia* (MIC {150 mg/mL}, MBC {300mg/ mL}) as shown in Figure 4.2.

4.1.8 Minimum inhibitory concentration (MIC) and minimum bactericidal Concentration (MBC) of *Leptidenia hastata* stem extract

Least MIC and MBC were recorded in *Leptidenia hastata* stem extract against *Klebsella pneumonia* (18.75 mg/mL) and (37 mg/mL) follow by *Escherichia coli* and *Pseudomonas aeruginosa* (37.5 mg/mL), (75 mg/mL) and *Salmonella bongori* (150mg/ mL) and (300 mg/ mL) as described in Figure 4.3.

4.1.9 Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of *Sacuridaca longipedunculata* stem extract

Securidaca longipedunculata stem extract had the least MIC and MBC against *Escherichia coli* (75mg/ mL) and (150mg/ mL) and the highest were recorded against *Pseudomonas aeruginosa, Salmonella typhi* and *Klebsella pneumonia* (150mg/ mL) and (300 mg/ mL) respectively (Figure 4.4).

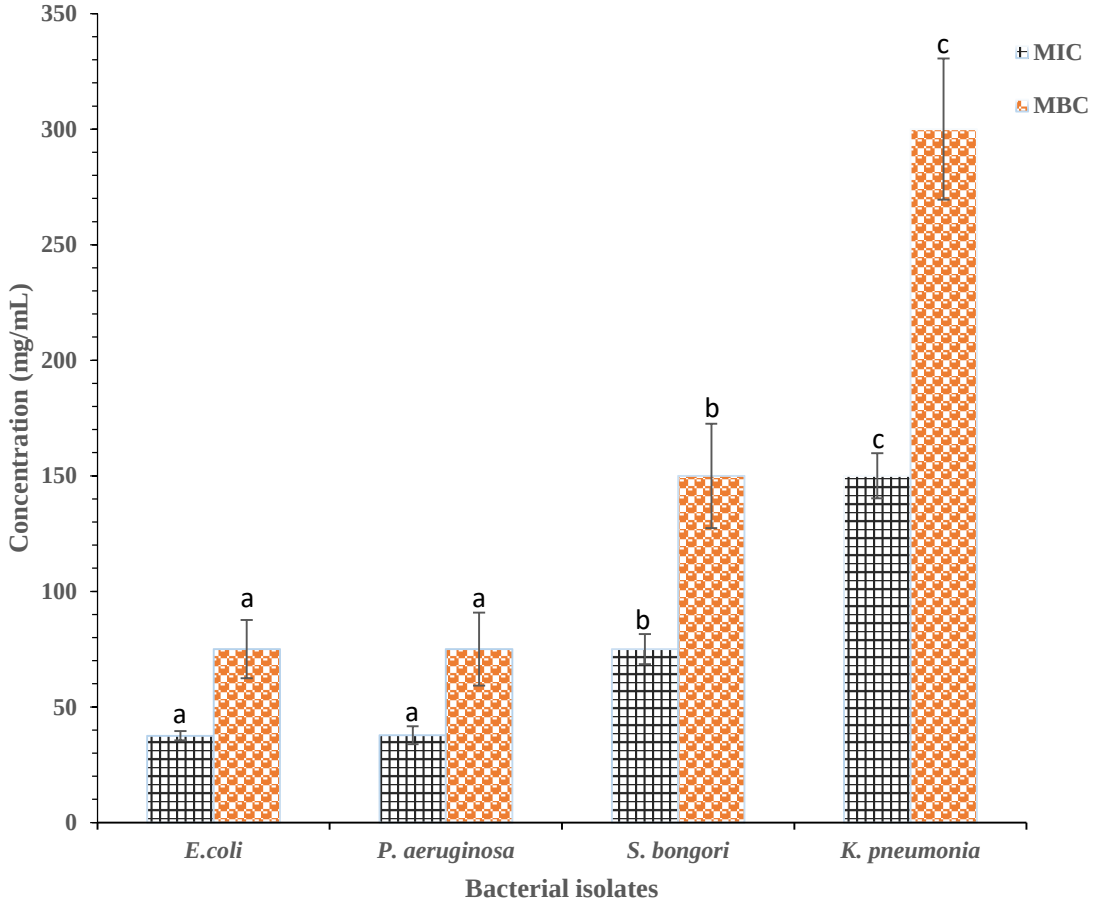

Figure 4.2: Minimum inhibitory concentration and Minimum Bactericidal concentration of crude methanol extract of *C. procera*

NB: Bars with the same color and the same alphabet have no significant difference at $p<0.05$

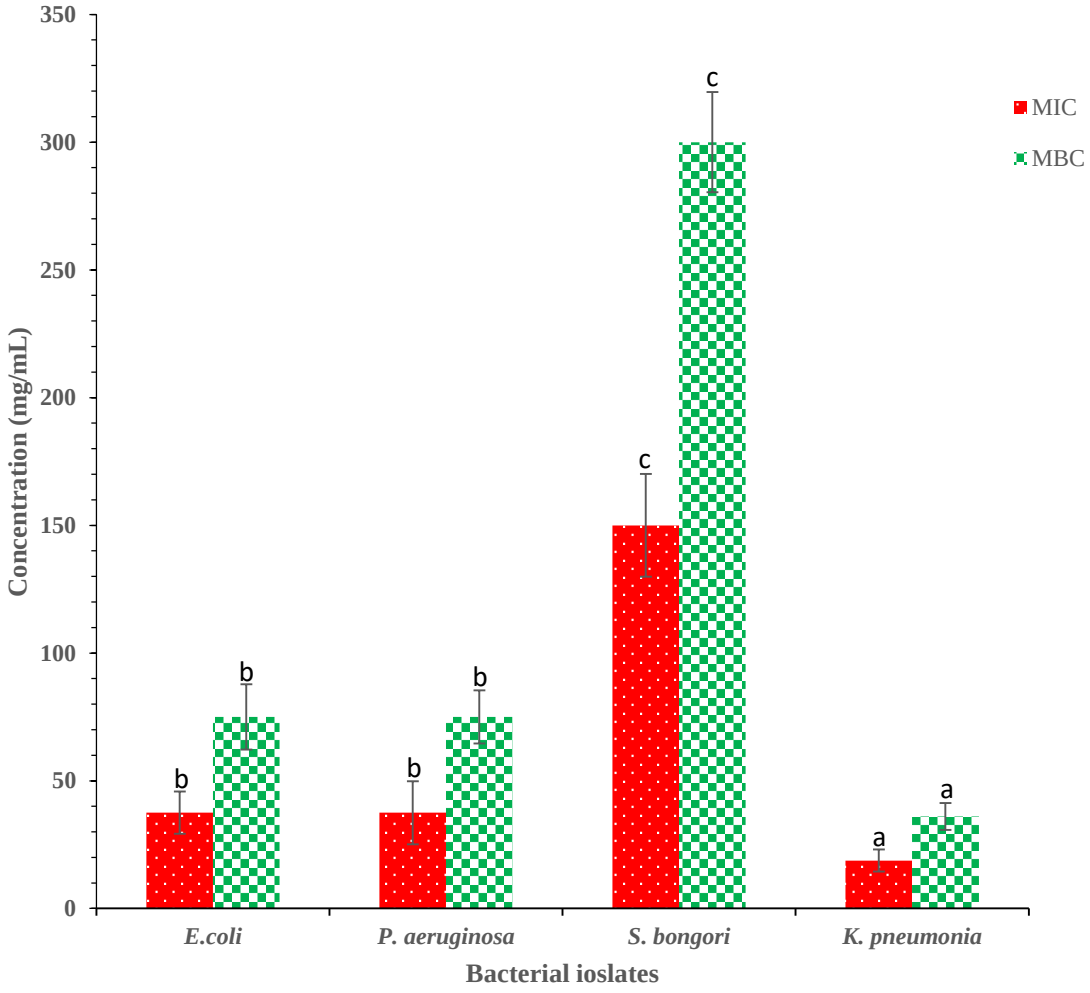

Figure 4.3: Minimum inhibitory concentration and Minimum Bactericidal concentration of crude methanol extract of *L. hastata*

NB: Bars with the same color and the same alphabet have no significant difference at $p<0.05$

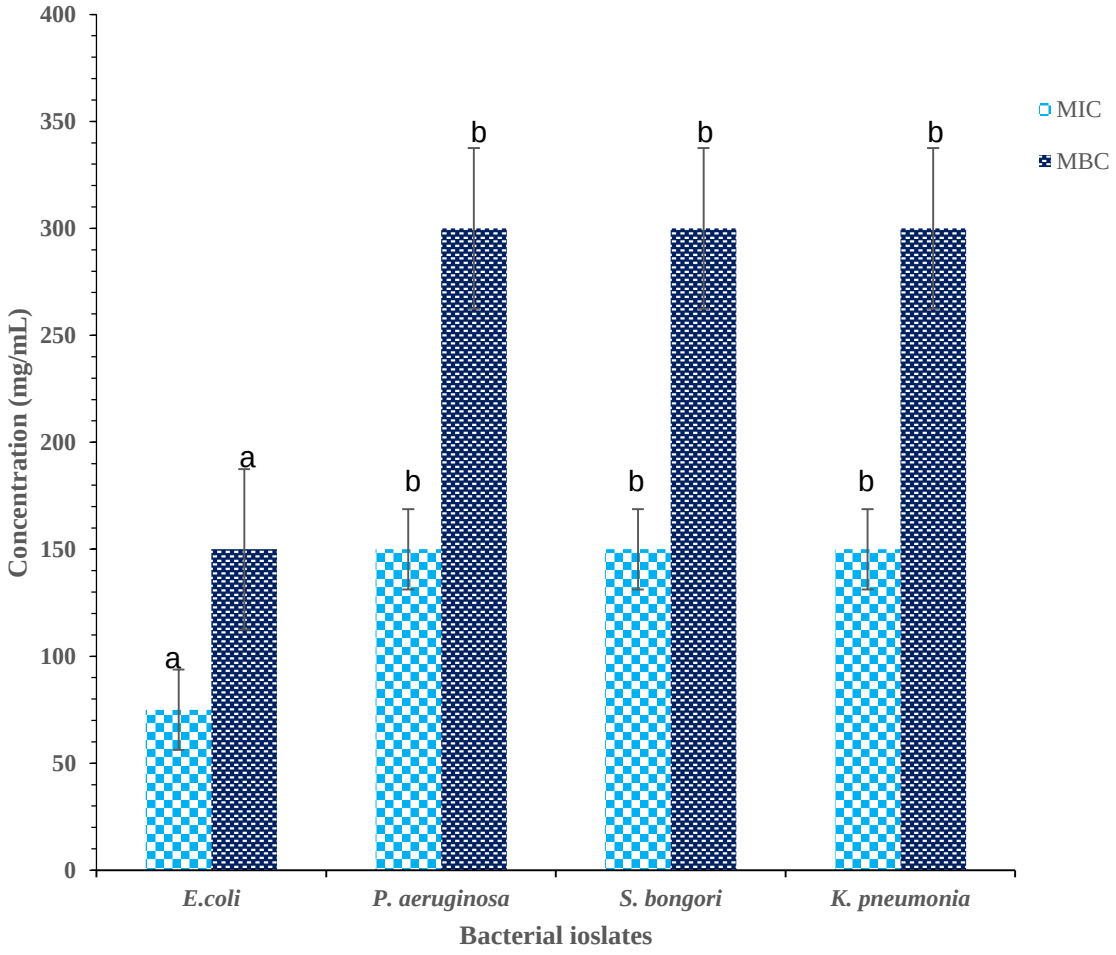

Figure 4.4: Minimum inhibitory concentration and Minimum Bactericidal concentration of crude methanol extract of *S. longipedunculata*

NB: Bars with the same color and the same alphabet have no significant difference at $p<0.05$

4.1.10 Percentage yield of partitioned fractions of plant crude extracts

Various yields were obtained in n-hexane (23 %), ethylacetate (41 %) and residual aqueous (35 %) fractions of *Calotropis procera* leaf extract while ethylacetate (30 and 27 %) and residual aquaouse (70 and 73 %) fractions were obtained from *Leptidenia hastata* stem and *Securidaca longipedunculata* stem extract (Table 4.8).

4.1.11 Antibacterial activity of partitioned fractions of methanol extract of *Calotropis procera* leaf

Narrow zones of inhibition were recorded for all the bacteria isolates across the various concentration in n-hexane fraction while ethyl acetate and residual aqueous shows an improve level of activity against the bacteria isolates than their crude extract (Table 4.9).

4.1.12 Antibacterial activity of the *Leptidenia hastata* stem and *Securidaca longipedunculata* stem extract fractions (ethyl acetate and residual aqueous extract) against the test organism

A significant zone of inhibition was observed across all the various concentration used in ethyl acetate and residual aqueous fraction of *Leptidenia hastata* stem and *Securidaca longipedunculata* stem against some bacteria isolates used in this study (Tables 4.10 and 4.11).

4.1.13 Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of partitioned fractions of plant crude extracts

Different values were recorded for the minimum inhibitory concentration and minimum bactericidal concentration of the partitioned fractions (Figures 4.5 – 4.11).

The values were lower than those obtained from the crude showing an improved in the activity of the extract on purification.

4.1.14 Gas chromatography and mass spectrometry analysis of the sub-fractions of the plants extracts

The Gas Chromatography and Mass Spectrometry analysis of the sub-fractions of the extracts shows the presence of various metabolites of the research plants ranging from fatty acids and their esters to carbohydrates which justifies usage in traditional medicines. This are shown from Tables 4.12-4.14.

4.1.15 Qualitative and quantitative phytochemical screening of plant extracts

Quantitative phytochemical screening of the extracts indicates the presence of the tested compounds in such as flavonoids, phenols, tannins Alkaloid and saponins except in *Leptidenia hastata* where tannins was absent (Table 4.15).

Table 4.8: Percentage yield of partitioned fractions of crude extract of plants

Samples		% Weight of fractions (g)		
	WDE (g)	n-hexane	Ethyl acetate	Aqueous
C. procera	15	3.5 (23)	6.2 (41)	5.3 (35)
L. hastate	15	-	4.5 (30)	10.5 (70)
S. longipedunculata	15	-	4.0 (27)	11.0 (73)

Key: WDE = weight of dry Extract

Table 4.9: Mean zones of inhibition of ethyl acetate and aqueous fraction extract of *Calotropis procera* leaf fractions against bacterial isolates

Organism	H150	H250	H300	E150	E250	E300	A150	A250	A300
					Concentration (mg/ mL)				
E. coli	-	5.00 ± 0.00^{a}	8.30 ± 0.30^{b}	7.10 ± 0.10^{b}	11.35 ± 0.15^{c}	13.00 ± 0.00^{d}	10.30 ± 0.2^{c}	13.05 ± 0.05^{c}	15.05 ± 0.35^{d}
P. aeruginosa	-	7.20 ± 0.20^{a}	9.90 ± 0.10^{a}	13.05 ± 0.05^{b}	15.40 ± 0.10^{bc}	17.25 ± 0.25^{c}	13.05 ± 0.05^{b}	15.40 ± 0.10^{bc}	17.25 ± 0.25^{c}
S. bongori	6.00 ± 0.00^{a}	9.00 ± 1.00^{b}	12.40 ± 0.10^{d}	11.55 ± 0.45^{c}	13.15 ± 0.25^{d}	15.10 ± 0.10^{e}	8.90 ± 0.50^{b}	11.60 ± 0.1^{c}	13.30 ± 0.30^{d}
K. pneumoniae	-	9.90 ± 0.40^{a}	13.10 ± 0.1^{c}	9.20 ± 0.20^{a}	12.20 ± 0.20^{b}	12.95 ± 0.50^{b}	8.20 ± 0.20^{a}	13.10 ± 0.1^{c}	14.20 ± 0.4^{c}

Keys: H: n-Hexane fraction, A: Residual aqueous fraction, E: Ethyl acetate fraction

Values are expressed in mean ± standard error of mean, values with the same superscript on the same row have no significance difference ($p > 0.05$), n= 3, - = No activity

Table 4.10: Mean zones of inhibition of ethyl acetate and aqueous fraction of *Leptidenia hastata* stem against bacterial isolates

Organism	E150	E250	E300	A150	A250	A300
	Concentration (mg/ mL)					
E. coli	11.55±0.15^{a}	12.10±1.70^{a}	18.15±0.55^{c}	11.25±0.45^{a}	13.30±0.30^{b}	14.55±0.85^{bc}
P. aeruginosa	14.15±0.45^{b}	19.30±0.9^{c}	25.80±0.20^{d}	9.80±0.80^{a}	15.10±1.10^{b}	20.30±1.3^{c}
S. bongori	-	8.75±0.45^{b}	10.40±0.90^{d}	6.80±0.50^{a}	8.20±0.80^{b}	10.00±0.6^{c}
K. pneumoniae	9.35±0.35^{b}	11.35±0.45^{c}	16.75±0.15^{d}	4.80±0.20^{a}	10.30±1.30^{b}	15.80±0.40^{d}

Keys: A: Residual aqueous fraction, E: Ethyl acetate fraction

Values are expressed in mean ± standard error of mean, values with the same superscript on the same row have no significance difference ($p > 0.05$), n= 3, - = No activity

Table4.11: Mean zones of inhibition of ethyl acetate and aqueous fractions of *Securidaca longipedunculata* stem against bacterial isolates

Organism	E150	E250	E300	A150	A250	A300
	Concentrations (mg/ mL)					
E. coli	4.30 ± 0.10^{b}	8.70 ± 0.2^{c}	10.95 ± 1.35^{d}	1.70 ± 1.70^{a}	10.80 ± 0.20^{d}	13.25 ± 0.55^{e}
P. aeruginosa	4.00 ± 0.60^{a}	8.25 ± 0.35^{b}	15.70 ± 0.10^{d}	10.95 ± 0.35^{c}	16.85 ± 0.15^{d}	20.00 ± 0.40^{e}
S. bongori	0.60 ± 0.60^{a}	1.30 ± 1.30^{a}	9.60 ± 1.10^{b}	-	4.15 ± 1.15^{a}	9.15 ± 0.55^{b}
K. pneumoniae	13.25 ± 0.35^{c}	17.60 ± 0.20^{d}	19.90 ± 0.10^{e}	5.85 ± 0.15^{a}	10.85 ± 0.05^{b}	12.80 ± 0.2^{c}

Keys: A: Residual aqueous fraction, E: Ethyl acetate fraction

Values are expressed in mean ± standard error of mean, values with the same superscript on the same row have no significance difference ($p > 0.05$), n= 3, - = No activity

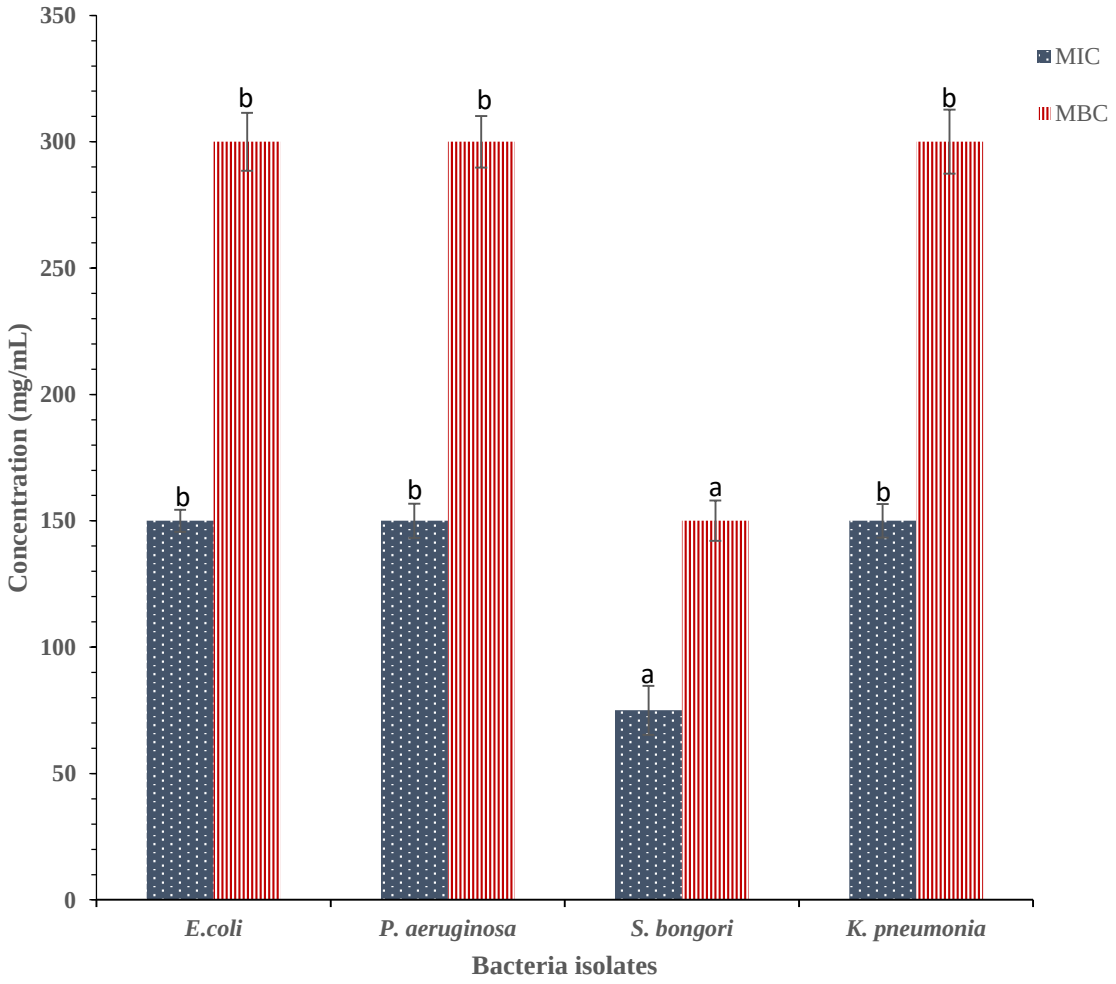

Figure 4.5: Minimum inhibitory concentration and Minimum Bactericidal concentration of n-hexane fraction of crude methanol extract of *C. procera*

NB: Bars with the same color and the same alphabet have no significant difference at $p<0.05$

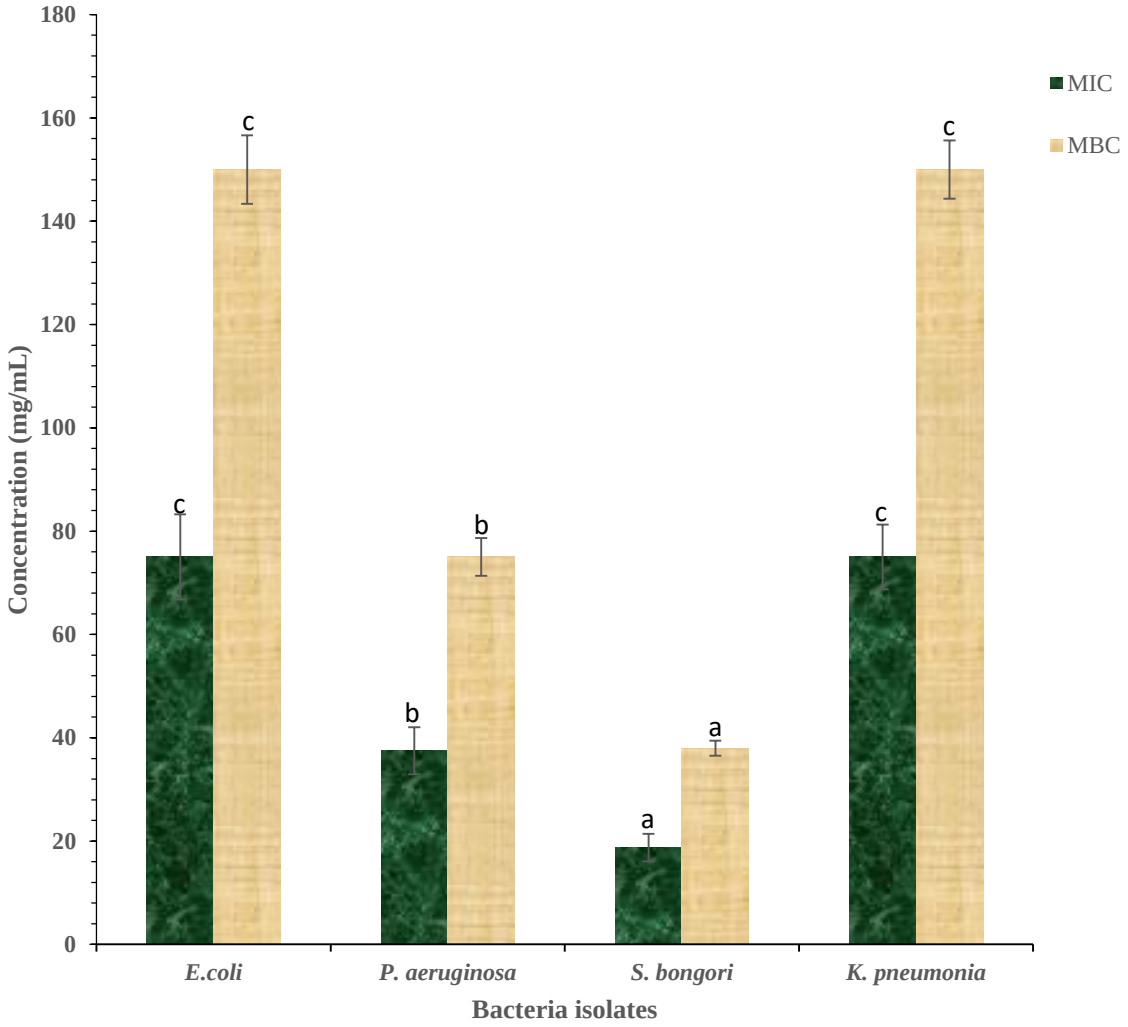

Figure 4.6: Minimum inhibitory concentration and Minimum Bactericidal concentration of ethyl acetate fraction of crude methanol extract of *C. procera*

NB: Bars with the same color and the same alphabet have no significant difference at $p<0.05$

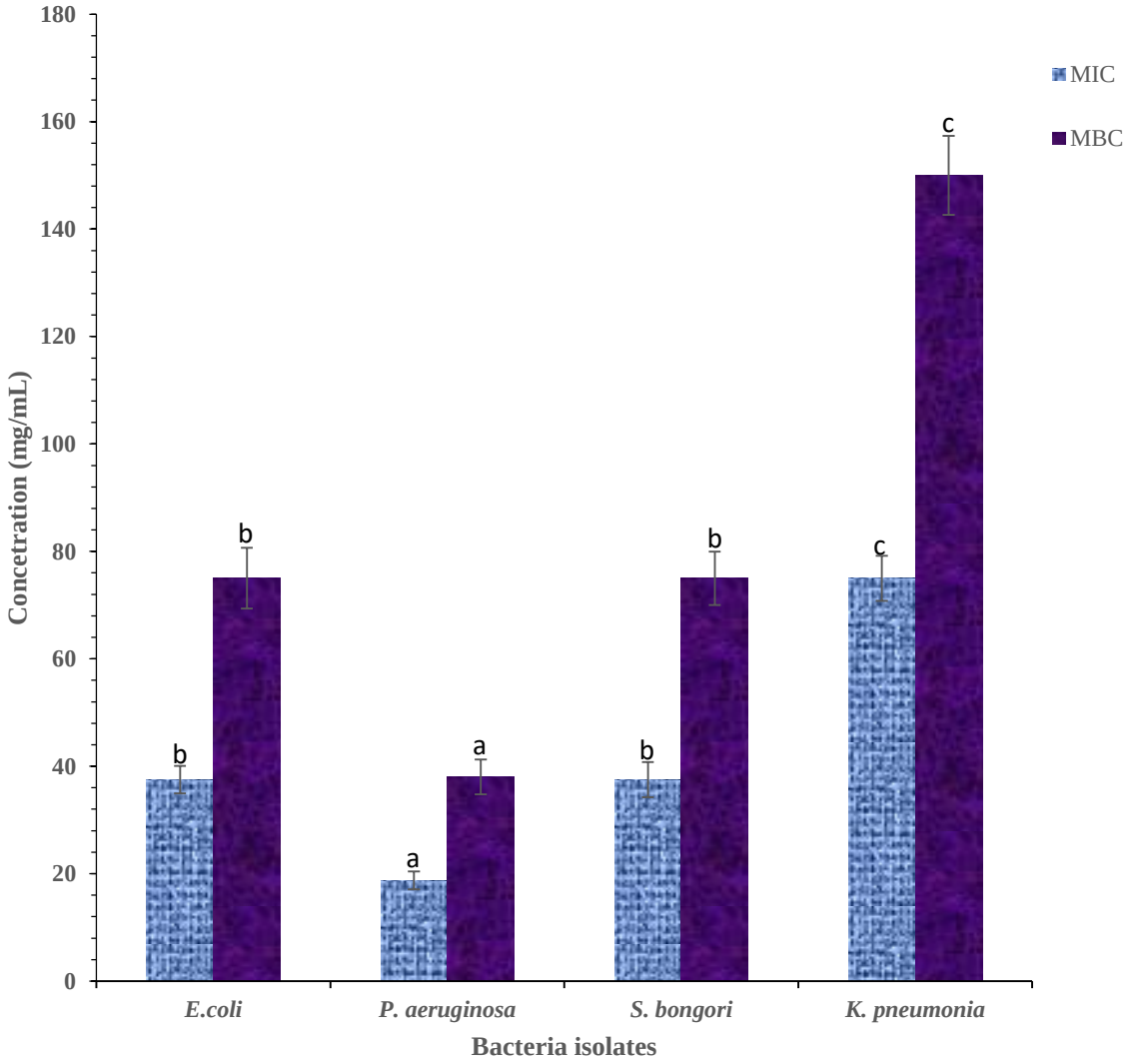

Figure 4.7: Minimum inhibitory concentration and Minimum Bactericidal concentration of aqueous fraction of crude methanol extract of *C. procera*

NB: Bars with the same color and the same alphabet have no significant difference at $p<0.05$

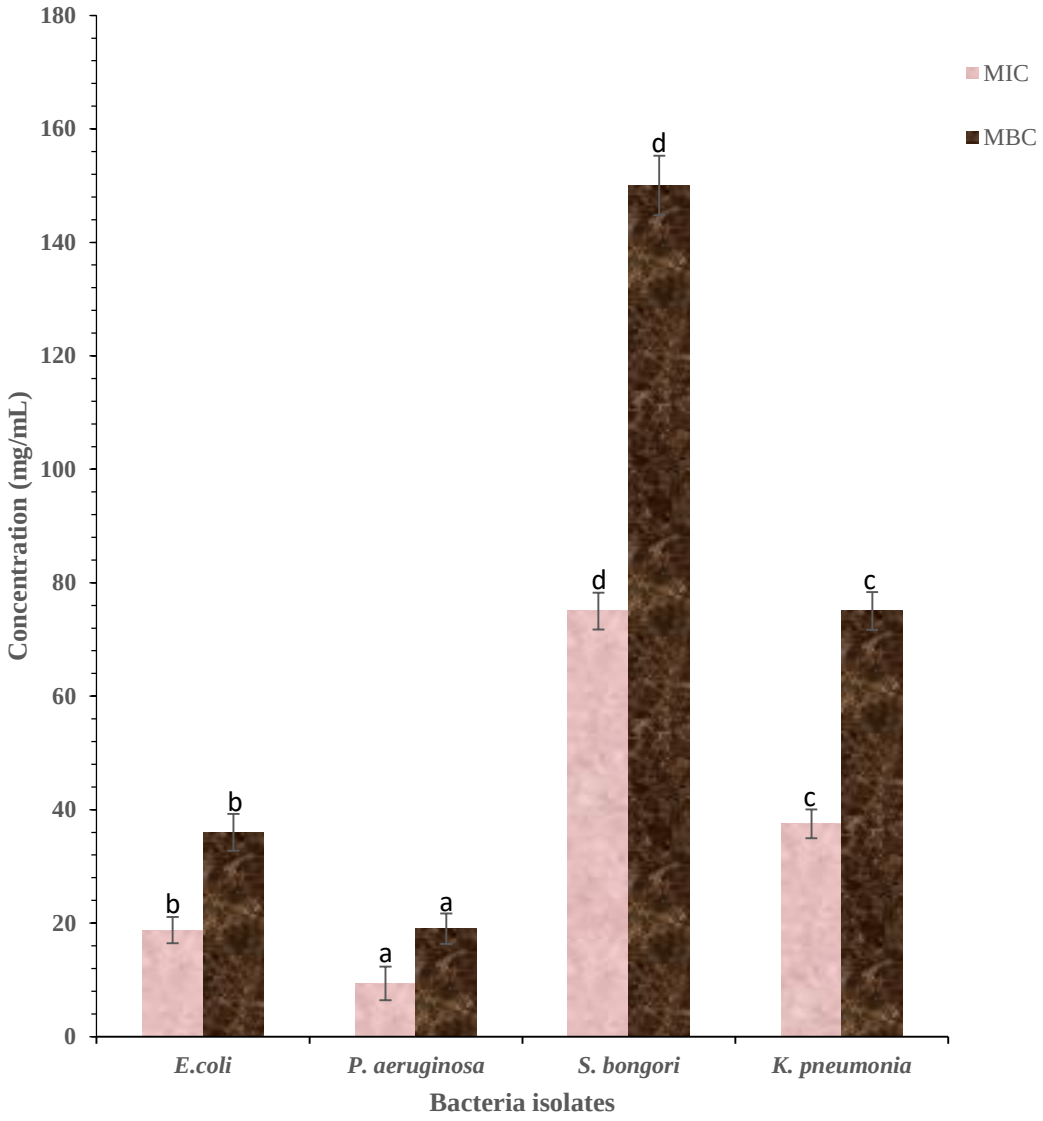

Figure 4.8: Minimum inhibitory concentration and Minimum Bactericidal concentration of ethyl acetate fraction of crude methanol extract of *L. hastata*

NB: Bars with the same color and the same alphabet have no significant difference at $p<0.05$

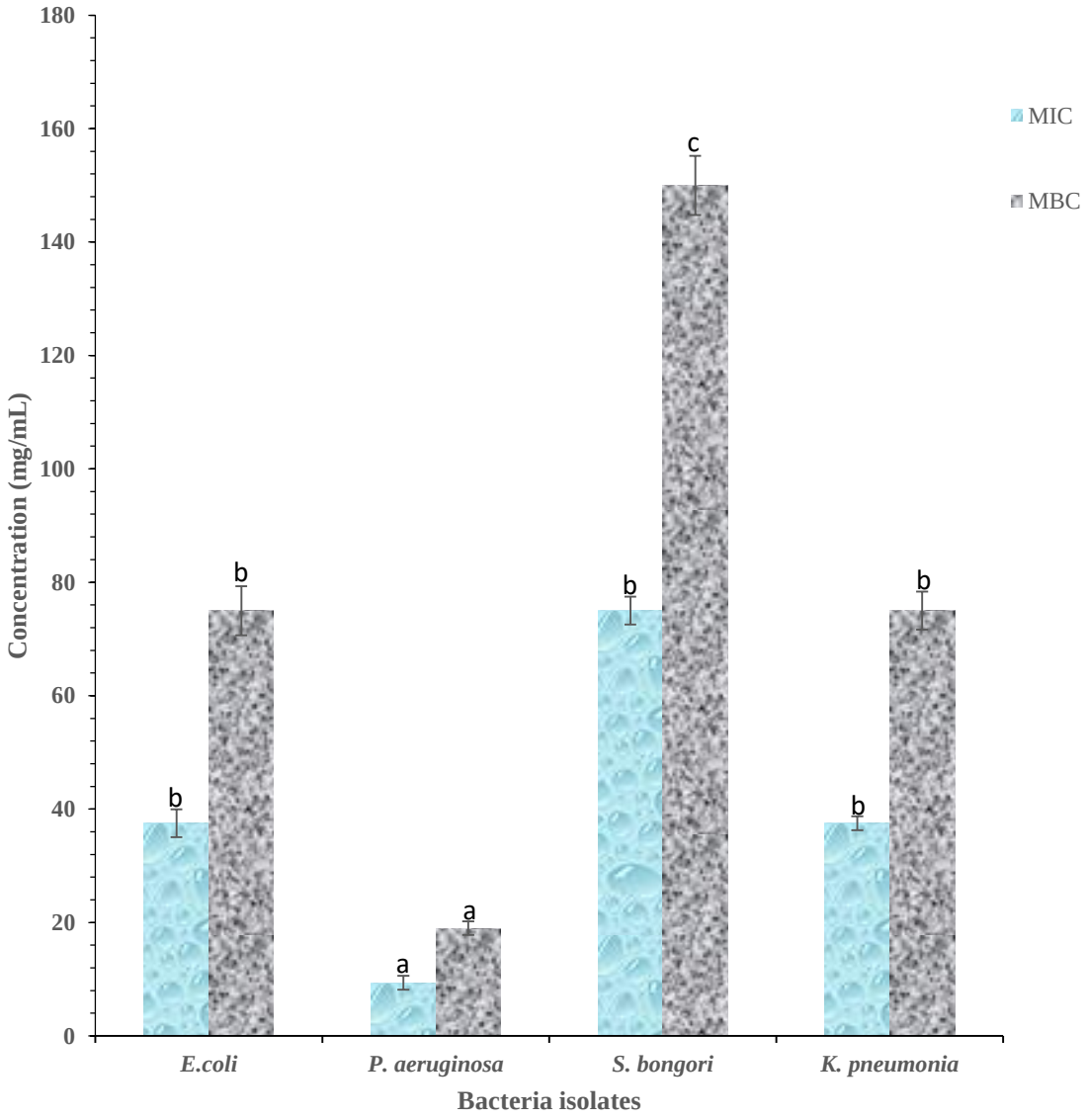

Figure 4.9: Minimum inhibitory concentration and Minimum Bactericidal concentration of residual aqueous fraction of crude methanol extract of *L. hastata*

NB: Bars with the same color and the same alphabet have no significant difference at $p<0.05$

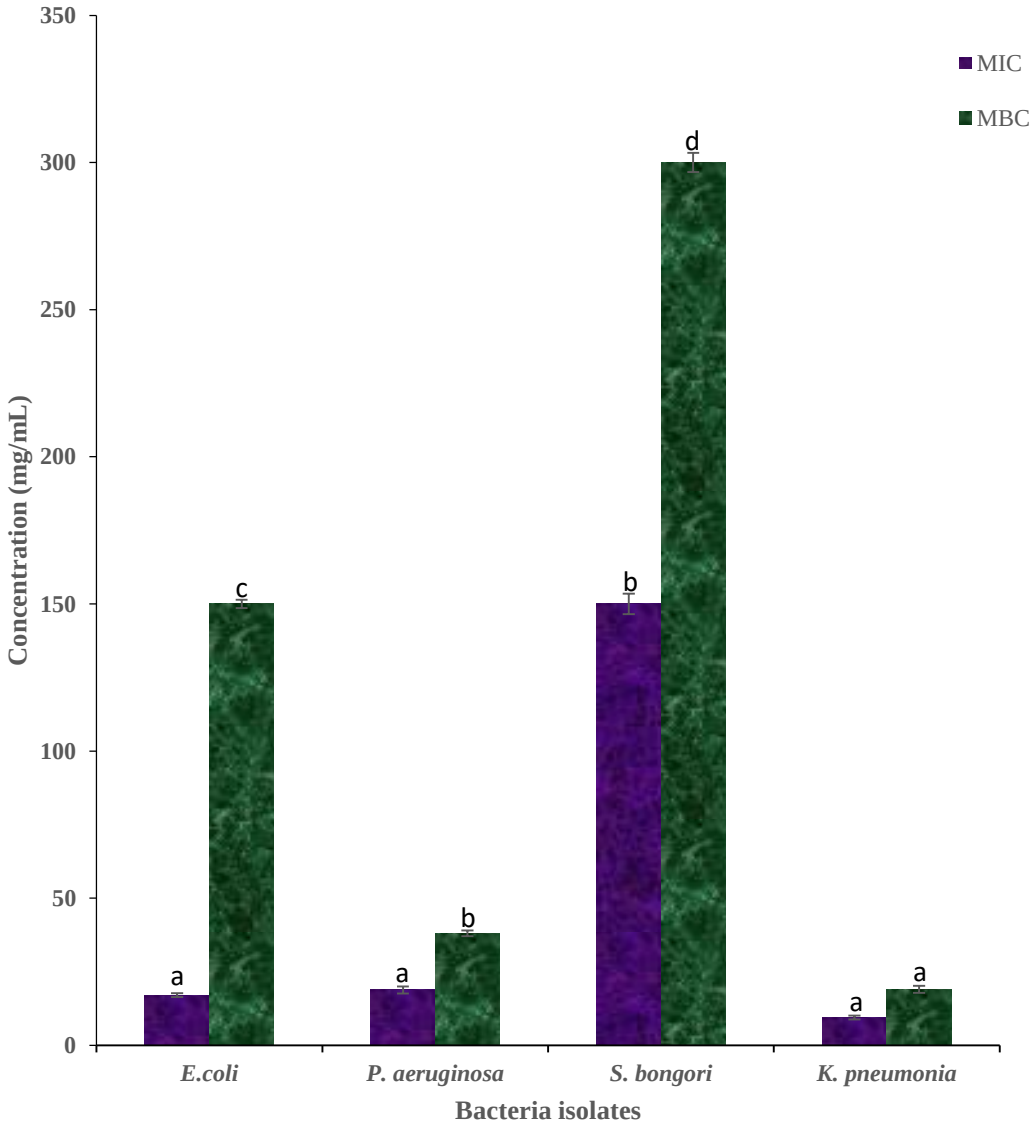

Figure 4.10: Minimum inhibitory concentration and Minimum Bactericidal concentration of ethyl acetate fraction of crude methanol extract of *S. longipedunculata*

NB: Bars with the same color and the same alphabet have no significant difference at $p<0.05$

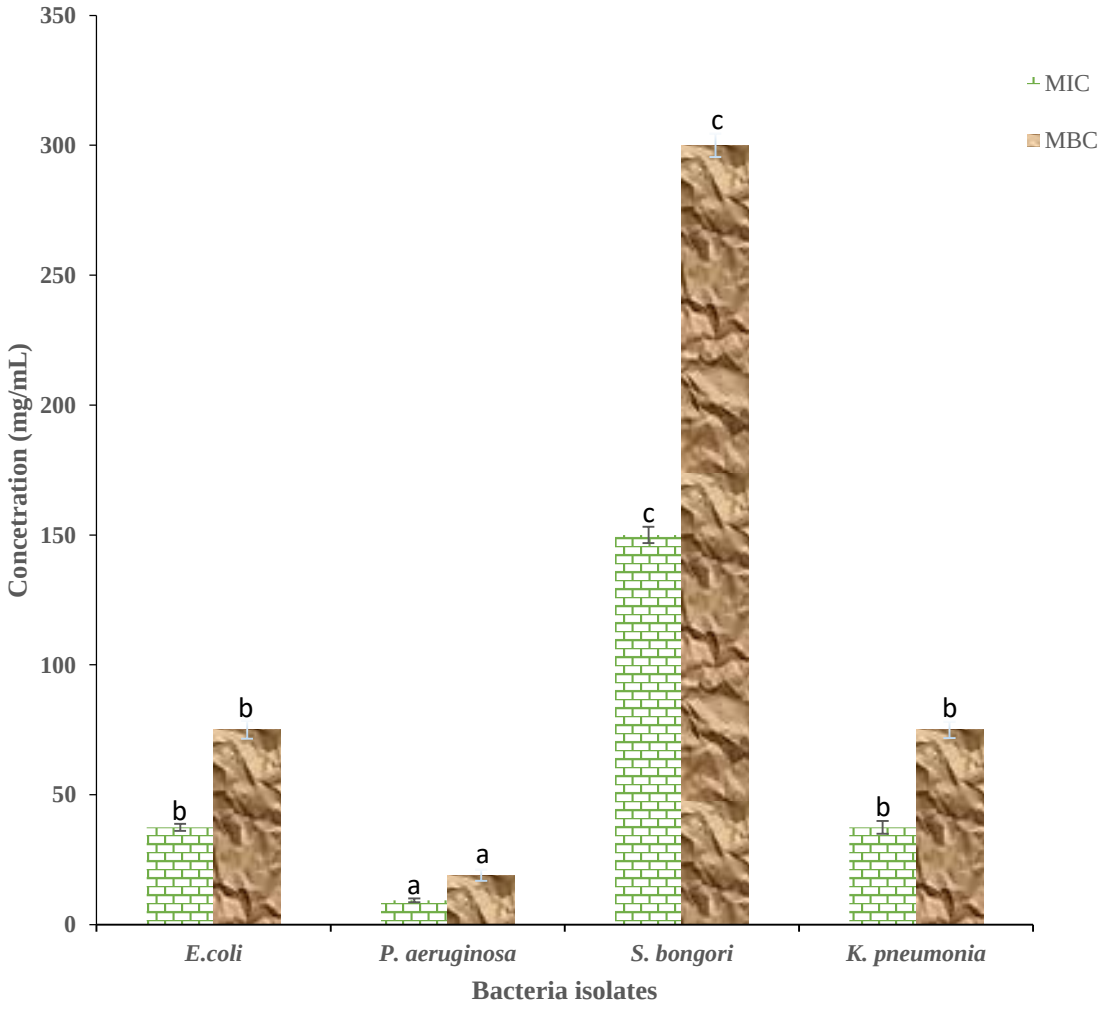

Figure 4.11: Minimum inhibitory concentration and Minimum Bactericidal concentration of residual aqueous fraction of crude methanol extract of *S. longipedunculata*

NB: Bars with the same color and the same alphabet have no significant difference at $p<0.05$

Table 4.12: GC-MS profile of sub-fraction extract from methanolic extract of *Calotropis procera* leaves

P-N	R-T	Compound names	M-F	M-W	% Conc.
		Ethyl acetate fraction			
5	16.118	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280	18.56
7	16.332	9-Octadecenamide, (Z)-	$C_{18}H_{35}NO$	281	28.71
11	18.732	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester	$C_{19}H_{38}O_4$	330	5.78
16	19.992	9,12-Octadecadienoic acid (Z,Z)-, 2,3- dihydroxypropyl ester	$C_{21}H_{38}O_4$	354	13.13
17	20.017	Propyleneglycol monoleate	$C_{21}H_{40}O_3$	340	4.99
		Residual aqueous fraction			
12	9.705	Glycerin	$C_3H_8O_3$	92	56.78
13	11.233	Propanal, 2, 3-dihydroxy-, (S)-	$C_3H_6O_3$	90	8.11
16	13.324	Nonanoic acid	$C_9H_{18}O_2$	158	5.58
20	16.113	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280	6.24
25	19.998	9,12-Octadecadienoic acid (Z,Z)-, 2,3-dihydroxypropyl	$C_{21}H_{38}O_4$	354	7.00
		n-Hexane fraction			
22	15.171	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284	13.11
23	15.404	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	11.53
25	15.813	9,9-Dimethoxybicyclo[3.3.1] nona-2,4-dione	$C_{11}H_{16}O_4$	212	7.37
26	16.025	Phytol	$C_{20}H_{40}O$	296	8.02

Table 4.12 cont.

27	16.278	Cyclopropaneoctanoic acid, 2-[[2-[(2- ethylcyclopropyl) methyl] cyclopropyl]	$C_{22}H_{38}O_2$	334	7.71
28	16.533	Dichloroacetic acid, tridec-2-ynyl ester	$C_{15}H_{24}Cl_2O_2$	306	5.29

P-K= Peak Number, R-T= Retention Time, M.F= Molecular formula, M-W= Molecular Weight, % Conc. = Percentage Concentration,

Table 4.13: GC-MS profile of sub-fraction extract from methanolic extract of *Leptidenia hastata* stem

P-N	R-T	Compound names	M-F	M-W	% Conc.
		Ethyl acetate fraction			
12	14.609	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	6.75
13	14.744	Phthalic acid, butyl hexyl ester	$C_{18}H_{26}O_4$	306	5.87
14	14.959	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	7.87
19	15.753	9,12-Octadecadienoic acid, methyl ester	$C_{19}H_{34}O_2$	294	5.03
22	16.145	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280	15.75
23	16.349	Octadecanamide	$C_{18}H_{37}NO$	283	6.25
30	18.742	Hexadecanoic acid, 2-hydroxy--(hydroxymethyl) ethyl ester	$C_{19}H_{38}O_4$	330	4.34
35	20.029	9,12-Octadecadienoic acid (Z,Z)-, 2,3-dihydroxypropyl Ester	$C_{21}H_{38}O_4$	354	18.70
		Residual aqueous fraction			
7	7.037	2,5-Dimethyl-4-hydroxy-3(2H)- furanone	$C_6H_8O_3$	128	5.84
10	7.803	4H-Pyran-4-one, 2,3-dihydro-3, 5-dihydroxy-6-methyl-	$C_6H_8O_4$	144	6.86

Table 4.13cont.

12	8.495	o-Tolylamino-acetic acid (4-nitro-benzylidene)–hydrazide	$C_{16}H_{16}N_4O_3$	312	6.37
16	11.638	3-tert-Butyl-4-hydroxyanisole	$C_{11}H_{16}O_2$	180	7.23
19	13.665	1,5-Anhydro-d-mannitol	$C_6H_{12}O_5$	164	25.08
21	14.880	n-Hexadecanoic acid	$C_{16}H_{32}O$	256	17.48
23	16.064	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	289	4.19

P-K= Peak Number, R-T= Retention Time, M.F= Molecular formula, M-W= Molecular Weight, % Conc.= Percentage Concentration

Table 4.14: GC-MS profile of sub-fraction extract from methanolic extract of *Sacuridica longipedunculata* stem

P-N	R-T	Compound names	M-F	M-W	% Conc.
		Ethyl acetate fraction			
23	13.124	4-((1E)-3-Hydroxy-1propenyl)-2-Methoxyphenol	$C_{10}H_{12}O_3$	180	4.20
32	14.752	1,2Benzenedicarboxylic acid, butyl octyl ester	$C_{20}H_{30}O_4$	334	9.30
33	15.021	n-Hexadecanoic acid-(2-nitro-2-heptenyl)-	$C_{16}H_{32}O_2$	256	15.05
39	16.181	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280	11.99
40	16.223	9-Octadecenoic acid, (E)-	$C_{18}H_{34}O_2$	282	5.43
45	19.997	9,12-Octadecadienoic acid (Z,Z)-, 2,3-dihydroxypropyl ester	$C_{21}H_{38}O_4$	354	6.11
		Residual aqueous fraction			
4	7.848	Erythritol	$C_4H_{10}O_4$	122	3.32
13	15.933	1,5-Anhydro-d- mannitol	$C_6H_{12}O_5$	164	93.10

P-K= Peak Number, R-T= Retention Time, M.F= Molecular formula, M-W= Molecular Weight, % Conc.= Percentage Concentratio

Table4.15: Quantitative phytochemical contents (mg/100g) of plant extracts.

Extracts	Flavonoids	Phenol	Tannins	Alkaloids	Saponins
SLS	49.820.63	201.62±1.98	114.25±3.71	655.56±3.05	160.20±1.80
LHS	80.34±6.11	372.98±0.99	ND	884.25±128.06	787.00±26.20
CP	62.21±3.56	509.68±10.26	87.75±1.25	380.92±12.66	35.28±0.50

Keys: SLS = Stem extract of *Securidaca longipedunculata,* LHS = Stem of *Leptidenia hastata,* CP = *Calotropis Procera,* ND= Not Detected

4.2 Discussion

4.2.1 The identity and characteristics of the isolates

The biochemical and molecular characterization of the bacteria isolates were carried out to confirm the true identity of the isolates. The DNA sequencing and blasting confirmed the isolates to be *Pseudomonas aeruginosa, Salmonella bongori Escherichia coli* and *Klebsiella pneumoniae* with accession number: MK572634.1, MN623691.1, CP074120.1 and AB642255.1 respectively. The phylogenetic tree of the bacteria isolates were also plotted to determine the source and the relationships among the organisms. All the isolates have a common origin showing common ancestry.

4.2.2 The antibacterial activities of the test plants

The three plants used in this studies: *Calotropis procera, Leptidenia hastata* and *Securidica longipedunculata* were selected based on their traditional usage in treating urinary tract infection, diarrhoea and wound infections. As extraction of plant showcase the biological active compounds, it also leads to fractionation and monitoring of their antimicrobial activity which may leads to the isolation and structural elucidation of the most potent compounds.

The study was aim at evaluating the *in vitro* antibacterial activity of *Calotropis procera* leaf, *Leptidenia hastata* and *Sacuridaca longipedunculata* stem. The percentage yield of the plant material extracted exhaustively with methanol was 7 % for *Calotropis procera* leaves, 6 % for *Securidaca longipedunculata* stem and 5.6 % for *Leptidenia hastata* stem, this agrees with the review and research of Abubakar *et al.* (2019) and Bello *et al.* (2011) who states that the yield of plant is affected by the nature and composition of the plant material that is

soluble in the solvent. Although extracting solvent contribute greatly to plants yields, as Kawo *et al.* (2009) reported, polar solvents tends to gives higher yield of active plant components than non-polar solvents.

The isolates used in determining the antibacterial activity of the extracts includes; *Escherichia coli, Pseudomonas aeruginosa, Salmonella bongori and Klebsiella pneumoniae*, all the isolates are gotten from hospital samples which includes urine, stool and wound swab. The antibacterial efficacy of the extracts and fractions were judged on the basis of their zones of inhibition of bacterial growth of the test organisms. The crude extracts a lower concentration (15 and 25 mg/ mL) were unable to inhibit the growth of the bacteria isolates, and this may be due to the percentage concentration of the plants phyto-components been too small to exert effect on the organisms.

For *C. procera* leaf and *L. hastata* stem at a concentration between 50-300mg/ mL exert effect on the growth of *E. coli* and *P. aeruginosa* with a zone of inhibition ranging from 8.00±0.30 – 19.60±0.20mm and 6.70±0.30-17.65±0.15 mm respectively. While *S. bongori* and *K. pneumoniae* (100-300mg/ mL) had a narrow zones of inhibition of 3.60±0.20 - 13.50±0.50mm and 4.35±0.25- 21.55±0.65mm.

On the other hand, *S. longipedunculata* extract could not inhibit the growth of *P. aeruginosa* and *S. bongori* at all the concentration rather an inhibitory activity of 2.70±0.70-13.15±0.15 mm is seen against *K. pneumoniae* and *E. coli* at 100-300mg/ mL concentration. The observed variation in the zones of inhibition by the extract across the bacteria isolates may be attributed the concentration of the phyto-constituents present in the extracts (Innalegwu *et al.*, 2021). Also, diffusion rate of the extract on the petri dish could also be a factor that maybe responsible for the observed level of activity as antibacterial agents with

high diffusion rates maybe more active (Ewansiha, 2020). The report of this study agrees with the findings of Hayat *et al.* (2020), who reported on the antibacterial activity of methanol extract of *C. procera* leaves against wide range of pathogenic organisms including those reported in this study. Akindele *et al.* (2017) also reported that extract of *C. procera* leaves shows better activity at higher concentration than at lower concentration when determining the activity of extract against vancomycin and methicillin resistant bacteria isolated from wound samples in hospital patients. Fraction of the extracts shows a narrow zone of inhibitions across all the organisms even at lower concentration of the fractions (15 mg/ mL, 25 mg/ mL, 50 mg/ mL and 100mg/ mL). The mean zones of inhibition of the isolates are a function of the relative antibacterial activity of the extracts. The zone of inhibition is the area that remains free of bacterial growth and the sizes of the zones of inhibition are usually related to the susceptibility of the extract (Innalegwu *et al.*, 2021).

Some of the fractions from the plant extract shows an improved activity against the isolates although depends on the solvent polarity. N-hexane fraction of *Calotropis procera* leaf shows an activity of 5.00±0.00-13.10±0.10 mm, ethyl acetate shows activity of 7.10±0.10-15.40±0.10 mm and residual aqueous with inhibition zone of 8.20±0.20-17.25±0.25 mm against the tested isolates all within the concentration range of 150-300mg/ mL. The findings of this study agrees with the report of Ali *et al.* (2014), who reported a good activity against *Salmonella typhi* and *Escherichia coli*. The residual aqueous fraction has significant activity against *Escherichia coli* and *Pseudomonas aeruginosa* at the concentration between 150-300 mg/mL and also against *Salmonella bongori* and *Klebsella pneumoniae* at 250 to 300 mg/ mL.

Ethyl acetate fractions obtained from of *Leptidenia hastata* show to exert effect against *Escherichia coli, Pseudomonas aeruginosa* and *Klebsella pneumoniae* ranging from 9.35±0.35-25.80±0.20 mm across the concentration of 150 to 300 mg/ mL, although for *Salmonella bongori* an inhibition zone of 8.75±0.45-10.40±0.90 mm the concentration of 150-300 mg/ mL. The activity observed from the residual aqueous ranges between 4.80±0.20-20.30±1.30 mm against all the tested isolates at the concentration of 150-300 mg/ mL. When compared with the crud extract, spectrum of activity shows an improvement against the isolates. As repoted by Abubakar *et al.* (2014), extract of *Leptidenia hastata* possess good antibacterial potentials with a broad spectrum of activity at 100 mg/ mL, 200 mg// mL and 300 mg/ mL against *Klebsella pneumoniae* and *Escherichia coli*. Imam *et al.* (2019) also reported a good spectrum of activity by the extract of *L. hastata* even at a lower concentration of 50mg/ mL. This variation in this study maybe as due to the type of solvent used, method of extraction and even the season the plant was collected. Ethyl acetate and residual aqueous fraction *L. hastata* also shows a significant activity *Escherichia coli* and *P. aeruginosa* while the fractions only inhibited *Salmonella* at 300 mg/ mL.

Ethyl acetate fraction of *Securidaca longipedunculata* stem extract shows a narrow zone of inhibition ranging from 1.30±1.30-19.90±0.10 mm against the tested isolates at a concentration of 150-300mg/ mL and an activity ranging from 1.70±1.70-20.00±0.40 mm was recorded from the residual aqueous fraction against the tested organisms. This finding disagrees with the report of Abubakar *et al.* (2018), who reported that the stem bark extract has activity against *E. coli, P. aeruginosa, S. typhi and K. pneumoniae* at lower concentration of 20 mg/ mL.

4.2.3 The MIC and MBC of the test organisms

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of a drug reveals the potency level of the drug i.e. the lower the MIC the more potent the drug. The MIC can be a guide on the choice of antimicrobial drugs used in treatment by predicting efficacy (Ewansiha *et al.,* 2020). If pharmacokinetic and pharmacodynamics (PKPD) principles are observed by careful selection of a particular antimicrobial drug administered at an appropriate dosage, it will lead to clinical cure, eradication of carrier status of a system, and prevention of resistance. The MIC and MBC of the crude extracts shows that all the extract recorded lower MIC and MBC values ranging between 17-300 mg/ mL against the bacteria isolates. Further purification of the extracts by subjecting it to partition fraction shows a decrease in the MIC and MBC values ranging between 9.38-300 mg/ mL. This could be true owing to the fact that standard drugs like the quinolones and aminoglycosides exert remarkable activity due to their pure nature and whose efficacy depends on concentration; whereas, the efficacy of some other drugs are time-dependent such as the beta-lactams (Ewansiha *et al.,* 2020). Purification of plant extract through various means such partitioning, column chromatography, high performance liquid chromatography etc have been used to determine the active compounds present in pant extract (Parvekar *et al.,* 2020). It has also been suggested that purification could lead to an increase or decrease in the activity of a given plant extract (Doughari, 2012). This is because some of the active component in plant extract act in a synergistically or antagonistic means hence a resolution of the components in the extract could lead to a decrease or and an increase in the level of activity (Parvekar 2020).

4.2.5 The findings from the GC-MS fractions of plant extract

Gas Chromatography Mass Spectrophotometric analysis of fractions obtained from the plant crude extracts revealed the presence of different phyto-constituents in various amount. The result analyzed from ethyle acetate fraction of *Calotropis procera* with high concentration ratio were five compounds, this includes 9,12-Octadecadienoic acid (Z,Z)- (18.56 %), 9-Octadecenamide, (Z)- (28.71 %), Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester (5.78 %), 9,12-Octadecadienoic acid (Z,Z)-, 2,3- dihydroxypropyl ester (13.13 %) and Propyleneglycol monoleate (4.99 %).

9,12-Octadecadienoic acid (Z,Z)- have been reported to be used in the treatment of atherosclerosis and hyperlipoidemia, anti-inflammatory, hypocholesterolemic, cancer preventive, hepatoprotective, nematicide, insectifuge(cide), antihistaminic, antieczemic, antiacne, 5-α reductase inhibitor, antiandrogenic, antiarthritic, anti coronary, antimicrobial (Adeoye-Isijola, *et al.,* 2018). An amide from oleic fatty acid called 9-Octadecenamide, (Z)- ($C_{18}H_{35}NO$) is also detected in high amount (28.71 %). This compound is naturally occurring metabolite of oleic acid and it has antibacterial activity as reported by Goraksh *et al.* (2017). It can also can be used in treating cannabinoid regulated depression, sleeping disorder and reducing psychological excitement or anxiety (Davic and Cascio, 2021). 2-palmitoylglcerol also known as Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester ($C_{19}H_{38}O_4$) was also found at a concentration of 5.78 %. it is a natural product of plant and algae metabolite which has various biological activity such as antioxidant, hypocholesterolemic, hemolytic 5-α reductase inhibitor, Intestinal histamine release inhibitor, pesticide, nematicide, and anitandrogenic as described by Adnan *et al.* (2019) and Laitha *et al.* (2015).

Propyleneglycol monoleate also known as Glycidil stearate ($C_{21}H_{40}O_{3)}$ was found at a concentration of 4.99 in the ethyle actate fraction of *Calotropis procera* leaves but it has no known record of biological activity.

In the residual aqueous fraction of *Calotropis procera* five compounds were also analyzed these are; Glycerin (56.78 %), Propanal, 2,3-dihydroxy-,(S)- (8.11 %), Nonanoic acid (5.58 %), 9,12-Octadecadienoic acid (Z,Z)- (6.24 %), and 9,12-Octadecadienoic acid (Z,Z)-, 2,3-dihydroxypropyl (7.00 %). Glycerin also known as Glycerols ($C_3H_8O_3$) is an intermediate in carbohydrate and lipid metabolism because excess carbohydrate is converted into long chain fatty acid which is esterified with three hydroxyl group in biological system. It interferes with immune reaction through histamines, and also enhances immune cells activity by increasing antibody production which is also known as allergen, this qualifies it to possess some antiviral properties as reported by Jiang *et al.* (2015) and antibacterial activity (Schlivert *et al.,* 2012). Above all this, the United States Food and Drug Administration (FDA) approved the use of this compound in treatment of wound and burnt injuries. Propanal, 2,3-dihydroxy-,(S)- ($C_3H_6O_{3)}$ also called glyceraldehyde is a monosaccharide sugar that is the simplest of all the aldoses and it is an intermediate compound in carbohydrate metabolism. It was reported to have antioxidant, antimicrobial, hypochloesterolemic (Raseem *et al.,* 2017) and also anticancer activity (Bakchiche, *et al.,* 2020; Elkhateeb *et al.,* 2020).

Nonanoic acid also known as pelargonic acid ($C_9H_{18}O_2$) is a nine-carbon saturated fatty acid that is seen in at extract at the concentration of 5.58 %. It was reported to be more potent than valproic acid in seizure treatment (Chang, *et al.,* 2013). Furthermore, this compound is said to have antifungal activity (Matejic *et al.,* 2018) and antibacterial activity (Akpuaka *et*

al., 2013; Sahin *et al.,* 2006). 9,12-Octadecadienoic acid (Z,Z)- ($C_{18}H_{32}O_2$) is one of the most abundant polyunsaturated fatty acid in human nutrition as said by it is important in treatment of atherosclerosis and hyperlipoidemia, Anti-inflammatory, hypocholesterolemic, cancer preventive, hepatoprotective, nematicide, insectifuge(cide), antihistaminic, antieczemic, antiacne, 5-α reductase inhibitor, antiandrogenic, antiarthritic, anti-coronary, antimicrobial (Adeoye-Isijola, *et al.,* 2018). 9,12-Octadecadienoic acid (Z,Z)-, 2,3-dihydroxypropyl ester also known as 2-linoleoylglycerol ($C_{21}H_{38}O_{4)}$ was detected and this compound has no or limited record of biological activity rather an industrial usage as emollient and emulsifying agent (Varadharaj and Kuppan 2015; Huang *et al.,* 2009). Although MOLBASE (chemical E-commerce platform) mentioned the compound to be used as amoebic control agent and metabolic biomarker in hepatotoxicant response.

In the n-Hexane fraction of *Calotropis procera*, six compounds were analyzed namely; Hexadecanoic acid, ethyl ester (13.11 %), n-Hexadecanoic acid (11.53 %), 9,9-Dimethoxybicyclo[3.3.1] nona-2,4-dione (7.37%), Phytol (8.02 %), Cyclopropaneoctanoic acid, 2-[[2- [(2- ethylcyclopropyl) methyl] cyclopropyl] (7.71 %) and Dichloroacetic acid, tridec-2-ynyl ester (5.29 %).

Hexadecanoic acid, ethyl ester known as stearic acid ($C_{18}H_{36}O_2$) is a saturated fatty acid known to have antibacterial, antifungal and cancer preventive activities as reported by (Elkhateeb *et al.,* 2020). n-Hexadecanoic acid also known as palmitic acid ($C_{16}H_{32}O_2$) is also a saturated fatty acid that is known to have antioxidant, nematicide, Hypo-cholestrolemic, 5-α reductase inhibitor, anti-inflammatory, antitumor, immunostimulant, chemo-preventive and lipo-oxygenase inhibitor (Adeoye-Isijola, *et al.,* 2018; Malik *et al.,* 2016). 9,9-Dimethoxybicyclo [3.3.1] nona- 2,4-dione ($C_{11}H_{16}O_4$) is a bicyclic organic

molecule with no known biological activity but has industrial uses particularly in the construction industries. Phytol is also called florasol ($C_{20}H_{40}O$) is the main precursor in the industrial synthesis of vitamin E and K1 and its biological activity includes; Anxiolytic, antimicrobial, cytotoxic, antioxidant autophagy activity, anti-nociceptive, and anti-inflammatory effect, immune and metabolism modulation (Babu *et al.,* 2017; Islam *et al.,* 2018).

Cyclopropaneoctanoic acid, 2-[[2- [(2-ethylcyclopropyl) methyl] cyclopropyl ($C_{22}H_{38}O_2$) is a decosatrienoic long chain omega-3 fatty acid is said to have antimicrobial, cancer preventive, hypocholestromic and hepatoprotective activity (Devakumar *et al.,* 2017). Dichloroacetic acid, tridec-2-ynyl ester ($C_{15}H_{24}C_{l2}O_2$) can be used in treating mastitis (Dinesh *et al.,* 2016). Though biological activity of this compound is not fully explored.

The ethyl acetate of sub- fractions of *Leptidenia hastata* consist of eight compounds and these includes; Hexadecanoic acid, methyl ester (6.75 %), Phthalic acid, butyl hexyl ester (5.87 %), n-Hexadecanoic acid (7.87 %), 9,12-Octadecadienoic acid methyl ester (5.03 %), 9,12-Octadecadienoic acid (Z,Z)- (15.75 %), Octadecanamide (6.25 %), Hexadecanoic acid, 2-hydroxy-(hydroxymethyl) ethyl ester (4.34 %) and 9,12-Octadecadienoic acid (Z,Z)-, 2,3-dihydroxypropyl Ester (18.70 %).

Hexadecanoic acid, methyl ester also refers as margaric acid ($C_{17}H_{34}O_2$) is a saturated fatty acid ester of essential oil that occurs in plant naturally. It is reported to have antioxidant, nematicide, Hypocholestrolemic, hemolytic 5-α reductase inhibitor and antiandrogenic activity (Easwaran and Ramani, 2014). Phthalic acid, butyl hexyl ester ($C_{18}H_{26}O_4$) is said to have antimicrobial activity (Ingole, 2016). n-Hexadecanoic acid also known as palmitic acid ($C_{16}H_{32}O_2$) is a saturated fatty acid that is known to have antioxidant, nematicide,

Hypocholestrolemic, 5-α reductase inhibitor, anti-inflammatory, antitumor, immunostimulant, chemopreventive and lipo-oxygenase inhibitor (Malik *et al.,* 2016; Adeoye-Isijola *et al.,* 2018). 9,12-Octadecadienoic acid, methyl ester ($C_{19}H_{34}O_2$) is a methyl ester fatty acid of linoleic acid is reported to have anticancer, anti-leukotriene and anti-inflammatory activity (Abubakar and Majinda, 2016).

9,12-Octadecadienoic acid (Z,Z)- ($C_{18}H_{32}O_{2)}$ It is one of the most abundant polyunsaturated fatty acid in human nutrition as said by it is important in treatment of atherosclerosis and hyperlipoidemia, Anti-inflammatory, hypocholesterolemic, cancer preventive, hepatoprotective, nematicide, insectifuge(cide), antihistaminic, antieczemic, antiacne, 5-α reductase inhibitor, antiandrogenic, antiarthritic, anti-coronary, antimicrobial (Adeoye-Isijola *et al.,* 2018). Octadecanamide ($C_{18}H_{37}NO$) is a fatty acid amide of stearic acid, although though it has no known record of biological activity. Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester ($C_{19}H_{38}O_4$) is a natural product of plant and algae metabolism which has various biological activity such as antioxidant, hypocholesterolemic, hemolytic 5-α reductase inhibitor, Intestinal histamine release inhibitor, pesticide, nematicide, and anitandrogenic as described by Adnan *et al.* (2019); Laitha *et al.* (2015). 9,12-Octadecadienoic acid (Z,Z)-, 2,3- dihydroxypropyl ester also known as 2-linoleoylglycerol ($C_{21}H_{38}O_4$) has no or limited record of biological activity rather an industrial usage as emollient and emulsifying agent (Varadharaj and Kuppan 2015; Huang *et al.,* 2009). Although MOLBASE (chemical E-commerce platform) mentioned the compound to be used as amoebic control agent and metabolic biomarker in hepatotoxicant response.

The residual aqueous fractions shows the present of seven compounds of high concentration and these includes; 2,5-Dimethyl-4-hydroxy- 3(2H)- furanone (5.84 %), 4H-Pyran-4-one, 2,3- dihydro-3, 5-dihydroxy-6-methyl- (6.86 %), o-Tolylamino-acetic acid(4-nitro-benzylidene) –hydrazide(6.37 %), 3-tert-Butyl-4-hydroxyanisole (7.23 %), 1,5-Anhydro-d-mannitol (25.08 %), n-Hexadecanoic acid (17.48 %) and 9,12-Octadecadienoic acid (Z,Z)-(4.19 %).

2,5-Dimethyl-4-hydroxy- 3(2H)- furanone ($C_6H_8O_3$) is a furaneol compound with inconclusive biological activities as researches are still in progress. 4H-Pyran-4-one, 2,3-dihydro-3, 5-dihydroxy-6-methyl- ($C_6H_8O_4$) was reported to have antioxidant activity by Malik *et al.* (2016) and antimicrobial activity (Verdini *et al.,* 2020). o-Tolylamino-acetic acid (4-nitro-benzylidene) –hydrazide ($C_{16}H_{16}N_4O_3$) and 3-tert-Butyl-4-hydroxyanisole ($C_{11}H_{16}O_2$) have no record of biological activities rather it has industrial activity as flavor. 1,5-Anhydro-d-mannitol ($C_6H_{12}O_5$) is a sugar alcohol that is reported to have anticancer activity by Alagammal *et al.* (2012) and antibacterial activity (Vambe *et al.,* 2020). n-Hexadecanoic acid also known as palmitic acid ($C_{16}H_{32}O_2$) is a saturated fatty acid that is known to have antioxidant, nematicide, Hypocholestrolemic, 5-α reductase inhibitor, anti-inflammatory, antitumor, immunostimulant, chemopreventive and lipo-oxygenase inhibitor (Adeoye-Isijola, *et al.,* 2018; Malik *et al.,* 2016). 9,12-Octadecadienoic acid (Z,Z)-($C_{18}H_{32}O_2$) is one of the most abundant polyunsaturated fatty acid in human nutrition as said by it is important in treatment of atherosclerosis and hyperlipoidemia, Anti-inflammatory, hypocholesterolemic, cancer preventive, hepatoprotective, nematicide, insectifuge(cide), antihistaminic, antieczemic, antiacne, 5-α reductase inhibitor, antiandrogenic, antiarthritic, anti coronary, antimicrobial (Adeoye-Isijola *et al.*, 2018).

The most abundant compounds found in the ethyl acetate sub- feaction of *Sacuridicat longipedunculata* were six namely; 4-((1E)-3-Hydroxy- 1-propenyl)-2- Methoxyphenol (4.20 %), 1,2-Benzenedicarboxylic acid, butyl octyl ester (9.30 %), n-Hexadecanoic acid-(2-nitro-2-heptenyl)- (15.05 %), 9,12-Octadecadienoic acid (Z,Z)- (11.99 %), 9-Octadecenoic acid, (E)- (5.43 %), and 9,12-Octadecadienoic acid (Z,Z)-, 2,3-dihydroxypropyl ester (6.11 %).

4-((1E)-3-Hydroxy- 1-propenyl)-2- Methoxyphenol ($C_{10}H_{12}O_3$), a phenolic compound that is reported to have antimicrobial, antioxidant and anti-inflammatory activities (Mathulakshimi *et al.*, 2012). 1,2-Benzenedicarboxylic acid, butyl octyl ester ($C_{20}H_{30}O_4$) is said to have antimicrobial and antifouling activity (Khalil *et al.*, 2014; Lakshmi and Rajalakshmi, 2011). n-Hexadecanoic acid also known as palmitic acid ($C_{16}H_{32}O_2$) is a saturated fatty acid that is known to have antioxidant, nematicide, Hypocholestrolemic, 5-α reductase inhibitor, anti-inflammatory, antitumor, immunostimulant, chemopreventive and lipo-oxygenase inhibitor (Malik *et al.*, 2016; Adeoye-Isijola, *et al.*, 2018). 9,12-Octadecadienoic acid (Z,Z)-($C_{18}H_{32}O_{2)}$ is one of the most abundant polyunsaturated fatty acid in human nutrition as said by it is important in treatment of atherosclerosis and hyperlipoidemia, Anti-inflammatory, hypocholesterolemic, cancer preventive, hepatoprotective, nematicide, insectifuge(cide), antihistaminic, antieczemic, antiacne, 5-α reductase inhibitor, antiandrogenic, antiarthritic, anti coronary, antimicrobial (Adeoye-Isijola, *et al.*, 2018). 9-Octadecenoic acid, (E)-($C_{18}H_{34}O_2$) which is an oleic fatty acid which occurs naturally in plant and animals has an Antimicrobial, cancer preventive anti-inflammatory and anti-tumor activity (Padma *et al.*, 2018; Vijayakumari and Raj, 2019). 9,12-Octadecadienoic acid (Z,Z)-, 2,3-dihydroxypropyl ester also known as 2-linoleoylglycerol ($C_{21}H_{38}O_{4)}$ has no or limited

record of biological activity rather an industrial usage as emollient and emulsifying agent (Huang *et al.,* 2009; Varadharaj and Kuppan 2015). Although MOLBASE (chemical E-commerce platform) mentioned the compound to be used as amoebic control agent and metabolic biomarker in hepatotoxicant response. The residual aqueous sub-fractions contain two compounds of high concentration which are; Erythritol (3.32 %) and Anhydro-d-mannitol (93.10 %).

Erythritol ($C_4H_{10}O_4$) is a sugar alcohol that has no known record of biological activities whist 1,5-Anhydro-d-mannitol ($C_6H_{12}O_5$) is a sugar alcohol that is reported to have anticancer activity by Alagammal *et al.* (2012) and antibacterial activity (Vambe *et al.,* 2020).

The presence of all these compounds in the frations of the crude extracts maybe attributed to the observed level of antibacterial properties depicted in this study and these compounds can serve as an analogue for the synthesis of new drug.

4.2.6 The qualitative and quantitative phytochemical composition of plant extracts

The qualitative and quantitative phytochemical composition of *C. procera* leaves *L. hastata* and *S. longipedunculata* stem revealed the presence of various amounts of flavonoids, phenols, tannins Alkaloids and saponins, excepts tannins which is absent in *L. hastata*. Umaru *et al.* (2018) also reported the unavailability of tannins from leaf of *L. hastata* and this may be attributed to the season and sit of collection of collection also the solvent and extraction method used. The best extraction method for extracting bioactive compounds from plants is cold maceration or reflux method. (Altemimi *et al.,* 2017). In this studies, *L. hastata* was found to have the highest amount of the analyzed phytochemicals with 80.34±6.11, 372.98±0.99, 0.00±0.00, 884.25±128.06 and 787.00±26.20 followed by *C.*

procera 62.21±3.56, 509.68±10.26, 87.75±1.25, 380.92±12.66 and 35.28±0.50. But the least is *S. longipedunculata* with 49.820±63, 201.62±1.98, 114.25±3.71, 655.56±3.05 and 160.20±1.80 all for Flavonoids, Phenols, tannins, alkaloid and saponin respectively.

The presence of these phyto-components confers the therapeutics potentials of the plants as reported by Doughari (2012). Flavonoids act by disrupting the cytoplasmic membrane energy metabolism and inhibiting nucleic acid synthesis of the bacterial cell (Ahmad *et al.,* 2015). Phenols are reported to have destructive effect on the bacterial membrane and inhibition of virulence factors such as enzymes and toxins (Majdanik *et al.,* 2018). Farha *et al.* (2020), reveals tannins to have the ability to disrupt the bacterial cell wall synthesis and fatty acid biosynthetic pathways. Alkaloid are said to exert effect on the microbial cell by inhibiting the DNA synthesis (Matsuura and Fett-Nato 2015). On the other hand, saponins disrupt the cell wall, cytoplasmic membrane and membrane bound proteins which causes a leakage on the cell content. (Dong *et al.,* 2020).

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

The pathogenic bacterial isolates used in this research are *Klebsiella pneumonia, Salmonella bongori, Pseudomonas aeruginosa* and *Escherichia coli* which were confirmed phenotypically and molecularly. Also the plants materials extract tested aginst the pathogens are *Calotropis procera* leaf, *Leptidenia hastata* stem and *Securidaca longipedunculata* stem. The Extracts, showed antibacterial activity against the tested isolates, and also a low level of MIC and MBC. Patitioning the plants extracts showed some increased level of antibacterial activity with more portency in the MIC and MBC when compared to the crude. Furthermore, the compounds isolated using GC-MS proofs the plants to be effective in treatment of ailment of both microbial and non-microbial origin. Many of the major compounds isolated from the GC-MS analysis have several therapeutic and biological potentials and proof to be medicinally valuable by possessing various pharmaceutical applications. All these may be attributed to the concentration of phytochemicals constituent presents in the plants. These findings strongly suggest that toxicological analysis of the identified bioactive compounds from the plants extract would be of necessity to develop safe drugs.

5.2 Recommendations

Based on the result obtained from this study, the following are recommended:

i. There is need to evaluate and analyzed other parts of the plant materials which are not used in this study to determine their antibacterial activity. This is because the

phyto-constituents which are the sole determinant of the therapeutic efficacy of plants varies from one location to another within the plant.

ii. Solvent of different polarity and method of extraction different from the one used in the study should be employed for extraction to fully harness the phytochemical qualities of the plant.

iii. Extract of these plant should also be screen against other bacterial and fungal pathogens so as to validate the efficacy of these plants against different microbial pathogens.

iv. Further purification and isolations of active compounds from this plant extract is also recommended to determine their mechanisms of actions against these bacterial pathogens.

REFERENCES

Abbo, L. M., Hooton, T. M. (2014). Antimicrobial stewardship and urinary tract infections *Antibiotics,* 2, 174-192.

Abhay, K., Singh, N. & Kumar, P. (2019). Purification and characterizations of Anti-Microbial protein from *Calotropis procera. International Journal of Current Microbiology and Applied Sciences*, 8(8), 1754-1761.

Aboh, M.I., Oladosu, P., Adeshina, G.O., Olayinka, B.O., Olonitola, S. & Atasie, N.H. (2019). Screening of selected medicinal plants for their antifungal properties. *African Journal of Clinical and Experimental Microbiology,* 20(1), 54-58.

Abubakar U. S., Abdullahi, M. S., Abdulrazak, A., Fatima, A., Joseph, M., & Sagir, H. (2018). Toxicity studies and phytochemical screening of aqueous and ethyl acetate stem bark extracts of Acute *Securidaca longipedunculata* Fresen (Polygalaceae). *International Journal of Pharmacological Research,* 8(10), 104-107.

Abubakar U.S., Da nmalam, U.H., Ibrahim, H. & Maiha B.B. (20019). A review on African voilet tree (*Securidaca longipedunculata)* a traditional drug with multiple medicinal uses. *Science Arena Publications Speceilty Journal of Chemistry,* 4 (3), 7-12

Abubakar, A.R & Haque, M. (2020). Preparation of medicinal plants: basic extraction and fractionation procedures for experimental purpose. *Journal of pharmacy & Bioallied Sciences*, 12(1) 1-10.

Abubakar, N.M. & Majinda, T.R.R. (2016). GC-MS analysis and preliminary antimicrobial activity of *Albizia adianthifolia* (schumach) and *Pterocarpus angolensis* (DC). *Medicines,* 3(3), 1-9.

Abubakar, S., Usman, A.B, Ismail, I.Z, Aruwa, G., Azizat, S.G., Ogbadu, G.H. & Onyenekwe, P.C. (2014). Nutrional and pharmacological potentials of *Leptidenia hastate* (pers.*)* Decne. Ethanolic leaves extract. *Journal of Food and Nutritional Research*, 2(1), 51-55.

Abubakar, U. S., Abdullahi, M. S., Hadiza, R. J., Joseph, M., Binta, I. K., & Habiba, G. U. (2018). Antibacterial activity of *Securidaca longipedunculata* stem bark against some clinical bacterial isolates. *Journal of Pharmacognosy and Phytochemistry,* 7(6), 1967-1970.

Adejuwon, A. O., Tsygankova, V. A., Adeosun, A. M., Falase, A. O., Obayemi, O. S. & Amusa, F. I. (2019). Phytochemical screening and antimicrobial efficacy of the root bark of Securidaca longipedunculata extracts, *American Journal of Research in Medical Sciences,* 5(1), 7–13.

Adeoye-Isijola, O.M., Olajuyigbe, O.O., Jonathan, S.G., Coopoosamy, R.M. (2018). Bioactive Compounds in Ethanol Extract if *Lentinus Squarrosulus* Mont - A Nigerian Medicinal Macrofungus. *African Journal of Traditional Complementary and Alternative Medicine*, 15(2), 42-50.

Adiele, R.C., Fakae, B.B. & Isuzu, I.U. (2013). Anthelmintic activity of Securidaca longepedunculata (Family: Polygalaceae) root extract in mice, in vitro and in vivo. *Asian Pacific Journal of Tropical Medicine,* 841-846.

Adnan, M., Chy, N.U., Mostafa Kamal, A.T.M., Azad, O.K., Paul, A., Uddin, S.B., Barlow, J.W., Faruque, M.O., Park, C.H. & Cho, D.H. (2019). Investigation of the biological

activities and characterization of bioactive constituent of *Ophiorrhiza rugosa* var. *prostrate* (D.Don) & Mondal leaves through in Vivo, in Vitro and in Silico approaches. *Molecules,* 24, 1376.

Adzitey, F. (2015). Antibiotic classes and antibiotic susceptibility of bacterial isolates from selected poultry; a mini review. *World Veterinary Journal* 5(3), 36-41.

Ahmad, A., Kaleem, M., Ahmed, Z. & Shafiq, H. (2015). Therapeutic potential of flavonoids and their mechanism of action against microbial and viral infections- A review. *International Journal of Food Research,* 77(2), 221-231.

Ahn, K. (2017). The worldwide trend using botanical drugs and startegies for developing global drugs. *BMB Reports,* 50(3), 111-116.

Ajiboye, B.O., Ibukun, E.O., Edobor, G., Ojo, A.O. & Onikanni, S.A. (2013). Qualitative and Quantitative Analysis of Phytochemicals in *Senecio biafrae* Leaf. *International Journal of Inventions in Pharmaceutical Sciences,* 1(5), 428-432.

Akindele, P. O., Fatunla, O. A. Ibrahim, K. A. & Afolayan, C. O. (2017). Antibacterial and Phytochemical Screening of *Calotropis procera* Leaf Extracts against Vancomycin and Methicillin Resistant Bacteria Isolated from Wound Samples in Hospital Patients. *Journal of Complementary and Alternative Medical Research,* 2(1), 1-14.

Akin-Osanaiye, B. C. & Okhomina, L. (2018). Phytochemical and Antibacterial Activity of Leaf Extractsof *Calotropis Procera* on some Selected Bacteria. *Direct Research Journal of Biololgy &. Biotechnology,* 4(2), 16-21.

Akpuaka, A.A., Ekwenchi, M.M., Dashak, D.A. & Dildar, A. (2013). Biological activities of characterized isolates of n-Hexane extract of *Azadirachta indica* A.Juss (Neem) leaves. *Journal of Nature and Science,* 11(5), 134-140.

Alagammal, M., Soris, P.T. & Mohan, V.R. (2012). GC-MS analysis of *Polygala rosmarinifolia* Wights & Arn. *Journal of Applied Pharmaceutical Sciences,* 2(4), 188 - 190.

Alara, O.R., Abdulrahman, N.H. & Olalere, O.A. (2020). Ethanolic extraction of flavonoids, phenolics and antioxidants from *Vernonia amygdalina* leaf using two level factorial design. *Journal of King Saud University Science,* 32(1), 7-16.

Al-Ghanayem, A.A., Al- Sobeai, S.M., Alhussaini, Joseph1, B. & Saadabi, A.M. (2017). Antibacterial activity of certain Saudi Arabian medicinal plants used in folk medicine against different groups of bacteria. *Nusantara Bioscience,* 9(4), 392-395.

Ali, A., Ansari, A., Qader, S.A., Mumtaz, M., Saied, S. & Mahboob, T. (2014). Antibacterial potential of *Calotropis procera* flower against various pathogens. *Pakistan Journal of Pharmaceutical Sciences*, 27(5), 1567-1569.

Alitonou, G.A Koudoro, A.Y., Dangou1, J.S., Yehouenou, B., Avlessi1, F., Adeoti, S., Menut,C., Dominique, C. & Sohounhloue, K. (2012). Volatile constituents and biological activities of essential oil from Securidaca longepedunculata Fers. growing in Benin. *Scientific Study & ResearchChemistry & Chemical Engineering, Biotechnology, Food Industry* 13(1), 033-042.

Al-Snafi, A.E. (2016). Antiparasitic effects of medicinal plants (part 1) - A review. *IOSR Journal of Pharmacy,* 6, (10), 51-66.

Altemimi A., Lakhssassi, N., Baharlouei, A., Watson, D. G. & Lightfoot, D.A. (2017). Review on Phytochemicals: extraction, isolation o and identification of bioactive e compounds from plants extracts. *Plant,* 6, (42), 1-20.

Alves, R.N. & Humberton, N.A. (2011). The faunal drug store: Animal based remedies used in traditional medicine in latin America. *Journal of Ethnobiology and Ethnomedicine,* 7(1), 9.

Amid, R., Salim, R.J. & Adenan, M. (2010). The Factors Affecting the Extraction Conditions for Newroprotective Activity of Centella asiatica evaluated by Metal Chelating Activity Assay. *Journal of Applied Science,* 10, 837-842.

Anand, U., Herrera, N.J., Altemimi, A. & Lakhsassi, N. (2019). A comprehensive review on Medicinal plants as antimicrobial therapeutics: Potential Avenue of Biocompatible Drug Discovery. *Metabolites*, 9 (11), 258.

Andino, A. & Hanning, I. (2015). *Salmonella enterica:* survival, colonization and virulence differences among serovars. *Hindawi, The Science World Journal,* Article ID 520179.

Anwar, G., Oryem-Origa, H. & Kamatenesi-Mugisha, M. (2014). Antibacterial and antifungal properties of some wild Nutraceutical Plant Species from Nebbi District, Uganda. *British Journal of Pharmaceutical Research*, 4(14), 1753-1761.

Asoso, O. S., Oladunmoye, K.O. & Ogundare, A.O. (2018). Release of sodium and potassium ions of Calotropis procera extract on selected pathogenic organisms. FACSalud UNEMI 2(2), 26-30.

Atanasov, A.G., Waltenberger, B., Pferschy-wenzig, E., Linder,T., Wawrosch, C., Uhrin, P., Veronica, T., Wang, L., Schwaiger, S., Heiss, E.H., Rollinger, J.M., Schuster, D., Breuss, J.M., Bochkov, V., Mihovilovic, M.D., Kopp, B., Bauer, R., Dirsch, V.M. & Stuppner, H. (2015). Discovery and resupply of pharmacologically active plant-derived natural product; A review. *Biotechnology Advances*, 33(8), 1582-1614

Atanasov, A.G., Zotchev, B.S., Dirsch, V.M. & Supuran, C.T. (2021). Natural product in drug discovery: advances and opportunities. *Nature Reviews and Drug Discoveries,* 20, 200-216

Azmi, F., Skwarczynski, M. & Toth, I. (2016). Towards the development of synthetic antibiotics: design inspired by natural antimicrobial peptides. *Current Medicinal Chemistry,* 23(35), 1-16.

Azwanida, N. N. (2015). A Review on the extraction methods use in medicinal plants, principle, strength and limitation. *Journal of Medicinal & Aromatic Plants*, 4(3), 1-6.

Babu, A., Anand, D. & Saravanan, P. (2017). Phytochemical analysis of *Ficus arnottiana* (Miq.) Leaf extract using GC-MS analysis. *International Journal of Pharmacognosy and Phytochemical Research,* 9(6), 775-779.

Bajpayee, K.K. (2015). Antifungal activities of bark extract of *Calotropis procera*. *Journal of Advances in Scientific Research,* 1(2), 108-110.

Bakchiche, B., Temizer, I.K., Guder, A., Cele mLi, O.G., Yegin,S.C., Bardaweel, S.K. & Ghareeb, M.A. (2020). Chemical composition and Biological activities of Honeybee products from Algeria. *Journal of Applied Biotechnology Reports,* 7(2), 93-103.

Balasundram, N., Sundram, K. & Saman, S. (2006). Phenolic Compounds in Plants and Agriindustrial by-Products: Antioxidant Activity, Occurrence, and Potential Uses. *Journal of Food Chemistry.* 99, 191- 203.

Barrajon-Catalan, E., Fernandez-Arroyo, S., Saura, D., Guillen, E., Fernandez-Gutierrez, A., Segura-Carretero, A. & Micol, V. (2010). Cistaceae aqueous extracts containing ellagitannins show antioxidant and antimicrobial capacity, and cytotoxic activity against human cancer cells. *Journal of Food Chemistry & Toxicology*, 48, 2273–2282.

Belete T.M. (2019). Novel target to develop new antibacterial agents and novel alternatives to antibacterial agents. *Journal of Human Microbiome* 11, 1-7.

Bello, A., Aliero, A.A., Saidu, Y. & Muhammad, S. (2011). Phytochemical screening, polyphenolic content and Alpha-Glucosidase inhibitory portential of *Leptidenia hastate. Nigerian Journal of Basic and Applied Science*, 19 (2), 181-186.

Bello, A., Aliero, A.A., Saidu, Y., Muhammad, S. (2011). *Hypoglycaemic and Hypolipidaemic Effects of Leptadeniahastata (Pers.) Decne in Alloxan Induced Diabetic Rats. The Nigerian Basic and Applied Science Journal*, 19 (2), 187-192

Bello, O.M., Ibitoye, C. & Adetunji C. (2019). Assessing antimicrobial agents of Nigeria flora. *Journal of King Saud University-Science,* 31, 1379-1383.

Bello, O.M., Zaki, A.A., Khan, S.I., Pius, S.F., Ali, Z., Khan, A.I., Usman, L.A. & Oguntoye, O.S. (2017). Assessment of selected medicinal plants indigenous to West Africa for antiprotozoal activity. *South African Journal of Botany*, 113, 200-211.

Benitez, R.B., Ortega-Bonilla, R.A. & Martin-Franco, J. (2016). Comparison of two sesame oil extraction methods; percolation and pressed. *Journal of Biotechnology en el sector Agropecuario,* 14(1), 12-18.

Bernstein, H., Bernstein, C. & Michod, R.E. (2018). Sex in microbial pathogens. *Journal of Infection, Genetics and Evolution,* 57, 8-25.

Borokini, T. I., Clement, M., Dickson, N. J., & Edagbo, D. E. (2013). Ethnobiological survey of traditional medicine practice for circulatory and nervous system related diseases in Oyo State, Nigeria. *Journal of Herbal Medicine*, 2(6), 111-120.

Bourgeois, A.L., Wierzba, T.F. & Walker, R.I (2016). Status of vaccine research and development for enterotoxigenic *Escherichia coli*. *Vaccine,* 34 (26), 2880–2886.

Bribi, N. (2018). Pharmacplogical activity of alkaloids: A Review. *Asian Journal of Botany*, 1, 1-7.

Bribi, N., Bouguezza, Y. & Maiza, F (2013). Evaluation of erythrocytes toxicity and antioxidant activity of alkaloids of fumaria capreolata. *International Journal of Pharma and Bioscience*, 4, 770-776.

Bruschi, P., Morganti, M., Mancini, M., & Signorini, M. A. (2011). Traditional healers and laypeople: aqualitative and quantitative approach to local knowledge on medicinal plants in Muda (Mozambique). *Journal of Ethnopharmacology,* 138(2), 543-563.

Cassells, A.C. (2012). Pathogen and biological contamination management in plant tissue culture: phytopathogens, vitro pathogens, and vitro pests. *Methods in Molecular Biology*, 877, 57–80.

Chang, F., Terbach, N., Plant, N., Chen, P., Walker, M.C. & William, R.S. (2013). Seizure control by ketogenic diet-associated medium chain fatty acid. *Journal of Neuropharmacology,* 69, 105-110.

Chang, h.Y., Lo, C.L. & Hung, Y.Y. (2019). Development of traditional and complementery medicine (TCM) scales for nurses: using structural equation modelling (SEM). *BMC Complementary and Alternative Medicine*, 19(1), 1-10.

Cheesbrough, M. (2006). *District Laboratory Practice in Tropical Countries*. 2nd edition, London: Cambridge University Press. Pp 38-64.

Cheong, S.H., Furuhashi, K., Ito, K.., Nagaoka, M., Yonezawa, T., Miura, Y. & Yagasaki, K. (2014). Daidzein promotes glucose uptake through glucose transporter 4 translocation to plasma membrane in L6 myocytes and improves glucose homeostasis in Type 2 diabetic model mice. *Journal of Nutritional Biochemistry*, 25, 136–143.

Chongs, M. E., Fuh, N. J., Olugbenga, O.O., Smart O. & Ngolo, L. J. (2018). Bacterialpthogens associated with urinary tract infaction among rural women in

Doma, Nasarawa State, Nigeria. *International Journal of Current Microbiology and Applied Sciences,* 7(8), 2823-2831

Clinical Laboratory Standard Institute (CLSI) (2012). *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically*; Approved Standard– Ninth Edition. Clinical and Laboratory Standards Institute, USA. 2012.

Coates, A.R.M., Halls, G. & Hu, Y. (2011). Novel classes of antibiotics or more of the same. *British Journal of Pharmacology,* 163, 184-194.

Cordero, H. I., Martin, M.A., Bravo, L., Goya, L. & Ramos, S. (2013). Epicatechin gallate induces cell death viap53 activation and stimulation of p38 and JNK in human colon cancer SW480 cells. *Journal of Nutrition & Cancer*, 65, 718–728

Costa, T.R., Felisberto, R.C., Meir, A., Prevost, M.S., Redzej, A., Trokter, M. & Waksman, G. (2015). Secretion systems in Gram-negative bacteria: structural and mechanistic insights. *Nature Reviews. Microbiology*, 13 (6), 343–59.

Cragg, G.M. & Newman, D.J. (2013). Natural products: a continuing source of novel drug leads. *Biochemistry Biophysics. Acta,* 1830(6), 3670-3695.

Croxen, M.A., Law, R.J., Scholz, R., Keeney, K.M., Wlodarska, M. & Finlay, B.B (2013). Recent advances in understanding enteric pathogenic Escherichia coli. *Clinical Microbiology Reviews*, 26 (4), 822–880

Dai, J. & Mumper, R. (2010). Plant phenolics: extraction, analysis and their antioxidant and anticancer properties. *Molecules*, 15, 7313-7352.

Dambatta, S.H., & Aliyu, B.S. (2011). A survey of major ethno medicinal plants of Kano North Nigeria, their knowledge and uses by traditional healers. *Bayero Journal of Pure and Applied Sciences,* 4, 28-34.

Daswani, P.G., Ghadge, A.A., Brijesh, S. & Birdi, T.J. (2011). Preparation of Decoction of Medicinal Plants: A Self-Help Measure? *Journal of Alternative and Complementary Medicine,* 17(12), 1099-1100.

Davic, A. & Cascio, M. (2021). Development of a microfluidic platform for trace lipid analysis. *Metabolits,* 11(3), 130.

De Smet, J., Hendrix, H., Blasdel, B.G., Danis-Wlodarczyk, K. & Lavigne, R. (2017). Pseudomonas predators: understanding and exploiting phage-host interactions*"*. *Nature Reviews. Microbiology*, 15 (9), 517–530.

Deepika, S. & Singh P. (2015). In – Vitro Fungitoxic Evaluation and Gcms Analysis of *Calotropis Procera. World Journal of Pharmaceutical Research*, 4(3), 1123-1135.

Deng, G.F., Shen, C., Xu, X.R., Kuang, R.D., Guo, Y.J., Zeng, L.S., Gao, L.L., Lin, X., Xie, J.F. & Xia, E.Q. (2012). Potential of fruit wastes as natural resources of bioactive compounds. *International Journal of Molecular Science*, 13, 8308–8323.

Deng, G.F., Xu, X.R., Guo, Y.J., Xia, E.Q., Li, S., Wu, S., Chen, F., Ling, W.H. & Li, H.B. (2012). Determination of antioxidant property and their lipophilic and hydrophilic phenolic contents in cereal grains. *Journal of Functional Foods*, 4, 906–914.

Devakumar, J., Keerthana, V. & Sudha, S.S. (2017). Identification of bioactive compounds by Gas Chromatography-Mass Spectrometry analysis of *Syzygium jambos* (L.) collected from western ghats region Coimbatore, Tamil Nadu. *Asian Journal of Pharmaceutical and Clinical Research,* 10(1), 364-369.

Dhanani, T., Shah, S., Gajbhiye, N.A. & Kumar, S. (2013). Effect of extraction methods on yield, phytochemical constituents and antioxidant activity of *Withania somnifera*. *Arabian Journal of Chemistry*, 9(3), 56-67.

Dinesh, M.D., Carmel, A., George, N., Ajma, N., Anjana, J.C. & Meenatchisundaram, S. (2016). Terminaliachebula-A potential natural herbal drug against mastitis isolate. IOSR *Journal of Agriculture and Veterinary Science*, 9(10), 64-69.

Do, Q. D., Angkawijaya, A. E. & Tran-Nguyen, P. L. (2014). Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of *Limnophila aromatica*, *Journal of Food and Drug Analysis*, 22(3), 296–302.

Dong, S., Yang. X., Zhao, L., Zhang, F., Hou, Z. & Xue, P. (2020). Antibacterial activity and mechanism of action saponins from *Chenopodium quinoa* Wild. Husks against foodborne pathogenic bacteria. *Journal of Industrial Crop and Product,* 149(112350), 1-10.

Doughari, J.H. (2012). *Phytochemicals: Extraction methods, Basic structure and mode of actionns as portential chemotherapeutic agents*. Phytochemicals a global perspective of their roles in nutrition and roles in Nigeria. Pp 1-34.

Dudek, N.K., Sun, C.L. & Burstein, D. (2017). Novel Mincrobiology Diversity and Functional Potential in the Marine Mammals Oral Microbiome. *Current Biology*, 27 (24), 3752–3762.

Dwivedi, G. & shridhar, D. (2007). History of medicine; sushurata-the clinician teacher per excellence. *Indian Journal of Chest Diseases and Allied Sciences,* 49, 243-244.

Easwaran, L., Ramani, A.V. (2014). Phytochemical examination and GC-MS studies of medicinal plant- *Naravelia zeylanica. International Journal of Research and Development in Pharmacy and Life Sciences*, 3(5), 1180-1188.

Ebrahim, N., Kershi, M. & Butnariub, M. (2014). Antioxidant Activity and Anthocyanin Content in Flower of Mirabilis jalab L. Collected from Yemen. *Journal of World Applied Sciences,* 29, 247-251.

Elkhateeb, W., El-Sayed, H., Fayad, W., Al-kolaibe, A.G., Imam, M. & Daba, G. (2020). *In vitro* anti-cancer and antifungal bio-efficiency of some microalgal extracts. *Egyptian Journal of Aquatic Biology and Fisheries,* 24(1), 264-273.

Elumalai, A. & Eswarial, M.C. (2012). Herbalism – A review. *International Journal of Phytotherapy*. 2(2), 96-105.

Emiru, T., Beyene, G., Tsegaye, W. & Melaku, S. (2013). Associated risk factors of urinary tract infection among pregnant women at Felege Hiwot Referral Hospital, Bahir Dar, North West Ethopia. *BMC Research Notes,* 6(292), 1-6.

Epps, S.V., Harvey, R.B., Hume, M.E., Phillips, T.D., Anderson, R.C. & Nisbet, D.J. (2013). Foodborne campylobacter infections metabolism, pathogenesis and reservoirs. *International Journal of Environmental Research and Public Health*, 10 (12), 6292–6304.

Estrada, O., Breen, J., Richard, M.S. & Cooper, A. (2018). Ancient Plant in DNA genomic era. *Nature Plant*, 4, 394-396.

Etebu, E. & Arikekpar, I. (2016). Antibiotics classification, mechanism of action with emphasis on molecular perspective. *International Journal of Applied Microbiology and Biotechnology*, 4, 90-97.

Ewansiha, J.U. (2020). Evaluation of Antibacterial Potency of *Citrus limon* (Lemon) Juice against Some Pathogenic Organisms as Alternative Source of Chemotherapy. *European Journal of Biology and Biotechnology*, 1(1), 1-8.

Ewansiha, J.U., Tilon, G., Kolawole, I.D. & Orji, S.L. (2020). Inhibitory potentials of coconut and carrot oil on some bacterial skin microflora. *African Journal of Biology and Medicinal Research*, 3(3), 15-21.

Ezenyi, I.C., Mbamalu, O.N. & Balogun, L. (2016). Antidiabetic potentials of Syzygium guineense methanol leaf extract. *Journal of Phytopharmacology*, 5, 150–156.

Eziah, V. Y., Buxton, T., & Owusu, E. O. (2013). Bioefficiency of Zanthoxylum xanthoxyloides and *Securidaca longepedunculata* against *Prostephanus truncates* (Horn) (Coleoptera: Bostrichidae) and *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae). *Journal of Biopesticides*, 6, 54-62.

Fabrega, A. & Vila, J. (2013). *Salmonella entrica* serovar Typhimurium skills to succeed in the host, virulence and regulation. *Clinical Microbiology Reviews*, 26 (2), 308–341.

Famuyide, I.M., Aro, A.O., Fasina, F.O., Eloff, J.N. & McGaw, L.J. (2019). Antibacterial activity and mode of action of acetone crude leaf extract of under investigated *Syzygium* and *Eugenia* (Myrtaceae) species on multidrug resistant porcine diarrhoeagenic *Escherichia coli*. *BMC Veterinary Research,* 15(162), 1-14.

Fang, H., Kang, J. & Zhang, D. (2017). Bacterial review and future perspective. *Microbial Cell Factories*, 16 (1), 15.

Farha, A.K., Yang, Q.Q., Kim, G., Li, H.B., Zhu, F., Liu, H.Y., Gan, R.Y. & Corke, H. (2020). Tannins as an alternative antibiotic. *Journal of Food Biosience*, 38 (100751), 1-14.

Fookes, M., Schroeder, G.N., Langridge, G.C., Blondel, C.J., Mammina, C., Connor, T.R., Seth-Smith, H., Vernikos, G.S., Robinson, K.S., Sanders, M., Maskell, D., Barrow, P. & Thomson, N.R. (2011). *Salmonella bongori* provides insight into the evolution of *Salmonellae. PLoS pathogens,* 7(8), e1002191.

Ganesh, R., Shrestha, D., Bhattachan, B., & Rai, G. (2019). Epidemiology of urinary tract infection and antimicrobial resistance in a pediatric hospital in Nepal. *BMC Infectious Disease,* 19(420), 1-2.

Ghezelbash, G.H.R., Parishani, M.R. & Fouani, M.H. (2015). Antimicrobial activity of *Salvia officinalis* acetone extract against pathogenic isolates. *Journal of Herbal Drugs,* 5(4), 215-218.

GianLuca, Q., Jacques D.M & Robert, L. (2012). Emerging and re-emerging infectious diseases: a continuous challenge for Europe. *European Respiratory Journal*, 40(6), 1312-1314.

Goraksh, J.H., Keshav, K.D., Raghunat, D.P., Tukaram, R.G. & Narendra, D.p. (2017). Phytochemical studies on *Nerium oleander L.* Using GC-MS. *Interbational Journal of Pharmacology and Phytochemical Research,* 9(6), 885-890.

Goyal, S., Kumar, S. Rawat, P. & Dhaliwal, N. (2013). Antifungal Activity of *Calotropis Procera* towards Dermatoplaytes. *International Journal of advances in Pharmacy, Biology and Chemistry* 2(3), 470-472.

Grynkiewicz, G. (2020). Phytochemicals between nutrition and medicine. *Acta Scientific Nutrition and Medicine*, 4(1), 24-29.

Guo, Y.J., Deng, G.F., Xu, X.R., Wu, S., Li, S., Xia, E.Q., Li, F., Chen, F., Ling, W.H. & Li, H.B. (2012). Antioxidant capacities, phenolic compounds and polysaccharide contents of 49 edible macro-fungi. *Journal of Functional Foods*, 3, 195–1205.

Halliwell, B. (2007). Dietary polyphenols; Good, Bad, or indifferent for your health? *Journal of Cardiovascular Research*, 73(2), 341-347.

Harvey, A.L., Edrada-Ebel, R.A. & Quinn, R.J. (2015). The emergence of natural product for drug discovery in the genomic era. *Nature reviews drug discovery*, 14(2), 111-129.

Hasan, M.M., Hossain, A., Shamim, A. & Rahman, M. (2017). Phytochemical and pharcological evaluation of ethanolic extract of *Lepisanthes rubiginosa* leaves. *BMC Complementary and Alternative Medicine*, 17(496), 1-11.

Hassan, U.F., Hassan, H.F., Baba, H., Shibdawa, M.A. & Lorfa, E.N (2017). Nutritional evaluation of *Leptadenia hastate* leaves. *International Journal of Advancements In Research And Technology*, 6(3), 4-6.

Hayat, B., Imdad, A., Shahab, U., Ibrahim, K., Anwar, S., Mahboob U. R., Abdul Majid, K., Abdul Bari, S., & Ayaz, A.K. (2020). Biological evaluation of antimicrobial activity of *Calotropis procera* against a range of bacteria. *Journal of Pharmacognosy and Phytochemistry*, 9(1), 31-35.

Hoiby, N., Ciofu, O. & Bjarnsholt, T. (2010). *Pseudomonas aeruginosa* biofilms in cystic fibrosis. *Future Microbiology*. 5 (11), 1663-1674.

Homann, M., Sansjofre, P., Van Zuilen, M., Heubeck, C., Gong, J., Killingsworth, B., Foster, L.S., Airo, A., Van Kranendonk, M.J., Ader, M. & Lalonde, S.V. (2018). Microbial life and biogeochemical cycling on land 3,220 million years ago. *Nature Geoscience*, 11 (9), 665–671.

Huang, Z.R., Lin, Y.K. & Fang, J.Y. (2009). Biological and pharmacological activities of squalen and its related compounds; potential uses in cosmetic dermatology. *Molecules*, 14(1), 540-552.

Hudson, C., Bent, Z., Meagher, R. & Williams, K. (2014). Resistance determinants and mobile Genetic Elements of an NDM-1-Encording *Klebsiella pneumoniae* strain. *PloS One* 9(6), e99209.

Huq, T.B., Rahma. M.S., Nure, M.A., Hossain, M.S., Sarwar, A., Islam, A., Das, M., Haque, M.E., Malik, T., Reza, M.W., Adhikary, B.C., Rahman M., Begum, T. & Begum, M.M. (2016). Evaluation of pharmacological activities of methanolic extract of *Ixora cuneifolia* leaves. *Journal of Clinical Phytoscience*, 2(22), 1-9.

Hussain, G., Rasul, A., Anwar, H., Aziz, N., Razzaq, A., Ali, M., Li, J. & Li, X. (2018). Role of plant derived alkaloids and their mechanisms in Neurodegenerative disorders. *International Journal of Biological Sciences*, 14(3), 341-35.

Ibrahim, H.A., Ali, G.Y., Halliru, S.N., Usaini, S. and Abdullahi, I.I. (2012). Ethno botanical Survey of the Wild Edible Food Plants Consumption among Local Communities 85 in Kano State, North-Western. *International Journal of Science and Technology*, 2, 713- 717

Imam, U.I., Abdallah, M.S. & Ali, M. (2019). Antibacterial activity of *Leptadenia hastate* leaves against some gastro intestinal isolates. *Achieves in Biomedical Engineering & Biotechnology*, 1(3) 2-5.

Ingole, N.S. (2016). Phytochemical analysis of leaf extract of *Ocimum americanum* (Lamiaceae) by GC-MS method. *World Scientific News*, 76-87.

Innalegwu, D.A., Oladunni, A.,Yebo, G.T., Mahmoud, M.F., Bosso, S.T. & Oluwafemi, A.S. (2021). Toxicological, Bioassay-Guided Fractionation and Antibacterial Activity of Methanol Stem Extract Of *Terminalia Microptera* On Oral Bacteria Pathogens. *Tropical Journal of Natura Product Research,* 5(2), 397-409.

Innalegwu, D.A., Oladunni, A.,Yebo, G.T., Mahmoud, M.F., Bosso, S.T. & Oluwafemi, A.S. (2021). Toxicological, Bioassay-Guided Fractionation and Antibacterial Activity of Methanol Stem Extract of *Terminalia Microptera* On Oral Bacteria Pathogens. *Tropical Journal of Natura Product Research,* 5(2), 397-409.

Islam, M.T., Ali, E.S., Uddin, S.J., Shaw, S., Islam, M.A., Ahmed, M.I., Chandra, S.M., Karmakar, U.K., Yarla, N.S., Khan, I.N., Billah, M.M., Pieczynska, M.D., Zengin, G., Malainer, C., Nicoletti, F., Gulei, D., Berindan-Neagoe, I., Apostolov, A., Xiao, J., Dey, P., Yele, S., Jozwik, A., Strzatkowska, J., Marchewka, J., Rengasamy, K.R.R., Horbanczuk, J., Kamal, M.A., Mubarak, M.S., Mishra, S.K., Shilpi, J.A. & Atanasov, A.G. (2018). Phytol: a review of biomedical activities. *Journal of Food and Chemical Toxicology,* 121, 82-94.

Ismaiel A. & Abd Alrheam, A. (2015). Biochemical effect of *Calotropis procera* on hepatotoxicity. *Journal of Biomedical Research and Therapy*, 2 (12) 446-453.

Jakhetia, V., Patel, R., Khatri, P., Pahuja, N., Garg, S., Pandey, A. & Sharma, S.A. (2010). *Cinnamon*: a pharmacological review. *Journal of Advanced Scientific Research,* 1(2), 19- 23.

James, P.B., Wardle, J., Steel, A. & Adams, J. (2018). Traditional, complementary and aleternative medicine use in sub-saharan Africa; a systematic review. *BMJ Global Health*, 3: e000895

Jantsch, J., Chikkaballi, D. & Hensel, M. (2011). Cellular aspects of immunity to intracellular Salmonella enterica. *Immunological Reviews*, 240 (1), 185–195.

Jayasri, M.A., Mathew, L. & Radha, A. (2009). A report on the antioxidant activities of leaves and rhizomes of *Costus pictus* D. Don. *International Journal of Integretive Biology*, 5(1), 20-26.

Jiang, Z., Yang, M., Du, J. & Zhang, H. (2015). Antibacterial and antiviral properties of glycerol monolaurate and its application in food. *Journal of the Chinese Cereals and Oils association*, 30(2), 142-146.

Jonathan, B. & Micheal, W.A. (2014). Chemistry; chemical con artist foil drug discovery. *Nature*, 513(7519), 481-483.

Josef, J. (2018). Design and discovery of new antibacterial agents; advances, perspectives and challenges. *Current Medicinal Chemistry*, 25(38), 4972-5006.

Kadiri, A. B., Agboola, O. M., & Fashina, F. O. (2013). Ethnobotanical survey andphyto-anatomicalstudies of some common plants used for the treatment of epilepsy in some rural areas of South westNigeria. *Journal of Pharmacognosy and Phytochemistry*, 2(4), 175-182.

Kang, H.K. & Park, Y. (2015). Glycopetide antibiotics: structure and mechanism of actions. *Journal of Bacteriology and Virology*, 45(2), 67-78.

Kawo, A.H., Mustapha, A., Abdullahi, B.A, Rogo, L.D., Gaiya, Z.A. & Kumurya, A.S. (2009). Phytochemical properties and antibacterial activities of the leaf extracts of calotropis procera (Ait. F). *Bayero Journal of Pure and Applied Sciences*, 2(1), 34-40.

Keshebo, D. L., Choundhury, M. K., & Dekebo, A. H. (2014). Investigation on toxicity, hypoglycemic effect of the root bark of *Securidaca longipedunculata* Fresen (Polygalaceae) and determination of heavy metals. *Annals of Biological Research*, 5(6), 15-19.

Keta, J.N., Manga, S.S., Keta, M.N. & Gudu, G.B. (2018). Comparative Study on Antifungal Effects of *Calotropis procera* and *Ficus gnaphalocarpa* Latex against *Tinea capitis. UMYU Journal of Microbiology Research,* 3(1), 39-44.

Khalil, M.N., Shalaby, A.E., Ali, A.I.M.D., Ali, M.E. & Aboul-Enein, M.A. (2014). Biological activities of secondary metabolites from *Emericella nidulans* EGCU312. *African Journal of Microbiology Research*, 8(20), 2011-2021.

Klockgether, J., Cramer, N., Wiehlmann, L., Davenport, C.F. & Tum mLer, B. (2011). *Pseudomonads aeruginosa* genomiic structuere and diversty. *Frontiers in Microbiology*, 2, 150-151.

Koche, D., Shirsat, R. & Kawale, M. (2016). An overview on major class of phytochemicals: there types and role in disease prevention. *Hislopia Journal*, 9(1/2), 1-11.

Koche, D., Shirsat, R., Syed, I. & Bhadenge, D.G. (2010). Phytochemical screening of eight ethnomedicinal plants from akola district. *International Journal of Pharma and Biosci*, 1(4), 256-259

Kolosova, N.G., Stefanova, N.A., Muraleva, N.A. & Skulachev, V.P. (2012). The mitochondria-targeted antioxidant SkQ1 but not N-acetylcysteine reverses aging-related biomarkers in rats. *Journal of Aging & Health*, 4, 686–694.

Kosalec, I., Cvek, J. & Tomic, S. (2010). Conterminant of medicinal herbs and herbal products. *Archieves of Industrial Hygiene and Toxicology*, 60(4), 485-490.

Laitha, S., Parthipan, B. & Mohan, V.R. (2015). Determination of bioactive components of *Psychotria nilgiriensis* Deb& Gang (Rubiaceae) by GC-MS analysis. *International Journal of Pharmacognosy and Phytochemical Research*, 7(4), 802-808.

Lakshmi, V.T.P. & Rajalakshmi, P. (2011). Identification of phyto-components and its biological activities of Aloe vera through the Gas chromatography- Mass Spectrometry. *International Journal of Pharmacy,* 2(5), 247-249.

Latif, A., Amer, H.M., Hamad, M.E., Alarifi, S.A. & Almahdi, F.N. (2014). Medicinal plant from Saudi Arabia and Indonesia; Invitro cytotoxicity evauation on Vero and HEp-2 cells. *Journal of Medicinal Plants Research*, 8(34), 1065-1073.

Lau, E. V., Gan, S. and Ng, H. K. (2010). Review Article; Extraction Techniques for Polycyclic Aromatic Hydrocarbons in Soils. *International Journal of Analytical Chemistry* 6, 1-9.

Li, B., Zhao, Y., Liu, C., Chen, Z. & Zhou, D. (2014). Molecular pathogenesis of *Klebsiella pneumoniae*. *Future Microbiology*, 9 (9), 1071–1081

Li, S.L., Lai, S.F., Song, J.Z., Qiao, C.F., Liu, X., Zhou, Y., Cai, H., Cai, B.C., & Xu, H.X. (2010). Decocting-induced chemical transformations and global quality of Du–Shen–Tang, the decoction of ginseng evaluated by UPLC-Q-TOFMS/MS based chemical profiling approach. *Journal of Pharmaceutical and Biomedical Analysis*, 53(4), 946–957.

Lim, J.Y., Yoon, J. & Hovde, C.J. (2010). A brief overview of Escherichia coli O157:H7 and its plasmid O157. *Journal of Microbiology and Biotechnology*, 20 (1), 5–14.

Liu, A.G., Volker, S.E. Jeffery, E.H. & Erdman, J.W.Jr, (2009). Feeding Tomato and Broccoli Powders Enriched with Bioactives Improves Bioactivity Markers in Rats. *Journal of Agriculture and Food Chemistry*,1, 22-28.

Liu, R.H. (2004). Potential synergy of phytochemicals in cancer prevention: mechanism of Action. *Journal of Nutrition*,134(12), 3479-3485

Lofty, R.A., Fahmy, D.M. & Ahmed, F.A. (2015). Qualitative and quantitative determination of secondary metabolites of 26 Medicinal plants from southeastern Egypt. *Egyptian Journal of Desert Research*, 65(2), 299-310.

Mafulul, S.G., Joel, E.B., Barde, L.A., Lenka, J.L., Ameh A.A. & Phililus, M.G. (2018). Extraction, Partial Purification and Characterization of Peroxidase from *Calotropis procera* Leaves. *Journal of Advances in Biology & Biotechnology,* 18(1), 1-10,

Mahmoud, R.K. (2012). Medical Plants Research Center, Shahrekord University of MedicalSciences, Shahrekord, *Iran. Journal of HerbMed Pharmacology* 1(1), 1–2.

Mahomoodally, F.M. (2013). Traditional medicines in africa: an appraisal of ten potent African medicinal plants. Hindawi Evidence bases complementary and alternative medicine, Article ID 617459.

Mailafiya, M.M., Pateh, U.U., Hassan, H.S., Sule, M.I., Bilah, A.H., Musa, T.L., Atinga, V. & Achika, J.I. (2020). Isolation of lupeol from the sterm bark of *Leptidenia hastate*

(Pers.) Decne. *Journal of Applied Sciences and Environmental Management*, 24(10), 1835-1838.

Maina, V.A., Peter I.D., Yahi, D., Sandabe, U.K. & Egwu, G.O. (2018). Effect of aqueous leaf extracts of of *Leptidenia hastate* pers (Decne) on spermiogram and testicular histology in albino rats (Wister rats). *Journal of Medicinal Plant Research*, 12(14), 164-169.

Majdanik, M.M., Kepa, M., Wojtyczka, R.D., Idzik, D. & Wasik, T.J. (2018). Phenolic compounds diminish antibiotic resistance of *staphylococcus aureus* clinical strains. *International Journal of Environmental Research and Public Health,* 15, 2321.

Mali, R.P., Rao, P.S. & Jadhav, R.S. (2019). A Review on Pharmacological Activities of *Calotropis Procera. Journal of Drug Delivery and Therapeutics*,9(3), 947-951.

Malik, B., Pirzadh, T.B., Tahir, I., Abdin, M.Z. & Ul-Rahman, R. (2016). Phytochemical studies on *Cichorium intybus* L. (chicory) from Kashmir Himalaya using GC-MS. *Journal of Pharmacy Research,* 10(11), 715-720.

Mamza, U.T., Sodipo, O.A., Khan, I.Z., Gulani, I.A. (2015). Phytochemical, antimicrobial and toxicity studies of ethanolic leaf extract of Phyllanthus amarus Thonn and Schum (Euphorbiacea). *International Journal of Green Herbal Chemistry*, 4, 21-30.

Mandal, S.C. & Mandal, M. (2011). Quality, Safety and Efficacy of herbal products through regulatory harmonization. *Drug Information Journal*, 45, 45-53.

Manoorkar, V.B., Mandge, S. V. & Gachande, B. D. (2015). Antifungal activity of leaf and latex extracts of *calotropis procera* against dominant seed-borne storage fungi of some oil seeds. *Journal of Bioscience Discovery*, 6(1), 22-26.

Matejic, J.S. Stojanovic- Radic, Z.Z., Ristic, M.S., Veselinovic, J.B., Zlatkovic, B.K., Marin, P.D. & Dzamic, A.M. (2018). Chemical characterization, *in vitro* biological activity of essential oil and extract of three *Eryngium* L. species and molecular docking of selected major compounds. *Journal of Food Science and Technology*, 55(8), 2910-2920.

Mathur, S. & Hoskins, C. (2017). Drug development: lesson from nature (Review). *Biomedical Reports*, 6, 612-614.

Matsuura, H.N & Fett-Nato, A.G. (2015). Plant alkaloid: Main features, toxicity and mechanism of action. *Springer Science + Business Media Dordrech 2015,* 1-3.

Mattson, M.P. & Cheng, A. (2006). Neurohormetic phytochemicals: low-dose toxins that induce adaptive neuronal stress responses. *Trends in Neurosciences*. 29(11), 632-639.

Menezes, R.F., Bergmann, A. & Thuler L.C. (2013). Alcohol consumption and risk of cancer; a systematic literature review. *Asian Pacific Journal of Cancer Prevention*, 14(9), 4965-4975.

Mincer, T.J. & Aicher, A.C. (2016). Methanol production by Broad phylogenetic array of marine phytoplankton. *PLoS One*, 11(3), 1-17.

Mir, M.A., Parihar, K., Tabasum, U & Kumari E. (2016). Estimation of alkaloid, saponings and flavonoid content in various extracts of *Crucus sativa. Journal of Medicinal Plant Studies*, 4(5), 171-174.

Mizielinska, M., Salachna, P., Ordon, M. & Lopusiewicz, L. (2017). Antimicrobial activity of water and acetone extract of some *Eucomis* taxa. *Asian Percific Journal of Tropical Medicine*, 10(9), 892-895.

Moellering, Jr. R.C. (2011). Discovering new antimicrobial agents. *International Journal of Antimicrobial Agent*, 37(1), 1-8.

Mogana, R., Adhikari, A., Tzar, M.N., Ra mLiza, R. & Wiart C. (2020). Antibacterial activities of the extracts, fractions and isolated compouns from Canarium patentinervium Miq. Against bacterial clinical isolates. *BMC Complementary Medicine and Therapies*, 20, 55, 4-6.

Mohammadhosseini, M. (2017). The ethnobotanical, phytochemical and pharmacological properties and medicinal application of essential oils and extract of different *Ziziphora* species. *Industrial Crops and Products*, 105, 164-192.

Mohanraj, R., Rakshit, J. & Nobre, M. (2010). Anti HIV-1 and antimicrobial activity of the leaf extracts of *Calotropis procera. International Journal of Green Pharmacy* 4(1), 242-246.

Moore, S.J. & Warren, M.J. (2012). The anaerobic biosynthesis of vitamin B12. *Biochemical Society Transactions*, 40 (3), 581–586.

Moreno-Quir´os, C.V., Sanchez-Medina, A., V´azquez- Hern´andez, M., Hern´andez Reyes, A.G., & Garc´ıa-Rodr´ıguez, R.V. (2017). Antioxidant, anti-inflammatory and antinociceptive potential of *Ternstroemia* sylvatica. *Asian Pacific Journal of Tropical Medicine*, 10(11), 1047–1053.

Moustafa, A.M.Y., Ahmed, S.H., Nabil, Z.I., Hussein, A.A., & Omran, M.A. (2010). Extraction and phytochemical investigation of *Calotropisprocera*: Effect of plant extracts on the activity of diverse muscles. *Pharmaceutical Biology*, 48(10), 1080–1190.

Murti, Y., Sharma, S. & Mishra, P. (2015). *In vitro* anthelminthic activity of *calotropis procera* (ait.) R. Br. Leaves. *Asian Journal of Pharmaceutical & Clinical Research*, 8 (6), 188-190.

Mushtaq, S., Abbasi, B.H., Uzair, B. & Abbasi, R. (2018). Natural product a reservoirs of novel therapeutic agents. *Journal of Experinmental and Clinical Sciences*, 17, 420-443.

Mustapha, A. A. (2013). Ethno-medico-botanical uses of *Securidaca longepedunculata* Fresen (Family-Polygalaceae) from Keffi Local Gover nment, Nasarawa State, *Nigeria.Journal of Natural Remedies,* 13(2), 133-137.

Mwangi, V.I., Mumo, R.M., Nyachieo, A. & Onkoba, N. (2017). Herbal medicine in the treatment of poverty associated parasitic diseases: A case of sub-Saharan Africa. *Journal of Herbal Medicine,* 10, 1–7.

Nascimento, G.G.F., Locatelli, J., Freitas, P.C. & Silva, G.L. (2000). Antibacterial activity of plant extracts and phytochemicals on antibioticresistant bacteria. *Brazilian Journal of Microbiology*. 31:247-256

Ndamitso, M. M., Mohammed, A., Jimoh, T. O., Idris, S., Oyeleke, S. B., & Etsuyankpa, M. B. (2013). Phytochemical and antibacterial activity of *Securidaca longepedunculata* on selected pathogens. *African Journal of Microbiology Research* 7(50), 5652-5656.

Neha, P., Vipra, P., Rajesh, S. P. & Kaushik C. P. (2018). Medicinal Use of Some Angiospermic Plant Species Growing in Punit-Van Sector- 18, Ghandinagar, Gujarat, India. *The world Journal of Engineering & Applied Science,* 4(1), 1-11.

Nenaah, E.G. & Ahmed, M.E. (2011). Antimicrobial activity of extracts and latex of *Calotropis procera* (Ait) and synergistic effect with reference. *Antimicrobials Research Journal of Medicinal Plant,* 5(6), 706 – 716.

Newman, D.J. & Cragg, G.M. (2016). Natural products as sources of new drugs from 1981 to 2014. *Journal of Natural Product*, 79(3), 629–661.

Ngonda, F., Magombo, Z., Mpeketula, P. & Mwatseteza, J. (2012). Evaluation of Malawian Vernonia glabra (Steetz) Vatke leaf and *Securidaca longepedunculata* (Fresen) root extracts for antimicrobial activities. *Journal of Applied Pharmaceutical Science*, 2(11), 26,

Nguta, J.M. (2019). In vivo antimalarial activity, toxicity, and phytochemical composition of total extracts from *Securidaca longepedunculata* Fresen. (polygalaceae) *Biomedical and Biotechnology Research Journal,* 3 (3), 196, 2019

Nitiema, L.W., Nikiema, F.W., Sérémé, D., Pierre A. E. & Sombié1, D. (2020). Antifungal Activity of Securidaca longepedunculata and Acacia gourmaensis Hydro-ethanolic extracts against Three Rice Seed-borne Fungi, *International Journal of Plant & Soil Science,* 32(2), 21-28.

O'Rourke, A., Beyhan, S., Choi, Y., Morales, P., Chan, A.P., Espinoza, J.L., Dupont, C.L., Meyer, K.J., Spoering, A., Lewis, K., Nierman, W.C. & Nelson, K.E. (2020).

Mechanism of action classification of antibiotics by Global transcriptome profiling. *Journal of Antimicrobial Agent and Chemotherapy,* 64(3), e01207-19.

Odetoye, T.E., Afolabi, T.J & onifade, K.R. (2016). Effect of extraction process parameters on the Quality characteristics of *parinari polyandra B.* Seed oil. *Nigerian Journal of Technological Development,* 13(2), 40-49.

Okoro CK, Kingsley RA, Connor TR, Harris SR, Parry CM, Al-Mashhadani MN, Kariuki S, Msefula CL, Gordon MA, de Pinna E, Wain J, Heyderman RS, Obaro S, Alonso PL, Mandomando I, MacLennan CA, Tapia MD, Levine MM, Tennant SM, Parkhill J, Dougan G (2012). International spread of human invasive *Salmonella bongori rium* pathovariant in sub-Saharan Africa. *Nature Genetics*, 44 (11), 1215–1221.

Oladeinde, B. H., Omoregie, R., Olley, M., Anunibe, J. A. & Onifade, A.A. (2013). A 5-year surveillance of wound infections at a rural tertiary hospitals in Nigeria. *Journal of African Health Science*, 13(2) 351-356.

Oladunmoye, M. K., & Kehinde, F. Y. (2011). Ethnobotanical survey of medicinal plants used in treating viral infections among Yoruba tribe of South Western Nigeria. *African Journal of Microbiology Research,* 5(19), 2991-3004.

Oni, P. I., Jimoh, S. O., & Adebisi, L. A. (2014). Management of indigenous medicinal plants in Nigeria using phenological information. *Journal of Medicinal Plants Research,* 8, 619-631.

Orsi, P.R., Bonamin, F., Severi, J.A., Santos, R.C., Vilegas, W., Hiruma-Lima, C.A. & Di Stasi, L.C (2012). Hymenaea stigonocarpa Mart. ex Hayne: A Brazilian medicinal plant with gastric and duodenal anti-ulcer and antidiarrheal effects in experimental rodent models. *Journal of Ethnopharmacology,* 143, 81–90.

Oyesola, A.O., Coolborn, A.F., Kolawole, O.M. & Bisola, M. (2016). Antimicrobial and Phytochemical Evaluation of *Calotropis procera* ("SODOM APPLE") against Human Pathogens. *British Microbiology Research Journal,* 11(3), 1-11.

Ozcan, T., Bayizit, A.A., Ersan, Y.L. & Delikanli, B. (2018). Phenolics in Human Health. *International journal of Chemical Engineering and Applications*, 5(5), 393-395.

Padma, M., Ganesan, S., Jayaseelan, T., Azhgumadhavan, S., Sasikala, P., Senthikumar, S. & Mani, P. (2018). Phytochemical screening and GC-MS analysis of bioactive compounds present in ethanolic leaves extract of *Silybum marianum* (L). *Journal of Drug Delivery and Therapeutics*, 9(1), 85-89.

Panda, K.S., Das, R., Leyssen, P., Neyts, J. & Luyten W. (2018). Assessing medicinal plants traditionally used in the chirang reserve forest, Northeast India for antimicrobial activity. *Journal of Ethnopharmacology,* 225, 220-233.

Pandey, A. & Tripathi, S. (2014). Concept of standardization, extraction and pre phytochemical screening strategies for herbal drug. *Journal of Pharmacognosy and Phytochemistry* 2 (5), 115-119.

Parihar, G. & Balekar, N. (2016). Calotropis procera: A phytochemical and Pharmacological Review. *Thai Journal of Pharmaceutical Science,* 40 (3), 115-131.

Parvekar, P., Palaskar, J., Metgud, S., Maria, R & Dutta, S. (2020). The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of silver nanoparticles gainst *Staphylococcus aureus*. *Biomaterial Investigation in Dentistry,* 7(1), 105-109

Pattanayak, S. (2018). Alternative to antibiotics from herbal origin- outline of a comprehensive research project. *Current Pharmacogenomics and Personalized Medicine*, 16(1), 9-60.

Pisoschi, A.M., Pop, A., Georgescus, C., Turcus, V., Olah, N. K & Mathe, E. (2018). An overview of natural antimicrobial role in food. *European Journal of Medicinal Chemistry,* 143, 922-935.

Praveen, G. & Rajesh G. (2019). Phytochemical screening and quantitative estimation of total flavonoids of *Ocimum sanctum* in different solvent extract. *The Pharma Innovation Journal,* 8(2), 16-21.

Preeti, C., Shamim, A. & Najam A.K. (2017). A Review on Medicinal Utility of *Calotropis Procera. World journal of Pharmaceutical and Medical Research,* 3(1), 335-342.

Rahigude, A., Bhutada, P., Kaulaskar, S., Aswar, M. & Otari, K. (2012). Participation of antioxidant and cholinergic system in protective effect of naringenin against type-2 diabetes-induced memory dysfunction in rats. *Journal of Neuroscience,* 226, 62–72.

Rao, N. (2003). Bioactive phytochemicals in Indian foods and their potentials in health promotions and disease preventions. *Asian Pacific Journal of Clinical Nutrition*, 12(1), 9-22

Raseem, H.H.A., Nour, A.H. & Yunus, R.M, (2017). GC-MS analysis of bioactive constituent of hibiscus flower. *Australian journal of Basic and Applied Sciences,* 11(7), 91-97.

Rashid, T. & Ebringer, A. (2007). Ankylosing spondylitis is linked to Klebsiella--the evidence. *Clinical Rheumatology,* 26 (3), 858–864

Ravanfar, R., Moein M., Niakousari, M. & Tamadoon A. (2018). Extraction and fractionation of anthocyanins from red cabbage; ultrasonic assisted extraction and conventional perculation method. *Journal of Food Measurement and Characterization,* 12(4), 2271-2277.

Sahin, N., Kula, I. & Erdogan, Y. (2006). Investigation of antimicrobial activities of nonanoic acid derivatives. *Fesenius Environmental Bulletin*, 15(2), 141-143.

Sanchez, G.V., Master, R.N., Clark, R.B., Fyyaz, M., Duvvuri, P., Ekta, G. & Bordon, J. (2013). Klebsiella pneumoniae antimicrobial drug resistance, United States, 1988-2010. *Emerging Infectious Diseases*, 19 (1), 133–136

Sanchez, J.L. (2015). Clinical Manifestations and Treatment of Lyme Disease. *Clinics in Laboratory Medicine*, 35 (4), 765–778.

Sanusi, J., Jibia, A.B., Runka, J.Y., Liadi, S., Abubakar, A.A. & Zurmi, R.S. (2015). Antimicrobial activity of aqueous and ethanol extracts of violet plant (*securidaca longipedunculata* fres) on tested pathogenic bacteria. *International Journal of Pharmaceutical Sciences and Research*, 6(8), 3276-3284.

Schlivert, P.M. & Peterson, M.L. (2012). Glycerol monolaurate antibacterial activity in Broth and Biofilm cultures. *PLoS One*, 7(7), 7-11. e40350

Seeland, M., Rzanny, M., Boho, D., Waldchen, J. & Mader, P., (2019). Image-based classification of plant genus and family for trained and untrained plant species. *BMC Bioinformatics*, 20(4), 1-3.

Sen, S., Chakraborty, R. & Devanna, B.N. (2011). An ethnobotanical survey of medicinal plants used by ethnic people in west and south district of Tripura, India. *Journal of Forestry Research*, 22(3), 471.

Serino, S., Costagliola, G. & Gomez, L. (2019). Lyophilized tomato plant material validation of a reliable extraction method for the analysis of vitamin C. *Journal of Food Composition and Analysis,* 81, 37-45.

Shamim, S. A. & Lubna, F. (2019). Pharmacological actions and therapeutic uses of *Aak* (*Calotropis procera*): A Review. *The Pharma Innovation Journal*, 8(2), 40-47.

Shapiro, E.D. (2014). Clinical practice, Lyme disease. *The New England Journal of Medicine*, 370 (18), 1724–31.

Sharma, P., Modi, G. & Singh, A. (2018). In Vitroantibacterial Activities Assessment of *Calotropis Procera* Leaf Extract. *International Journal of Current Research in Life Sciences*, 7(5), 2117-2120.

Shobowale, O.O., Ogbulie, N.J., Itoandon, E.E., Oresegun, M.O. & Olatope, S.O.A. (20013). Phytochemical and Antimicrobial Evaluation of Aqueous and Organic Extracts of *Calotropis procera* Ait Leaf and Latex. *Journal of Nigerian Institute of Food Science and Techonology*, 31 (1), 77 – 82.

Shukitt-Hale, B., Miller, M.G., Chu, Y.F., Lyle, B.J. & Joseph, J.A. (2013). Coffee, but not caffeine, has positive effects on cognition and psychomotor behavior in aging. *Journal of Aging Studies*, 35, 2183–2192.

Smith, H.C., Larsen, H.O., Pouliot, M. (2012). People, plants and Health; a conceptual framework for assessing changes in medicinal plants consumption. *Journal of Ethnobiology & Ethnomedicine* 8, 43-50.

Sreelatha, S., Dinesh, E. & Uma, C. (2012). Antioxidant properties of Rajgira (Amaranthus paniculatus) leaves and potential synergy in chemoprevention. *Asian Pacific Journal of Cancer Prevention,* 13, 2775–2780.

Staub, P.O., Casu, L. & Leonti, M. (2016). Back to the roots: a quantitative survey of herbal drugs in Dioscorides De Materia Medica (ex Matthioli, 1568). *Journal of Phytomedicine,* 23(10), 1043-1052.

Stogner, J.M., Eassey, J.M., Baldwin, J.M. & Miller, B.L. (2014). Innovation alcohol use; assessing the prevalence of alcohol without liquid and non- oral rout of alcohol administration. *Drug and Alcohol Dependence*, 142, 74-78.

Sulieman, A.E., Issa, F.M. & Elkhalifa, E.A. (2007). Quantitative determination of tannin content in some sorghum cultivars and evaluation of its antimicrobial activity. *Research Journal of Microbiology*, 2(3), 284-288.

Sultan, A. (2015). Steroids: a diverse class of secondary metabolites. *Journal of Medicinal Chemistry*, 5(7), 310-317.

Sung, M. & Park, M. (2013). Nutritional modulators of ulcerative colitis: Clinical efficacies and mechanistic view. *World Journal of Gastroenterology*, 19, 994–1004.

Tabuti, J. R., Kukunda, C. B., Kaweesi, D., & Kasilo, O. M. (2012). Herbal medicine use in the districtsof Nakapiripirit, Pallisa, Kanungu, and Mukono in Uganda. *Journal of Ethnobiology and Ethnomedicine,* 8(1), 35-46.

Talero, E., Avila-Roman, J. & Motilva, V. (2012). Chemoprevention with phytonutrients and microalgae products in chronic inflammation and colon cancer. *Journal of Current Pharmaceutical Design*, 18, 3939–3965.

Tambunan, A.P., Bahtiar, A. & Tjandrawinata, R.R. (2017). Influence of extraction parameters on the yield, phytochemical, TLC-Densitomeric Quantification of Quercetin, and LC-MS profile and how to standardize different batches for long term from *Ageratum conyoides L.* leaves. *Journal of Pharmacognosy,* 9(6), 767-774.

Tan, S.Y. & Tatsumura, Y. (2015). Alexander Fleming (1881-1955): Discoverer of penicillin. *Singapore Medical Journal,* 56(7), 366-367.

Tauheed, A.M., Suleiman, M. M., Mamman, M. & Lawal, I.A. (2016). Ex vivo trypanostatic effect of stem-bark extracts of Securidaca longipedunculata (Fres. Holl) against *Trypanosoma brucei brucei*. *African Journal of Biotechnology* 15(51), 2789-2794.

Tchabo, W., Ma, Y., Kwaw, E. Meng Wu, L.X. & Apaliya M.T. (2018). Impact of extraction parameters and their optimization on the Nutraceuticals and antioxidant

properties of aqueous extract mulberry leaf. *International Journal of Food Properties, 21*(1), 354-466

Thakur, L., Ghodasra, U., Patel, N. & Dabhi, M (2011). Novel approaches for stability improvement in natural medicines. *Pharmacognosy Review* 5(9), 48.

Thomas J. Elpel (2013). Botany in a day, a pattern method of plant identification. An herbal field guide to plant families. sixth edition. HOPS Press LLC Atlanta ,USA. PP 235.

Thouri, A., Chahdoura, H., El Arem, A. Hichri O.A., Hassin, R. & Achour, L. (2017). Effect of solvents extraction on phytochemical components and biological activities of Tunisian date seeds (var. Korkobbi and Arechti). *BMC Complementary and Alternative Medicine,* 17 (248), 1-10.

Truong, D.H., Nguyen, D.H., Ahn Ta, N.T., Bui, A. Ha Do, T. & Nguyen, H.C. (2019). Evaluation of the use of different solvent for phytochemical constituents, Antioxidants and *Invitro* anti-inflammatory Activities of *Serverinia buxifolia. Journal of Food Quality,* Hindawi, article ID 8178294, 1-9.

Tshisikhawe, M. P., Van Rooyen, M. W., & Bhat, R. B. (2012). An evaluation of the extent and threat of bark harvesting of medicinal plant species in the Venda Region, Limpopo Province, South Africa. *Phyton-International Journal of Experimental Botany,* 81, 89-100.

Tulashie, S.K., Appiah, A.P., Torku, G.D., Darko, A.Y. & Wiredu, A. (2017). Determination of methanol and ethanol concentration in local and foreign alcoholics drinks and food products (Banku, Ga kenkey, Fantekenkey and Hausa koko) in Ghana. *International Journal of Food Contmination*, 4, 14-19

Turner, J.V., Kustrin, A.S. & Glass B.D. (2007). Molecular aspect of phytoestrogen selective binding at estrogen receptors. *Journal of Pharmaceutical Science*, 96(8), 1879-1885.

Uddin, S.N., Akond, M.A., Mubassara, S. & Yesmin MN (2008). Antioxidant and Antibacterial activities of *Trema cannabina. Middle-East Journal of Scientific Research*. 3, 105-108.

Ullah, N. & Ali Khan, F. (2016). An introduction to natural products and phytochemicals with special reference to it antimicrobial activity. *Journal of Life Science*, 13(10) 103-109.

Umapathy, E., Ndebia, E.J. & Meeme, A. (2015). An Experimental evaluation of *Albuca seroseae* aqueous extract on membrane stabilization, protein denaturation and white blood cell migration during acute inflammation. *Journal of Medicinal Plant Research,* 3(2), 789-795.

Umar, I.A., Ndidi, U.S., Abdullahi, A.S., Ohilebo, A. & Oluwafunminiyi, E.T. (2018). In vitro anti-trypanosomal activities of methanolic extract of selected Nigerian plants. *Journal of Herbs, Spices & Medicinal Plants*, 24(1), 52-63.

Umaru, I.J., Badruddin, A.F. & Assim, B.Z & (2018). Effect of *Leptidenia hastate* Hexane leaf extracts against heamatological, biochemical and indomethacin induced ulcer in rats. *European journal of Pharmaceutical and Medical Research*, 5(5), 34-41.

Umaru, I.J., Badruddin, A.F., Assim, B.Z & Umaru, H.A. (2017). Antimicrobial properties of *Leptadeniahastata (Pers.) Decne* leaves extracts. *International Journal of Pharmacy and Pharmaceutical Sciences*, 10(2), 149-152.

Umaru, I.J., Badruddin, A.F., Assim, B.Z & Umaru, H.A. (2017). Antibacterial and cytotoxicity action of chloroform crude extract of *Leptadenia hastata (Pers.) Decne*. *Journal of Clinical Biochemistry*, 4(1), 139.

Umaru, I.J., Badruddin, A.F., Assim, B.Z & Umaru, H.A. (2018). Antifungal activity of *Leptadenia hastata* leaves extract. *International Journal of Pure and Applied Bbioscience*, 5(6), 14-18.

Umaru, I.J., Badruddin, A.F., Assim, B.Z & Umaru, H.A. (2018). Phytochemical, antifungal and antibacterial potential of *Leptadenia hastate* stem bark extract. *Medcrave Journal of Toxicology*, 4(4), 263-268.

Umaru, I.J., Umaru H.A. & Umaru, K.I. (2020). Zinc Oxide Nanoparticles Biosynthesis using Leptadenia hastata Leaf Extracts and their Potential as Antimicrobial Agents. *International Journal of Innovative Science and Research Technology*, 5(4), 94-96.

Upadhyay, R.K. (2015). Antimicrobial Activity of Fruit Latexes from Ten Laticiferous Plants. *American Journal of Plant Sciences*, 6, 483-499.

Vambe, M., Aremu, A.O., Chukwujeku, J.C., Gruz, J., Luterova, A., Finnie, J.F. & Van Staden, J. (2020). Antibacterial, Mutagenic properties and Chemical characterization of Sugar Bush (*Protea caffra* Meisn.): A South African Native Shrub Species. *Plants*, 9, 1331.

Varadharaj, V. & Kuppan, M. (2015). Identification and determination of bioactive phytochemical constituents from the hydro-alcoholic extract of *Achyranthes aspera* whole plant by Chromatography-Mass Spectrometry analysis. *Asian Journal of Pharmaceutical and Clinical Research*, 8(6), 125-129.

Verdini, L., Setiawan, B., Sinaga, T., Sulaeman, A. & Wibawan, W.T. (2020). Phytochemical profile of cinnamon extract (*Cinnamomum burmanii blume)* from three regions of Sumatra Island Using GC-MS. *European Journal of Molecular & Clinical Medicine*, 7(2), 4557-4568.

Verma, R., Satsangi, G. P. & Shrivastava, J. N. (2011). Susceptibility of a weed *Calotropis procera* against clinical isolates of dermatophytes. *Journal of Medicinal Plants Research*, 5(19), 4731-4739.

Verma, R.K. & Yadav, M. (2017). Taxonomical studies of calotropis species in ChuruDistrict of Rajasthan. *World Journal of Pharmaceutical Research*, 6(8), 2462-2472.

Vijayakumari, J. & Raj, T.L.S. (2019). GC-MS analysis of secondary metabolites from aceton and chloroform extract of *Dicranopteris linearis* (burm.F.) underw. *International Research Journal of Biological Sciences,* 8(9), 39-43.

Vishnukant, T., & Vidyut K. S. (2017). Chemical profile and Folk-lore uses of *Calotropis procera* (Willd). R.Br. *Indian Journal of Applied & Pure Biology,* 32(2), 289-292 (2017).

Vuuren, S.V., Williams, V.L., Sooka, A., Burger, A. & Harr, L.V. (2014). Microbial contamination of traditional medicinal plants sold ate the Faraday muthi market, Johannesburg, South Africa. *South African Journal of Botany*, 94, 95-100.

Waheed, N., Jabeen, K., Iqbal, S. & Javaid, A. (2016). Biopesticidal Activity of *Calotropis procera* against *Macrophomina phaseolina. African Journal of Traditional Complement & Alternative Medicine*, 13(6), 163-167.

Wiese, J. & Imhoff, F.J. (2019). Marine bacteria and fungi as promising source for antibiotics. *Journal of Drug Development Research*, 80(1), 24-27.

Xie, S., Sinha, R.A., Singh, B.K., Li, G.D., Han, W.P. & Yen, P.M. (2013). Resveratrol induces insulin gene expression in mouse pancreatic alpha-cells. *Cell &Bioscience*, 3, 47-52.

Xing, J., Cheng, Y., Chen, P., Ruan, L.R. & Wang, L. (2019). Effect of high pressure homogenization on the extraction of sulforaphane from broccoli (*Brassica oleracea*) seeds. *Powder Technology*, 358, 103-109.

Xiong, Y., Zhou, L., Zhao, Y., Liu, Y., Liu, X., Zhu, G., Yan, Z & Liu, Z. (2018). Effect of different extraction methods on the extraction rates of five chemical ingredients of swertia musssotii france by UPLC-ESI-MS/MS. *Materials Science and Engineering* 5301(1), 1-24.

Xu, Y., Fan, M., Ran, J., Zhang, T., Sun, H., Dong, M., Zhang, Z. & Zheng, H. (2016). Variation in phenolic compounds and antioxidant activity in apple seeds of seven cultivars. *Saudi Journal of Biological Science,* 23, 379–388.

Yadav, N., Yadav, R. & Goyal, A. (2014). Chemistry of terpenoids. *International journal of Pharmaceutical Science Review and Research*, 27(2), 272-278.

Yang, D.C., Blair, K.M. & Salama, N.R. (2016). Staying in shape; the impact of cell shape on bacterial survival in diverse environments. *Microbiology and Molecular Biology Reviews*, 80 (1), 187–203.

Yaniv, Z. & koltai, H. (2018). Calotropis procera, Apple of Sodom Ethnobotanical Review and Medicinal Activities. *Israel Journal of Plant Sciences*, 65(1), 55-61.

Yen, G.C., Chen, C.S. & Chang, W.T. (2018). Antioxidant activity and anticancer effect of ethanolic and aqueous extracts of the roots of *Ficus beecheyana* and their phenolic components, *Journal of Food and Drug Analysis*, 26(1), 182–192.

Yingngam, B., Monschein, M. & Brantner, A. (2014). Ultrasound-assisted extraction of phenolic compounds from Cratoxylum formosum ssp. Formosum leaves using central composite design and evaluation of its protective ability against H2O2-induced cell death. *Asian Pacific Journal of Tropical Medicine* 7, 497-505.

Zhang, Q., Lin, L. & Ye, W. (2018). Techniques for extraction and isolation of natural products: a comprehensive review. *Journal of Chinese Medicine*, 13 (20), 2-26.

Zhang, Y.L. & Liu, D.M. (2011). Flavonol kaempferol improves chronic hyperglycemia-impaired pancreatic beta-cell viability and insulin secretory function. *European Journal of Pharmacology*, 670, 325–332.

Zhao, Y., Chen, S., Wang, Y. C., Wang, J. & Lu, J. (2018). Effect of drying processes on prenylflavonoid content and antioxidant activity of *Epimedium koreanum* Nakai," *Journal of Food andDrug Analysis*, 26 (2), 796–806,

Zia-Ul-Haq, M., Riaz, M., DeFeo, V., Jaafar, H.Z.E. & Moga, M. (2014). Rubus fruticosus L. constituents, biological activities and health related uses. *Molecules*, 19(8), 10998–1029.

Złotek, U., Mikulska, S., Nagajek, M. Swieca, M. (2018). Effect of different solvents and number of extraction steps on the polyphenol content and antioxidant capacity of basil leaves (*Ocimum basilicum* L.) extracts. *Saudi Journal of Biological Sciences*, 23(5), 628–633.

APPENDIX

Appendix A: Molecular Identification of the Gene Sequence Amplified from the Isolates

> MK572634.1 *Pseudomonas aeruginosa* strain IDZU7

AAAGCGCTGGCAGCAGGGGCCTTCAACACATGCAAGTCGAGCTTATGAAGGGA
GCTTGCCTTGGATTCAGCGGCGGACGGGTGAGTAATGCCTAGGAATCTGCCTGG
TAGTGGGGGATAACGTCCGGAAACGGCCGCTAATACCGCATACGTCCTGAGGG
AAAAAGTCGGGGATCTTCGGACCTCACGCTATCAGATGAGCCTAGGTCGGATTA
GCTAGTTGGTGGGGTAAAGGCCTACCAAGGCGACGATCCGTAACTGGTCTGAGA
GGATGATCAGTCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCA
GCAGTGGGGAATATTGGACAATGGGCGCAAGCCTGATCCAGCCATTGCCGCGTG
TGTGAAGAAGGTCTTCGGATTGTAAAGCACTTTAAGTTGGGAGGAAGGGCAGTA
AGTTAATACCTTGCTGTTTGACGTTACCAACAGAATAAGCACCGGCTAACTTCG
TGCCAGCAGCCGCGGTAATACGAAGGGTGCAAGCGTTAATCGGAATTACTGGG
CGTAAAGCGCGCGTAAGTGGTTCAGCAAGCTTGATGTGAAATCCCCGGGCTCAA
CCTGGGAACTGCATCCAAAAGCTACTGAGCTAGAGTACGGTAGAGGTGGTAGA
ATTTCCTGTGTAGCGGTGAAATGCGTAGATATAGGAAGGAACACCAGTGGCGA
AGGCGACCACCTGGACTGTACTGACACTGAGGTGCGAAAGCGTGGGGAGCAAA
CAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCGACTAGCCGGTTG
GGATCCTTGAGATCTTAGTGGCGCACGTAACGCGATAAGTCGACCGCCTGGGGA
GTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGG
TGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCTGGCCTTGACA
TGCTGAGAACTTTCCAGAGATGGATTGGTGCCTTCGGGAACAGAGACACAGGTG
CTGGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGTAAC
GAGCGCAACCCTTGTCCTTAGTTACCAGCACCTCGGGTGGGCACTCTAAGGAGA
CTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAGTCATCATGGCCC
TTACGGCCAGGGCTACACACGTGCTACAATGGTCGGTACAAAGGGTTGCCAAGC
CGCGAGTGGGAGCTAATCCCATAAAACCGATCGTAGTCCGGATCGCAGTCTGCA
ACTCGACTGCGTGAAGTCGGAATCGCTAGTAATCGTGAATCAGAATGTCACGGT
GAATACGTCCCCGGGCCTTGTACACACCGCCCGTCACACCATGGGAGTGGGTTG
CTCCAGAAGTAGCTAGTCTAACCGCAAGGGGG

> MN623691.1 *Salmonella bongori* strain IDZU8

AAATTGAAAAGTTTGGTATGGCTCAGATTGAACGCTGGCGGCAGGCCTAACACA
TGCAAGTCGAACGGTAACAGGAAGCAGCTTGCTGCTTCGCTGACGAGTGGCGG
ACGGGTGAGTAATGTCTGGGAAACTGCCTGGTGGAGGGGGATAACTACTGGAA
ACGGTGGCTAATACCGCATAACGTCGCAAGACCAAAGAGGGGGACCTTCGGGC
CTCTTGCCATCAGATGTGCCCAGATGGGATTAGCTTGTTGGTGGGGTAACGGCC
CACCAAGGGACGATCCCTAGCTGGTCTGAGAGGATGACCAGCCACACTGGAAC
TGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAAT
GGGCGCAAGCCTGATGCAGCCATGCCGCGTGTATGAAGAAGGCCTTCGGGTTGT
AAAGTACTTTCAGCGGGGAGGAAGGTGTTGTGGTTAATAACCACAGCAATTGAC
GTTACCCGCAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATAC

GGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGT
CTGTCAAGTCGGATGTGAAATCCCCGGGCTCAACCTGGGAACTGCATCCGAAAC
TGGCAGGCTTGAGTCTCGTAGAGGGGGGTAGAATTCCAGGTGTAGCGGTGAAAT
GCGTAGAGATCTGGAGGAATACCGGTGGCGAAGGCGGCCCCCTGGACGAAGAC
TGACGCTCAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAG
TCCACGCCGTAAACGATGTCGACTTGGAGGTTGTGCCCTTGAGGCGTGGCTTCC
GGAGCTAACGCGTTAAGTCGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAAC
TCAAATGAATTGACGGGGGCCCGCACAAGCCGGGGAGTATGTGGTTTAATTCGA
TGCAACGCGAAGAACCTTACCTGGTCTTGACATCCACGGAAGAATCCAGAGATG
GATTTGTGCCTTCGGGAGCCGTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCG
TGTTGTGAAATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATCCTTTGTTG
CCAGCGGTCCGGCCGGGAACTCAAAGGAGACTGCCAGTGATAAACTGGAGGAA
GGTGGGGATGACGTCAAGTCATCATGGCCCTTACGACCAGGGCTACACACGTGC
TACAATGGCGCATACAAAGAGAAGCGACCTCGCGAGAGCAAGCGGACCTCATA
AAGTGCGTCGTAGTCCGGATTGGAGTCTGCAACTCGACTCCATGAAGTCGGAAT
CGCTAGTAATCGTGGATCAGAATGCCACGGTGAATACGTTCCCGGGCCTTGTAC
ACACCGCCCGTCACACCATGGGAGTGGGTTGCAAAAGAAGTAGGTAGCTTAAC
CTCCGGGG

> CP074120.1 *Escherichia coli* strain IDZU9

CATGATCATGGCTCAGATTGAACGCTGGCGGCAGGCCTAACACATGCAAGTCGA
ACGGTAACAGGAAGAAGCTTGCTTCTTTGCTGACGAGTGGCGGACGGGTGAGTA
ATGTCTGGGAAACTGCCTGATGGAGGGGGATAACTACTGGAAACGGTAGCTAA
TACCGCATAACGTCGCAAGACCAAAGAGGGGGACCTTCGGGCCTCTTGCCATCG
GATGTGCCCAGATGGGATTAGCTAGTAGGTGGGGTAACGGCTCACCTAGGCGAC
GATCCCTAGCTGGTCTGAGAGGATGACCAGCCACACTGGAACTGAGACACGGTC
CAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCT
GATGCAGCCATGCCGCGTGTATGAAGAAGGCCTTCGGGTTGTAAAGTACTTTCA
GCGGGGAGGAAGGGAGTAAAGTTAATACCTTTGCTCATTGACGTTACCCGCAGA
AGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGCAAG
CGTTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGTTTGTTAAGTCAGA
TGTGAAATCCCCGGGCTCAACCTGGGAACTGCATCTGATACTGGAAGCTTGAGT
CTCGTAGAGGGGGGTAGAATTCCAGGTGTAGCGGTGAAATGCGTAGAGATCTG
GAGGAATACCGGTGGCGAAGGCGGCCCCCTGGACGAAGACTGACGCTCAGGTG
CGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAA
CGATGTCGACTTGGAGGTTGTGCCCTTGAGGCGTGGCTTCCGGANNTAACGCGT
TAAGTCGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGA
CGGGGGCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGATGCAACGCGAAGA
ACCTTACCTGGTCTTGACATCCACGGAAGTTTTCAGAGATGAGAATGTGCCTTC
GGGAACCGTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTTGTGAAATGT
TGGGTTAAGTCCCGCAACGAGCGCAACCCTTATCCTTTGTTCCCAGCGGTCCGG
CCGGGAACTCAAAGGAGACTGCCAGTGATAAACTGGAGGAAGGTGGGGATGAC
GTCAAGTCATCATGGCCCTTACGACCAGGGCTACACACGTGCTACAATGGCGCA

TACAAAGAGAAGCGACCTCGCGAGAGCAAGCGGACCTCATAAAGTGCGTCGTA
GTCCGGATTGGAGTCTGCAACTCGACTCCATGAAGTCGGAATCGCTAGTAATCG
TGGATCAGAATGCCACGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTC
ACACCATGGGAGTGGGTTGCAAAAGAAGTAGGTAGCTTAACTTCGGGGG

> AB642255.1 *Klebsiella pneumoniae* strain IDZU10

AAAGGTTTTTCCCGGCCAGATTGAACGCTGGCGGCAGGCCTAACACATGCAAGT
CGAGCGGTAGCACAGAGAGCTTGCTCTCGGGTGACGAGCGGCGGACGGGTGAG
TAATGTCTGGGAAACTGCCTGATGGAGGGGGATAACTACTGGAAACGGTAGCT
AATACCGCATAACGTCGCAAGACCAAAGTGGGGGACCTTCGGGCCTCATGCCAT
CAGATGTGCCCAGATGGGATTAGCTAGTAGGTGGGGTAACGGCTCACCTAGGCG
ACGATCCCTAGCTGGTCTGAGAGGATGACCAGCCACACTGGAACTGAGACACG
GTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAAG
CCTGATGCAGCCATGCCGCGTGTGTGAAGAAGGCCTTCGGGTTGTAAAGCACTT
TCAGCGGGGAGGAAGGCGTTAAGGTTAATAACCTTGGCGATTGGCGTTACCCGC
AGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGC
AAGCGTTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGTCTGTCAAGTC
GGATGTGAAATCCCCGGGCTCAACCTGGGAACTGCATTCGAAACTGGCAGGCTA
GAGTCTTGTAGAGGGGGGTAGAATTCCAGGTGTAGCGGTGAAATGCGTAGAGA
TCTGGAGGAATACCGGTGGCGAAGGCGGCCCCCTGGACAAAGACTGACGCTCA
GGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCG
TAAACGATGTCGATTTGGAGGTTGTGCCCTTGAGGCGTGGCTTCCGGAGCTAAC
GCGTTAAATCGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAA
TTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGATGCAACGCG
AAGAACCTTACCTGGTCTTGACATCCACAGAACTTAGCAGAGATGCTTTGGTGC
CTTCGGGAACTGTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTTGTGAA
ATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATCCTTTGTTGCCAGCGGTT
AGGCCGGGAACTCAAAGGAGACTGCCAGTGATAAACTGGAGGAAGGTGGGGAT
GACGTCAAGTCATCATGGCCCTTACGACCAGGGCTACACACGTGCTACAATGGC
ATATACAAAGAGAAGCGACCTCGCGAGAGCAAGCGGACCTCATAAAGTATGTC
GTAGTCCGGATTGGAGTCTGCAACTCGACTCCATGAAGTCGGAATCGCTAGTAA
TCGTAGATCAGAATGCTACGGTGAATACGTTCCCGGGCCTTGTACACACCGCCC
GTCACACCATGGGAGTGGGTTGCAAAAGAAGTAGGTAGCTTAACCTTAGGGG