ANTIBACTERIAL ACTIVITIES AND PHYTOCHEMICAL COMPOSITION OF STEM BARK AND SEEDS EXTRACTS OF THREE BOTANICALS ON THREE SELECTED PATHOGENIC BACTERIA

BY

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MTECH/SLS/2018/8994

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FEDERAL UNIVERSITY OF TECHNOLOGY MINNA, NIGER SATE

NOVEMBER, 2022

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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

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ABSTRACT

The increasing rate of development of resistance to commonly used antibiotics have necessitates the need to re-evaluate some of the plants that have been previously studied due to changes in ecosystem and geographical variations. The aim of this study was to investigate the antibacterial activities and phytochemical constituents of *Boswella dalzielii* (stem), *Enantia chlorantha* (stem), and *Abrus precatorius* (seeds) extract. The plant materials were extracted using cold merceration technique and weight percentage yield were recorded for each plant extract. The plants extracts were prepared at different concentration of 50, 100, 150, 200, 250, and 300 mg/ml against *Escherichia coli, Pseudomonas aeruginosa* and *Salmonella bongori*. Antibacterial activity and quantitative phytochemical analysis of the extracts were carried out using agar well diffusion method and spectrophotometry respectively. Bioactive compounds present in the fractions were determined using Gas Chromatography-Mass Spectrometry (GC-MS). The minimum inhibitory concentrations (MICs) and minimum bactericidal concentration (MBC) of the extracts and fractions were also determined using the broth micro-tube dilution technique. A significant antibacterial activity was observed against *E. coli* for *B. dalzielii* stem (7.50±0.00-18.45±00.25 mm), *E. chlorantha* stem (5.35±0.65-15.90±0.10 mm), and *A. precatorius* seed (8.75±0.25-14.00±1.00 mm) and the lowest activity was observed against *P. aeruginosa* for the three plant 6.70±0.30-12.80±0.20, 4.15±1.15-5.60±0.40, 9.80±0.50 mm respectively. The n-Hexane fraction shows a narrow zone of inhibition for all the three plants extract, the aqueous fraction has a significant activity against *E. coli* for *B. dalzielii* stem (11.50±1.50-19.50±0.50 mm), *E. chlorantha* stem (5.55±0.45-15.50±45.5 mm) and *A. precatorius* seed (7.75±1.25-12.60±0.40 mm) and a minimal activity was observed against *P. aeruginosa* for the three plant extracts as 8.50±0.50-11.50±0.50mm, 8.55±1.55-11.75mm, 4.50±0.30-6.90±0.10mm respectively. The ethyl acetate fraction was observed to have a significant activity against *E. coli* for *B. dalzielii* stem (9.00±1.00-17.50±0.50mm), *E. chlorantha* stem (5.40±1.05-10.05±0.05mm) and *A. precatorius* seed (5.95±0.50-6.95±0.25mm). The MIC and MBC of the extract ranged between 35.5 to 300 mg/mL against the bacterial isolates. The GC-MS analysis of the *B. dalzielii* fraction revealed 6 compounds for aqueous fraction, 6 compounds for ethyl acetate and 5 compound *for n-Hexane* with 9,12-octadecadienoic acid (z,z)-,2,3-dihydroxypropyl (50.05%), 9,12-octadecadienoic acid (z,z)- (20.50) and 9,12-octadecadienoic acid (z,z)- (5.46%) with the highest concentration respectively. 5 compounds for *A. precatorius* with 1,2,3-Benzenetriol (ethylacetate fraction at 30.83%) and 1,5-anhydro-d-mannitol (residual aqueous fraction at 31.77%) with the highest concentration respectively and 4 compounds for *E. chloranta* with 9,12-octadecadienoic acid (z,z)-2,3-dihydroxypropyl (ethylacetate fraction at 28.05%) had the highest concentration. The quantitative phytochemical screening of the plants extracts revealed the presences of phenols, flavonoids, saponins, alkaloids and tannins with highest phenol content observed with *B. dalzielii* (989.04±0.60) and the lowest was observed with *E. chlorantha* (416.07±0.07). The extracts of stem bark of *Boswella dalzielii, A. precatorius* seed and *E. chlorantha* stem barks exhibited antibacterial and contained many active compounds.

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CHAPTER ONE

1.0 INTRODUCTION

1.1 Background to the Study

Pathogenic microorganisms have unfavorable effects on the quality and safety of life (Sylvester *et al*., 2016). The emergence of modern medicine opens up much more knowledge about human pathologies and the mode of intervention to fight its discomforts (Fifa *et al*., 2019). According to World Bank and World Health Organization (WHO), millions still pushed into extreme poverty because of health expenses, half the world lacks access to essential health services. Added to this are other factors including the low coverage of medical personnel, the lack and poor distribution of health infrastructure (Nazifi *et al.,* 2017). Synthetic chemicals are widely used against the microorganisms. However, most of the pathogenic bacteria have developed resistance to modern antibiotics leading to multi drug resistance among bacteria (Anyim *et al.,* 2010).

Development of Antimicrobial Drug Resistance (ADR) is a natural phenomenon (Aleksic and Knezevic, 2018). However, certain human actions accelerate the emergence and spread of ADR. Inappropriate therapeutic use of antimicrobial drugs, and use in agriculture, fish, poultry and animal farming, leads to the emergence and selection of resistant strains. In addition, poor control practices and infection prevention further contribute to emergence and spread of ADR. Prominent organizations like WHO, have collaborated to promote best practices to avoid the emergence and spread of antibacterial resistance. All attempts are in progress to promote optimal use of antibiotics both in humans and animals to address problem of growing Antimicrobial resistance (AMR).

Traditional medicine, also known as botanical medicine or phytomedicine refers to the use of herbs for their therapeutic and medicinal value. They are derived from plants or plant

extracts containing therapeutic substances (Akhlash *et al*., 2019). Alternatively, Traditional medicine is the use of plants' seeds, berries, roots, bark or flowers for medicinal purpose and this have been long practiced outside conventional medicine (Saranraj and Sivasakthi, 2019). The herbal medicine practice is generally known as complementary and alternative medicine (CAM). Many plant extracts and essential oils are relatively easy to obtain, have low mammalian cell toxicity, and degrade quickly in water and soil, making them relatively easy to use and environment friendly antibiotic alternatives (Temitayo *et al*., 2020). Antimicrobials of plant origin have therapeutic potential and they are useful in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials (Musa *et al*., 2016).Plants produce chemical compounds or bioactive components often referred to as phytochemicals (phyto in Greek translation meaning 'plant') or phyto constituents are responsible for protecting the plant against microbial infections or infestations by pests (Doughari *et al.,* 2019).The beneficial medicinal effects of plant materials typically result from the combinations of secondary products which are more present in the plant (Saranraj and Sivasakthi, 2019). In plants, these compounds are mostly secondary metabolites (phytochemical compounds) such as alkaloids, steroids, tannins and phenol compounds, flavonoids, steroids, resins fatty acids gums which are capable of producing definite physiological action on body. Compounds extracted from different parts of the plants can be used to cure diarrhea, dysentery, cough, cold, cholera and fever (Odeghe *et al.,* 2012).

Medicinal plants are assuming greater importance in the primary health care of individuals and communities in many developing countries (Abubakar and Ibrahim, 2019). There has been an increase of demand in international trade because of very effective, cheaply available, supposedly have no side effects and used as alternative to allopathic medicines.

Medicinal plants are believed to be much safer and proved elixir in the treatment of various ailments (Midéko *et al.,* 2017).

Herbal drugs are used by physicians for hundreds of years as indigenous systems of medicine and approximately 80% of the world populations still use them for primary health care (Gupta *et al.,* 2013). There are innumerable uses of herbal medicines; therefore, there is a need to revamp research to develop alternative antimicrobial drugs for the treatment of infectious diseases (Ukwuani and Igbokwu, 2015).

Boswellia dalzielii (family *Burseraceae*), commonly known as frankincense tree; is a tree plant, abundantly found in north-western Nigeria (Musa *et al*., 2016). It is known as "Arrabi or Hano" among the Hausa speaking people of Northern Nigeria (Iyabo *et al,* 2018), the tree can grow up to 12m high with a pale brown and smooth stem bark, peeling off in thin ragged papery patches (Nazifi *et al*., 2017). The bark is reddish brown, and its exudates are a whitish fragrant resin (Nazifi *et al*., 2017). It is the West African species of the frankincense-producing plants - *B. carteri*, *B. frereana* and *B. serrata*. This plant is very popular among the locals as a potent source of ethnomedicine (Zerbo *et al.,* 2013). The decoction of the stem bark is used to treat septic sores, rheumatism, gastrointestinal ailments, and venereal diseases (Ohadoma *et al.,* 2014).

Phytochemical studies of the plant revealed the presence of tannins, saponins, cardiac glycosides, flavonoids, steroids, and terpenes (Hassan *et al*., 2009), while alkaloids were found to be absence (Midéko *et al*., 2017), However, in spite of its numerous applications in traditional medical practice in the sub region, the West African species is less known and studied compared to its more popular congeners. In addition, the aqueous (dialyzed) extract of the dried gum resin from Cameroon has been shown to possess anti-inflammatory activity in male rats (Hassan *et al*., 2009). The methanol and aqueous

extracts also showed broad-spectrum of antibacterial and antifungal activities (Okoro *et al.,* 2014).

Enantia chlorantha (family- *Annonaceae*) locally known as Awopa (Yoruba), Oso pupa or Dokita (Igbo), is a tree of the high rainforest of up to 30m high and 70cm girth, with along clear bole. This tree grows in dense shade and may be recognized by its bright yellow slash and conspicuous black fruits (Maximilienne *et al*., 2018). Its distribution is limited to South Nigeria, West Cameroons and Fernando Po, however may also be found in Gabon, Angola and Zaire (Oheme *et al*., 2018).

The plant is mainly used in the treatment of malaria fever in Nigeria. Studies have reported the ethno-pharmacological uses of *E. chlorantha* as a hepatoprotective, antiviral, antimalarial, antibacterial and antiulcer agent (Oheme *et al*., 2018). According to Ayoade *et al.,* (2017), stem bark of *E. chlorantha* extract is used to treat jaundice and urinary tract infections.

Abrus precatorius belongs to family *fabaceae*. It grows in tropical climates such as India, Sri Lanka, Thailand, the Philippine Islands, South China, tropica Africa and the West Indies. It also grows in all tropical or subtropical areas (Varaprasad and Varahalarao, 2009). Its commonly known as jequirity, crab's eye, rosary pea, precatory pea or bean, john crow bead. The most poisonous part of the plant is the seed. The seed contains the toxic poison abrin which is close relative to ricin. Ingested seeds can affectthe gastrointestinal tract, the liver, spleen, kidney, and the lymphatic system. Infusion of seed extracts can cause eye damage after contact. The most poisonous parts of the plant involved in poisoning are the small, scarlet seeds that have a black eye at the hilum. The roots, stems, and leaves also contain glycyrrhizin (Chiamaka and Emeka, 2020). The seeds were also used to treat diabetes and chronic nephritis. The plant is also used in some traditional medicine to treat scratches and sores, and wounds caused by dogs, cats and

mice and are also used with other ingredients to treat leucoderma (Abou El-Khair *et al*., 2010). They are ground with lime and applied on acne sores, boils, and abscesses. The plant is also traditionally used to treat tetanus, and to prevent rabies. Boiled seeds of *A. precatorius* are eaten in certain parts of India (Abou El-Khair *et al*., 2010).

1.2 Statement of the Research Problem

The disadvantages of antibiotics used are manifested in the development of resistance, side-effects, and opportunistic pathogens. The emergence and spread of antibacterial resistance in all geographical areas, including in bacteria that cause hospital- and community-acquired infections, is, however, jeopardizing the effectiveness of potentially life-saving treatments (Janmejaya, 2014). The threat includes the spread of multidrug resistant bacteria, severe adverse reaction, high cost and infections with no therapeutic options have been reported (Woon and Fisher, 2016). The rise in resistance not only impedes the ability to treat bacterial infections in humans and animals but has broader societal and economic effects that ultimately threaten achievement of the Sustainable Development Goals.

Emergence of resistant strains of pathogenic microorganisms has also continued to pose a major health concern about the efficacy of several drugs, most importantly and antibiotics in current use (Kokilam and Vasuki, 2014). Causes of the widespread and development of antibiotic resistance are multifactorial, including the specific nature of the relationship of bacteria to antibiotics, the usage of antibacterial agent, host characteristics, environmental factors, often inappropriate use of antibacterial agents such as; broad-spectrum drugs and incomplete compliance with basic infection control practices such as hand washing (Zerbo *et al.,* 2013).

A reason for limiting the use of antibiotic agents for genuine therapeutic indications is their ability sometimes to induce severe or fatal adverse reactions (Alison *et al.,* 2012). Generalized adverse events are common to most antibiotics (gastrointestinal distress with any oral antibacterial drug), but certain antibiotics are associated with specific effects such as hypersensitivity, effect on commensal flora, Drug interactions and toxicity. Some adverse events are mild, for instance yellowing of the teeth for tetracyclines (Alison *et al.,* 2012), increased intestinal peristalsis related to erythromycin therapy.

Antibiotics, particularly those with a wide spectrum of activity, alter the normal flora of the body, allowing colonization by and multiplication of resistant and opportunistic pathogens (Moses *et al*., 2019). This may then cause secondary infection such as candida vaginitis in a healthy woman, or fungal and systemic infection in a highly susceptible patient such as a transplant recipient on immunosuppressive therapy (Janmejaya, 2014; Alison *et al.,* 2012).

1.3 Aim and Objectives of the Research

This study was designed to investigate the antibacterial activities and phytochemical composition of stem bark and seeds extract of *Boswella dalzielii, Enantia chlorantha,* and *Abrus precatorius* on three selected pathogenic bacteria.

The objectives of the study were to:

i isolate and identify pathogenic bacteria.

ii determine the Molecular characterization of Bacterial isolates.

iii determine the antibacterial activities of the plants extracts.

iv determine the Minimum Inhibitory Concentrations (MIC) of the plants extracts and Minimum Bactericidal Concentrations (MBC) of the plants extracts

v fractionate the crude extract from (iii) above

vi determine the compounds present in the purified fraction using GC-MS analysis.

vii determine the quantitative phytochemical constituents of plants extracts

1.4 Justification for the Research

The increasing rate of development of resistance to commonly used antibiotics has led to the search for newer, more effective, affordable and readily available sources with less side effects, in particular, from local medicinal plants (herbs) as the cost of production of synthetic drugs is high and they produce adverse effects compared to plant derived drugs (Amir *et al*., 2009).

The demand for the herbal drugs has increased in recent times, as many plants or herbs are scientifically proven to contain bioactive compound(s) and as alternatives to harmful synthetic drugs that cause side effects to biological system and environment (Collignon *et al.,* 2015). The herbal drugs have been used for treatment of many infectious diseases in humans as well as in animals all over world since ancient times (Henley-Smith *et al.,* 2013; Kubmarawa *et al.,* 2013).

Antimicrobial substances of natural origin are environmentally friendly and have found application as biological control agents (Emmanuel *et al.,* 2015). However, some medicinal plants/herbs have not found wider application and sometimes are referred as forgotten plants (Abubakar and Ibrahim, 2019). This necessitates the need to re-evaluate some of the plants that have been previously studied due to changes in ecosystem and geographical variations which may alter the chemical composition of these plants.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 *Boswella dalzielii*

Boswellia dalzielii (*B. dalzielii*) hutch is from the family of *Burseraceae* and the genus Boswellia. It is a tree plant of about 13m high and abundantly found in North -western Nigeria; it's very common among the locals as a potent source of ethnos medicine (Figure 2.1). *Boswellia dalzielii* is commonly known in English as the frankincense tree while in Ghana, it is generally called pianwogu (Onoriose *et al*., 2012). In Nigeria, the Hausa speaking people calls it Hano or Harrabi, Fulfulde calls it juguli, and the Kanuri people call it kafi-dukkan. While in Niger, the fula-fulfulde calls it adakehigorki (Emmanuel *et al.,* 2015).

Traditionally, the combination of a decoction of the dried-crushed bark (*B. dalzielii*) is used to treat malaria, yellow fever, stomach ailments, and many childhood diseases (Ververidis *et al.,* 2007). The bark has antiseptic, healing, and antifungal potential and is used externally to treat sores, ulcers and dental caries (Kubmarawa *et al.,* 2013). It's also used to treat rheumatism, asthma, wounds, diarrhea, gastrointestinal disorders, appendicitis, dizziness, palpitations, pleurisy, leprosy, and bloating in cattle (Henley-Smith *et al.,* 2013; Kubmarawa *et al.,* 2013;).

The roots and barks are used for antidote to arrow poisons. In Nigeria (specifically in Adamawa state), the fresh bark is eaten to cause vomiting after a few hours to relieve symptoms of giddiness and palpitations (Azwanida, 2015). The extract from it leaves is used for the treatment of diarrhea in poultry and humans (Alemika *et al*., 2018).

2.2 *Enantia chlorantha*

E.*chlorantha* Oliv [*Annonaceae*] is an ornamental tree of up to 30 m high, with dense foliage and spreading crown. The stem is fluted, the bark fissured geometrically and with

bright yellow wood (Figure 2.3). The leaves display up to 20 pairs of prominent lateral veins and parallel secondary nerves. It is commonly called "Dokitainigbo" and Awopka in yoruba, in Nigeria (Toyin and Ayoade, 2008).

E. chlorantha is used traditionally in the treatment of malaria and other ailments of the body such as cough and wounds (Maximilienne *et al.,* 2018). The antiviral, antibacterial and antipyretic properties of *E. chlorantha* had also been documented. It has been shown to confer cytoprotection on the mucosa lining of the gastrointestinal tract through its antiulcerative effect.

2.3 *Abrus precatorius*

Abrus precatorius belong to family *Fabaceae*. It grows in tropical climates such as India, Sri Lanka, Thailand, the Philippine Islands, South China, tropical Africa and the West Indies. It also grows in all tropical or subtropical areas (Chiamaka and Emeka, 2020). The most poisonous part of the plant is the seed (Figure 2.2). The seed contains the toxic poison abrin (Manisha *et al.,* 2013) which is close relative to ricin. Ingested seeds can affect the gastrointestinal tract, the liver, spleen, kidney, and the lymphatic system. Infusion of seed extracts can cause eye damage after contact (Manisha *et al.,* 2013). The roots, stems, and leaves also contain glycyrrhizin. It is a beautiful, much-branched, slender, perennial, deciduous, woody, prickly twining or climbing herb. Stem cylindrical, wrinkled, bark smooth-textured, brown.

Abrus precatorius is traditionally used to treat tetanus, and to prevent rabies. Leaves, roots and seeds are used for medicinal purposes. The plant is used in some traditional medicine to treat scratches and sores and wounds caused by dogs, cats and mice, and are also used with other ingredients to treat leucoderma (Sonali and Shonkor, 2020). The leaves of the herb are used to cure fever, cough and cold. They have anti-suppurative properties. They

are ground with lime and applied on acne sores, boils and abscesses (Varaprasad and Varahalarao, 2009). Decoction of leaves is taken orally for cough and flu (Manisha *et al*., 2013; Mensah *et al*., 2011). The roots of Abrus herb are used to treat jaundice and haemoglobinuric bile. Paste of roots is administered to cure abdominal pains, tumors and also for abortion (Bapat and Sane, 2012).

2.4 Antibacterial activity of the research plant

2.4.1 Antibacterial activity

Recently, there has been a lot of attention focused on producing medicines and products that are from natural source. Several stem bark, root, leaves and leaf extracts have been found to have antimicrobial activity against microorganisms (Nas and Ali, 2017). The antimicrobial properties of plants have been investigated by a number of researchers worldwide and the biological evaluation of plants extracts is vital to ensure their efficacy and safety (Funke and Tortara, 2009). These factors are of importance if plant extracts are to be accepted as important agents for the treatment of infectious diseases especially in light of the emergence of drug-resistant microorganisms (Umar *et al.,* 2018).

According to Moses *et al.* (2015); Umar *et al.* (2018), the stem bark of *Boswellia dalzielii* has activity against both Gram-positive and Gram-negative bacteria (broad spectrum activity). Some of the Gram-negative bacteria include; *Klebsiella pneumoniae, Escherichia coli, Proteus mirabilis* and *Pseudomonas aeruginosa,* while Gram-positive bacteria include *Staphylococcus aureus, Staphylococcus epidermidis, Bacillus cerens and Bacillus megaterium.* In general, the plant has activity against Gram-positive bacteria than Gram-negative bacteria and this is due to the complex nature of Gram-negative bacteria cell wall which makes entry of drugs difficult. However, this justifies the use of this plant in traditional medicine for the treatment of bacterial infections.

2.4.2 Antifungal activity

Fungi are pathogenic microorganisms causing a number of skin infections, nail or hair, minor infections of mucous membranes or systemic infections causing progressive often fatal disease (Zaki *et al*., 2014).

According to most Researchers, the crude extract, fractions and sub-fractions of the stem bark of *Boswellia dalzielii* has activity against *Candida albicans* and only ethyl acetate and aqueous fractions has activity against *Penicillium notatum* (Moses & Yvonne, 2015). However, no activity was recorded for the methanol extract, fractions and sub-fractions of stem bark of *Boswellia dalzielii* against *Aspergillus niger* (Yves *et al*., 2017).

2.4.3 Antiviral activity

Since ancient times, medicinal plants have been used in the treatment of diseases and infection, including viral infections (Anganette *et al*., 2019). Viral infections are almost always followed by a secondary bacterial infection, thus, resulting in the world's most transmissible diseases (Temitayo *et al.,* 2020). The high cost of available antiviral drugs and their toxic side effects, viral resistance coupled with viral latency and conflicting efficacy in recurrent infection in immunocompromised patients has made viral disease a major and continuous burden for researchers (Ngono *et al*., 2011).

According to Temitayo *et al.* (2020); Oheme *et al*. (2018), the antiviral assay of the stem bark of *B. dalzielli* confirm that the plant has antiviral potential against Newcastle diseases virus (NDV).

2.5 Phytochemical Contents

Phytochemicals (from the Greek word phyto, meaning plant) are biologically active, naturally occurring chemical compounds found in plants, which provide health benefits for humans. They protect plants from disease and damage and contribute to the plants

color, aroma and flavor (Geetha and Nallani, 2018). In general, the plant chemicals that protect plant cells from environmental hazards such as pollution, stress, drought, UV exposure and pathogenic attack are called as phytochemicals. Recently, it is clearly known that they have roles in the protection of human health, when their dietary intake is significant (Ida *et al*., 2018). More than 4,000 phytochemicals have been cataloged and are classified by protective function, physical characteristics and chemical characteristics (Isman, 2013). Plant-derived substances have recently become of great interest owing to their versatile applications (Isman, 2013). Medicinal plants are the richest bio-resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs (Geetha and Nallani, 2018).

2.5.1 Classification of Phytochemical Compounds

Phytochemicals are classified as primary or secondary metabolites, depending on their role in plant metabolism.

2.5.1.1 Primary metabolites

Primary metabolism is important for growth and development of plants include the common sugars, aminoacids, proteins, purines and pyrimidines of nucleic acids, chlrophyll's.

2.5.1.2 Secondary metabolites

Secondary metabolites in plant play a major role in the survival of the plant in its environment (Joseph *et al*., 2013). Attractions of pollinators, natural defense system against predators and diseases, etc., are examples of the roles of secondary metabolites (Akhtar *et al.*, 2018).

The secondary metabolites formed also are an important trait for our food plants (taste, colour, scent, etc.) and ornamental plants. Moreover, numerous plant secondary

metabolites such as flavonoids, alkaloids, tannins, saponins, steroids, anthocyanins, terpenoids, rotenoids etc. have found commercial application as drug, dye, flavour, fragrance and insecticide (Akhtar *et al.*, 2018). Plant produces these chemicals to protect itself but recent research demonstrates that many phytochemicals can protect humans against diseases including cancer, cardiovascular disease, arthritis and diabetic (Joseph *et al.,* 2013).

The phytochemical screening of methanolic extract of *Boswellia dalzielii*stem bark revealed the presence of alkaloid, coumarin, flavonoid, tannin, anthocyanin, carbohydrates, anthraquinone and terpenoid. (Abubakar and Ibrahim,2019).

Phytochemical studies had revealed the presence of alkaloids in E. *chlorantha.* The alkaloids include palma-tine, columbamine and pseudocolumbamine. The alkaloids are thought to be the active components, also present are simple sugars and saponins (Toyin and Ayoade, 2008).

2.6 Plant Material

Figure 2.1: *Boswellia dalzielii* tree Source**:** (Kubmarawa *et al.,* 2013)

Figure 2.2: *Abrus precatorius* Seed Source**:** (Toyin and Ayoade, 2008).

Figure 2.3*: Enantia chlorantha* stem bark Source: (Maximilienne *et al.,* 2018).

2.7 Research Organism

2.7.1 *Pseudomonas aeruginosa*

Pseudomonas aeruginosa is a Gram-negative, motile, and non-fermenting organism, belonging to the *Pseudomonadaceae* family. The pathogenic organism has a rod-shaped and blue-green pigmented bacterium (Alaa, 2015). By 1960s, *P. aeruginosa* emerged as an important human infectious agent (Kosanic *et al.,* 2013; Alaa, 2015). In 1961, it was observed that this organism cause both severe acute and chronic infections (Alaa, 2015). Despite the use of drugs for several decades, it still remains one of the most difficult to treat organisms (Mussaret and Teresa, 2014).

2.7.1.1 Nosocomial infection due to p. aeruginosa

Nosocomial infections (hospital-acquired infection) are those not present or incubating at the time of hospital admission, but usually develop post-admission (Gupta *et al.,* 2013). The major predisposing factors responsible in the acquisition of this infection in the hospital are antibiotic therapy, mechanical ventilation, chemotherapy and surgery (Tan *et al.,* 2010). It is worth noting however that difficulties in treatment of such infections and the associated morbidity and mortality, have made *P. aeruginosa* on of the most feared hospital pathogens (Tan *et al*., 2010).

2.7.1.2 Burn wound infections

Pseudomonas aeruginosa causes an invasive infection in burn patients. Approximately 75% of all deaths in patients with severe burn are related to sepsis from invasive burn wound infection (Gupta *et al.,* 2013). In addition to wounded skin injury, inhalation injury is common in burn patients. However, leads to edema and sloughing of the respiratory tract mucosa and thus making these patients more prone to upper respiratory tract infections and also *Pseudomonas aeruginosa* pneumonia (Thuong *et al*., 2013).

2.7.1.3 Bacteremia

Pseudomonas aeruginosa is among the leading causes of nosocomial bacteremia and thus, leads to sepsis. During the 1960s polymyxins and aminoglycosides were the only options for treatment of *Pseudomonas aeruginosa* bacteremia but were found to be ineffective for these infections (Rios *et al*., 2015).

2.7.1.4 Hospital-associated pneumonia

Pseudomonas aeruginosa uses the human respiratory tract as a favorable environment for adaptation. *P.aeruginosa* has the ability to cause both acute nosocomial pneumonia and chronic infections in the lung (Rios *et al*., 2015).

2.7.1.5 Ventilator-associated pneumonia

It is the most common multidrug resistance agent involved in this disease and recovery rate of *Pseudomonas aeruginosa* is increased with increased duration of mechanical ventilation (Thuong *et al.,* 2013).

2.7.2 *Escherichia coli*

Escherichia coli (*E. coli)* are Gram-negative bacilli of the family *Enterobacteriaceae* (Oheme *et al*., 2018). They are facultative anaerobes and nonsporulating. *E. coli* are considered to be the cause of most food-borne illnesses in humans (Mian *et al*., 2018). *Escherichia coli* is mostly found in the intestinal tract of human which is also found in infants within few hours after birth and thus, it is considered as one of the first facultative organism to colonize the human gut (Sejal and Leonard, 2020; Mian *et al*., 2018). Maturation of the bacteria takes several years and thus, confined to the lumen of gut and to the external layer of the intestinal mucous (Mian *et al*., 2018).

Escherichia coli can be classified in to two types; pathogenic *Escherichia coli* and non-pathogenic *Escherichia coli* (Sejal and Leonard, 2020). The non-pathogenic *Escherichia coli* is known as commensal *Escherichia coli* and thus, present as normal microflora of intestine which are harmless, inhibit the growth of harmful bacteria and produce vitamins in the intestine (Mansan-Almeida *et al.,* 2013).

The pathogenic *Escherichia coli* can be further divided into intestinal diarrheagenic *Escherichia coli* which causes diarrhea and extraintestinal *Escherichia coli* (ExPEC) which causes wide range of illnesses in humans such as the neonatal meningitis, chronic urinary tract infections, septicemia and hemolytic uremic syndrome (Sejal and Leonard, 2020; Mian *et al*., 2018; Mansan-Almeida *et al.,* 2013). The clinical Significance of *Escherichia coli* are;

2.7.2.1 Septicemia and meningitis in neonates

Neonates are susceptible to septicemia and meningitis. The *Escherichia coli* responsible for meningitis and sepsis is called neonatal meningitis–associated *Escherichia coli* Neonatal septicemia or meningitis caused by *E coli* cannot be distinguished clinically from infection caused by other micro-organisms (Mansan-Almeida *et al.,* 2013).

Signs of septicemia include fevers, vomiting, diarrhea, heart rate abnormalities, apnea, cyanosis, temperature instability, respiratory distress, lethargy, irritability, jaundice and abdominal distention.

2.7.2.2 Diarrheal infections

Different strains of *E coli* are associated with a number of distinctive diarrheal illnesses. Among these are the enterotoxigenic *E coli* (ETEC), enteroinvasive *E coli* (EIEC), and Shiga toxin–producing *E coli* (STEC).

2.7.2.3 Urinary tract infections

These *E. coli* strains are the cause of over 80% of community-acquired and 30% of nosocomial-acquired urinary tract infections (UTIs). Infections in children are often due to blockages in the urinary tract, resulting in pools of stagnant urine ((Rios *et al*., 2015). These strains reside in the colon and then can be introduced into the urethra which consists of bloody diarrhea or occult positive diarrhea ((Rios *et al*., 2015). Severe abdominal pain is typical, and fever occurs in a third of the cases (Mian *et al*., 2018).

2.7.3 *Salmonella*

Salmonella belongs to the family *Enterobacteriaceae*. It is a rod-shaped gram-negative facultative anaerobe bacterium (Schroll *et al*., 2010). The genus salmonella currently comprises two species; *S. bongori* and S. enteric. *S. bongori* have been reported to infect humans (Vadhana *et al.,* 2015). The species is predominantly associated with cold-blooded animals whereas serovars causing disease in humans and other warm-blooded animals

mostly belong to *S. enteric* subspecies enteric. Salmonellosis is more prevalent in developing parts of the world like Asia, Africa, and South America. South Asia is at highest risk for infections that are nalidixic acid-resistant or multidrug-resistant (chloramphenicol, resistant to ampicillin, and trimethoprim-sulfamethoxazole) (Schroll *et al.*, 2010). In humans, salmonellosis can be in two forms; gastroenteritis which is non-typhoidal and enteric fever which can be typhoid or paratyphoid (Vadhana *et al.,* 2015).

In areas of endemicity, patients admitted to hospital are usually school-aged children or young adults between 5 and 25 years of age, and both sexes are affected equally (John *et al.*, 2015). Many patients do not require admission to hospital, due to mild disease, self-medication, or being treated in health stations, clinics, or as hospital outpatients. These community-managed cases may be of nonspecific illness that is not recognized clinically as enteric fever, especially among children under 5 years of age (John *et al.*, 2015). Patients can have influenza like symptoms, a dull frontal headache, malaise, anorexia, a dry cough, sore throat, and occasionally epistaxis (John *et al.*, 2015).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

The map below shows the location of Chanchaga local government area, Niger state. Chanchaga is the Local Government Area (LGA) where Minna the capital of Niger state is located. Chanchaga is located between latitudes 9°30′ to 9°40′ North of the equator and longitudes 6°30′ to 6°40′ East of the Greenwich meridian. Chanchaga LGA the main study area is completely encircled by Bosso LGA (fig 3.1).

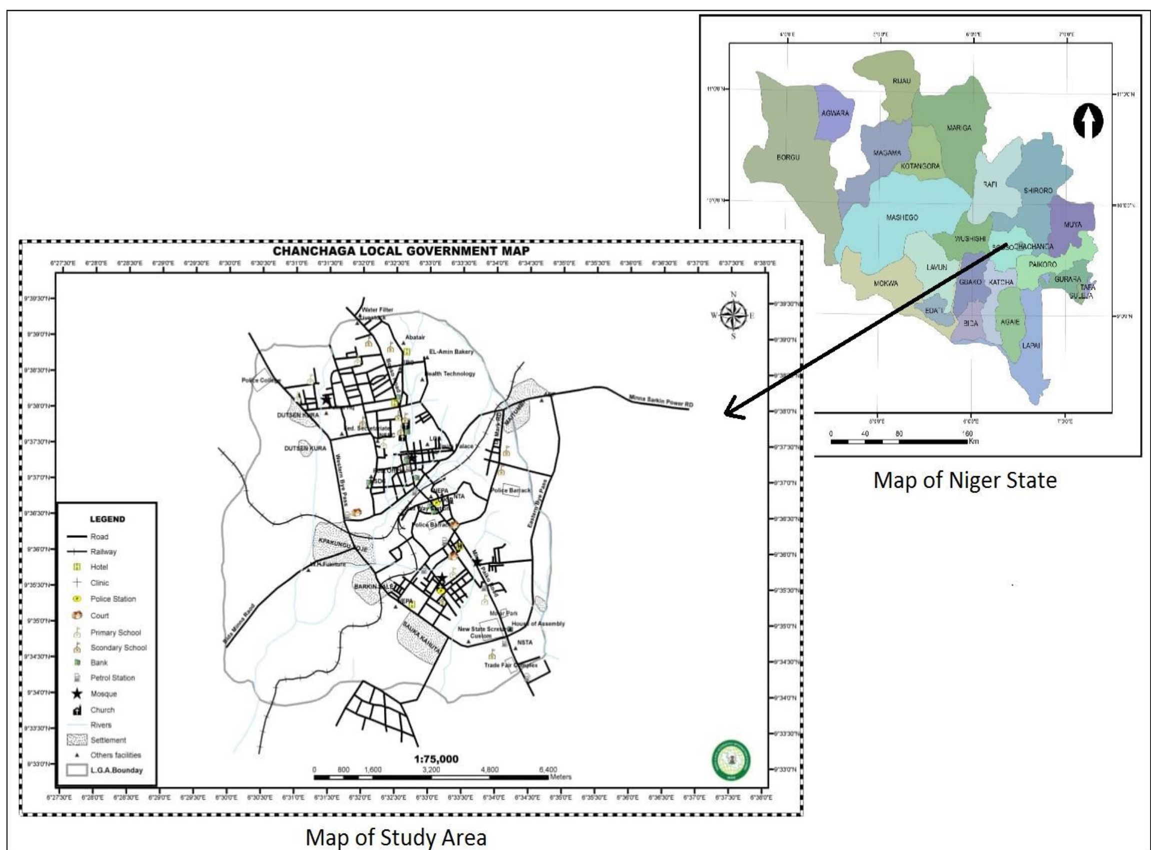

Figure 3.1: Map showing Chanchaga local government area in Niger state.

Source: (Ravi *et al.*, 2015)

3.2 Ethical Consideration

Ethical approval was obtained from the ethics and publication committee of General hospital Minna, Niger State, in order to permits the collection of bacterial isolates.

3.3 Sample Collections

3.3.1 Collection and Identification of plants materials

Fresh stem bark of *Boswellia dalzielii* was collected from it habitat at Kontagora (Niger state), *Abrus precatorius* seed was collected from its habitat in Minna (Niger State), and *Enantia chlorantha* stem bark was collected from Lagos Nigeria. Each of the plant material collected were washed under running tap water, air- dried at room temperature in the laboratory and then pulverized and homogenized to fine powder and stored in airtight glass containers at room temperature in dark until required for use as described by Iyabo *et al.,* (2018).

3.4 Preparation of Plants Extracts/Concentrations

This was carried out in accordance with the method described by Sonali & Shonkor (2020), *Boswellia dalzielii* (stem), *Enantia chlorantha* (stem) *and Abrus precatorius (*seed) stems were extracted using cold maceration technique. One hundred (100g) of the dried powder were soaked in Methanol and water respectively in sterile bottle and allowed to stay at room temperature for 72 hours, while undergoing vigorous shaking at regular interval. The mixture was filtered through muslin cloth and then re-filtered by passing through Whattman No. 1 filter paper. The filtrates were concentrated by complete evaporation of the solvent on water bath at 45°C. The extracts were subsequently transferred into clean sterile airtight glass container and stored in the refrigerator at 4°C until use.

The weights of each of the extracts were obtained and the percentage yields were calculated using Equation 3.1

$$\% \text{ Yield} = \frac{\text{weight of the extract}}{\text{weight of dry sample}} X\ 100$$

(Equation 3.1)

3.5 Isolation and Identification of the Bacterial Isolates

A broth containing the isolates were incubated at 37°C for 24 hours and a loop full from the broth is further sub-cultured onto the agar media which includes the SSA, EMB, and NA and incubated at 37°C for 24 hours. Colonies from the agar plates were sub cultured on to nutrient agar and incubated for another 24 hours at 37°C so as to obtain pure isolates. The pure isolates obtained were identified and characterized by Gram's staining and other biochemical test as follows:

3.5.1 Gram Staining

Gram staining was performed for all isolated colonies according to the standard procedure.

3.5.2 Microscopy

The stained glass was dried and an oil immersion was added to the smear and covered with cover slip. The smear was viewed under microscope under 100x oil immersion lens.

During the microscopical viewing, the colony that appears appears dark purple in colour were considered as Gram positive organism while those that appears pale to dark red in colour were considered as Gram negative organism, also their cellular morphological shape was noted (Ravi *et al.*, 2015).

3.5.3 Catalase test

A drop of 3% hydrogen peroxide was added to a bacterial colony on a sterile glass slide and mixed well. Production of air bubble was observed for a minute. Production of air bubble indicated catalase positive and no bubble indicated catalase negative (Ravi *et al.*, (2015).

3.5.4 Methyl Red (MR) test

A loop full of the colonies from the Nutrient Agar plate was picked and inoculated in to the Methyl Red-Vorges Proskaur (MR-VP) broth and the broth was incubated for five days at 37^0C. After the incubation periods a methyl-red reagent was added to the tube and it was observed for change in colour. When the culture medium turns red after addition of methyl red, the organism is MR Positive while when yellow culture medium indicates the organism is MR Negative (Mansan-Almeida *et al.,* 2013).

3.5.5 Vorges Proskaur (VP) test

A loop full of the colonies from the Nutrient Agar plate was picked and inoculated in to the MR-VP broth. The broth was incubated for five days at 37^0C. After the incubation periods a Barritt reagent was added to the tube and it was observed for change in colour. When the culture medium turns red after addition of methyl red the organism is VP Positive while yellow culture medium indicates the organism is VP Negative (Mansan-Almeida *et al.,* 2013).

3.5.6 Simmons citrate test

Isolates were tested to determine the utilization of citrate as the sole source of carbon for metabolism. Tubes of citrate media were inoculated by streaking the slant with bacteria and incubated at 37^0C for 24 hours. Results were recorded for change in color of citrate media (Ravi *et al*., 2015).

3.5.7 Oxidase test

Oxidase test was performed with1% solution of N, N, N1,N1-Tetra methyl-phenyldiamine-dihydrochloride which was socked in a piece of filter paper. A portion of the colony of the test organism was picked up with a sterile tooth pick and touched on to the paper with impregnated reagent. A dark purple color development within 5-10 second

was considered positive and no change of color was interpreted as a negative result for oxidase (Ravi *et al*., 2015).

3.5.8 Indole test

Indole test was carried out using pepton water broth by inoculation of a loop full of the colonies into it. The broth was incubated for five days at 37^0C. After the incubation period, few drops of Kovacs reagent were added into the broth and were shook gently. The presence of the red or pinkish to player indicates the organism to be Indole positive while the absence of it indicates the organism to be indole negative (Roseline *et al*., 2014).

3.5.9 Urease test

Urea agar slant was prepared in a bijou bottle and the test organism was inoculated heavily on the slant surface. The cap of the bottle was left partly loose and the slant was incubated for 5-7 days at 37^0C. Development of pink colour within the 7 days incubation period indicate the organism to be urease positive whilst absent of pink colour within the incubation period indicate organism is urease negative (Woon *et al*., 2016).

3.7 Molecular Characterization of Bacteria Isolates

3.7.1 Bacteria DNA Extraction

Deoxyribonuleic acid (DNA) was extracted using the protocol stated by fifa *et al*. (2019). Briefly, Single colonies grown on medium were transferred to 1.5 ml of liquid medium and cultures were grown on a shaker for 48 hours at 28 ºC. After this period, cultures were centrifuged at 4600g for 5 minutes. The resulting pellets were re-suspended in 520 µl of TE buffer (10 mMTris-HCl, 1mM 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 hour 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 minutes at 65 ºC and kept on ice for 15 minutes. An equal volume of chloroform: isoamyl alcohol (24:1) was added,

followed by incubation on ice for 5 minutes and centrifugation at 7200g for 20 minutes. The aqueous phase was then transferred to a new tube and isopropanol (1: 0.6) was added and DNA precipitated at –20 ºC for 16 hours. DNA was collected by centrifugation at 13000g for 10 minutes, washed with 500 µl of 70% ethanol, air-dried at room temperature for approximately three hours and finally dissolved in 50 µl of TE buffer.

3.7.2 Bacteria Polymerase Chain Reaction

PCR sequencing preparation cocktail consisted of 10 µl of 5x GoTaq colourless reaction, 3 µl of 25mM MgCl2, 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 8µl 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 minutes; 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 minutes. And chill at 4oC.GEL (2,3)

3.7.3 Integrity of the amplified product

The integrity of the amplified about 1.5 Mb 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 4µl 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

The amplified fragments were ethanol purified in order to remove the PCR reagents. Briefly, 7.6 µl of Na acetate 3M 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 minutes. 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 minutes 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 10-15 minutes, then re-suspend with 20 µl of sterile distilled water and kept in -20oC prior to sequencing. The purified fragment was checked on a 1.5% Agarose gel ran on a voltage of 110V 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 of the amplified product

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 Big Dye terminator v3.1 cycle sequencing kit. Bio- Edit software and MEGA 6 were used for all genetic analysis.

3.8 Determination of the Antibacterial Activity of Extracts and Fractions

3.8.1 Standardization of the Bacterial Culture

According to the method described by Iyabo *et al*. (2018), two milliliters (0.2 mL) of overnight cultures of the test organism were transferred into 20 mL of sterile nutrient broth and the culture was incubated for 2-3 hours at 37^0C to standardize the culture to 106 CFU/mL McFarland. A loopful of the standardized inoculum was used for the antibacterial assay.

3.8.2 Antibacterial activities of the Crude Plants Extracts

Agar well diffusion method was carried out to determine the antimicrobial activity of each extracts as described by Ravi *et al*. (2015) and Gupta *et al*. (2013) Mueller Hinton agar plates was prepared for *Pseudomonas aeruginosa, Salmonella bongori,* and *Escherichia coli.*

Forty grams (40 g) of the Nutrient agar was dissolved into 1L of distilled water in a conical flask. The mixture was sterilized in the autoclave at 121^0C for 15minutes and the medium was allowed to cool to 45^0C. Sterile molten Muller Hinton agar (20 mL) at 45^0C was dispensed into sterile Petri dishes and allowed to set. A sterile cork borer of diameter 6 mm was used to bore equidistant wells onto the agar plates. One drop of the molten agar was used to seal the bottom of the bored wells to prevent the extract from sipping beneath the agar. Sterile cotton swab sticks were used to streak on the surface of the agar plates with the standardized test organisms and 100 µL of the extract (15, 25, 50, 100, 150, 200, 250 and 300 mg/ml) of methanol extract of the plant were added separately to the bored wells and 5 mg/mL of the standard drug (Ciprofloxacin and Amoxicillin) were used as positive control while dimethyl sulfoxide (DMSO) served as the negative control. Thirty minutes pre-diffusion time was allowed after which the plates were incubated at 37^0C for 24 hours. The zones of inhibition were then measured in millimeters. The above method

was carried out in duplicates and the mean of the result was taken. Test with ten millimeters (10 mm) zones of inhibition was considered sensitive to the plant extract.

3.9 Determination of Minimum Inhibitory Concentration (MIC)

The method of Iyabo *et al*. (2018) was employed with slight modification using spectrophotometer to determine the MIC of extract against the organisms. A series of two-fold dilutions of each extract ranging from 300 mg/mL to 38.85 mg/mL was made in nutrient broth. Zero-point one milliliter (0.1 mL) of each of the standardized test organisms (0.5 McFarl and turbidity standards) was added to each dilution. Two controls were maintained for each batch. These included tubes containing extract and growth medium without inoculum and tube containing the growth medium and inoculum (organism control). The tubes were incubated at 37^0C for 24 hours. At the end of the incubation period, the optical density of the cultures in the test tubes was read using a spectrophotometer at a wavelength of 600 nm. The MIC was determined by subtracting the absorbance of the negative control from the absorbance of the test and comparing the result with the absorbance of the positive control using the formula:

Absorbance of Test (T) – Absorbance of Control (C0) = Absorbance of positive control (C1)

The concentration/test tube where a significant reduction in absorbance was observed was recorded as the MIC.

3.10 Determination of Minimum Bactericidal Concentration (MBC)

The minimum bactericidal concentration (MBC) was determined by sub-culturing the cultures with the lowest optical density beginning with the test tube containing the minimum inhibitory concentration and above onto a freshly prepared nutrient agar medium. The cultures were incubated for 24 hours at 37^0C, after incubation, the culture

concentration without visible growth was regarded as the minimum bactericidal concentration.

3.11 Fractionation/Partitioning of the Crude Extracts

Methanol extract of the plants was partitioned as described by Iyabo *et al*. (2018). The extracts (25 g) was dissolved in 100 mL of distilled water and was partitioned into n-hexane, ethyl acetate and distilled water in increasing order of polarity (n-hexane < ethyl acetate< distilled water) using separate funnel. The resultant fractions were evaporated to dryness at a reduced temperature of 40°C in the water bath and the weight of each of the fractions was taken. The fractions were then subjected to antibacterial sensitivity test. MIC and MBC were also carried out to detect the active fraction (s).

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

The GC-MS analysis of aqueous, ethyl acetate and n-Hexane sub-fraction of *Boswella dalzielii* stem, *Enantia chlorantha* stem and *Abrus precatorius* seed methanol extract was 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). For GC-MS detection, 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 rate1ml/min and an injection volume of 2μL was employed (split ratio of 10:1) injector temperature-250ºC; ion source temperature 280ºC.

The oven temperature was programmed from 110ºC (Isothermal for 2 min.) with an increase of 10ºC/min to 200ºC then 5ºC/min to 280ºC/min, ending with a 9 min isothermal at 280ºC. Mass spectra were taken at 70 eV; a scanning interval of 0.5s 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 its average peak area to the total

areas. Software adapted to handle 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).

3.13 Determination of Phytochemical Constituents of the Plants

The methanol extract of *Boswellia dalzielii* stem, *Enantia chlorantha* stem *and Abrus precatorius* seed were analyzed quantitatively for the presence of phenols, flavonoids, alkaloids, tannins, and saponins using spectrophotometric method.

3.14 Data Analysis

Data generated were expressed as mean value±standard error of mean (SEM). Among groups, comparisons of means were performed by two way ANOVA test for statistical significance of differences at $p<0.05$. Mean values were separated by Duncan multiple Range Test (DMRT). All data were evaluated using the statistical package SPSS version 25.0.0.0

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Characterization and Identification of Bacterial Isolates

The bacterial isolated were *Escherichia coli*, *Pseudomonas aeruginosa*, and *Salmonella bongori.* Table 4.1 and 4.2 indicated morphological and biochemical tests conducted.

4.2 Molecular Characterization of Bacteria Isolates

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 (Gen Bank Accession Number: MK572634.1, MN623691.1 and CP074120.1for *Pseudomonas aeruginosa, Salmonella bongori, and Escherichia coli* respectively) (Table 4.3). The phylogenetic tree of the isolates shows the relationship among the organisms and their origin using the NCBI data (figure 4.1)

Table 4.1 Cultural growth characteristics on media, microscopic morphological characteristics and Gram staining Reaction

Organism	GC & M	MCM	Gram staining
Escherichia coli	Greenish metallic sheen colonies on EMB	Rod shaped bacilli	Negative
Pseudomonos aeruginosa	Large opaque flat colonies with green	Rod shaped bacilli	Negative
Salmonella bongori	Colourless colonies with black center on SSA	Rod shaped bacilli	Negative

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

Table 4.2: Biochemical characteristics of bacteria isolates

Organism	Indole	Methyl-red	Voges-proskaur	Citrate	Catalase	motility	oxidase	Urease
Escherichia coli	+	+	-	-	+	+	-	-
Pseudomonas aeruginosa	-	-	-	+	+	+	+	-
Salmonella bongori	-	+	+	-	+	+	-	-

Key: + = Positive and - = Negative

Table 4.3: Molecular Characterization of Bacteria Isolates

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

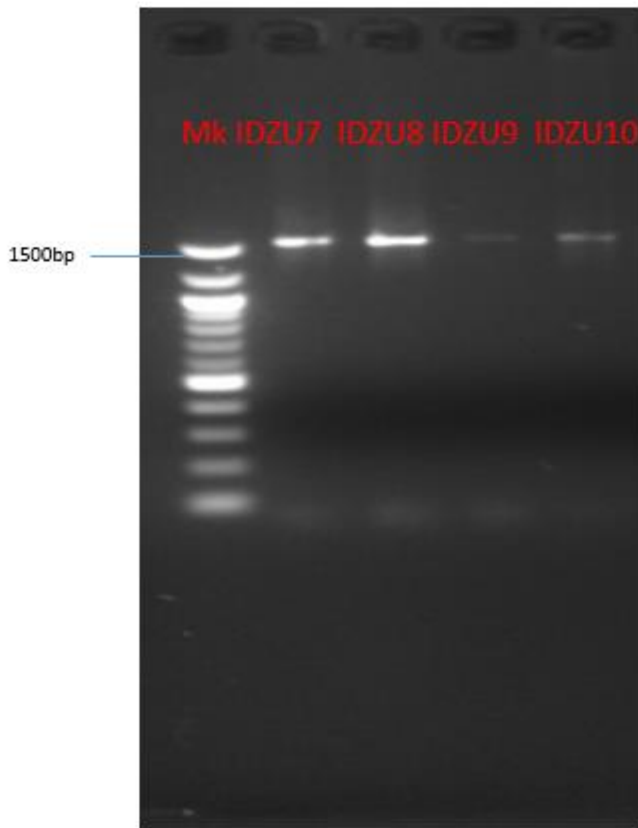

Plate I: PCR amplification of the DNA region of the bacteria isolates using the 27F and 1525R primer

Keys: MK = 1500bp DNA ladder, IDZU7, IDZU8, IDZU9, IDZU10 = PCR reaction mixture with DNA of the samples to be identified.

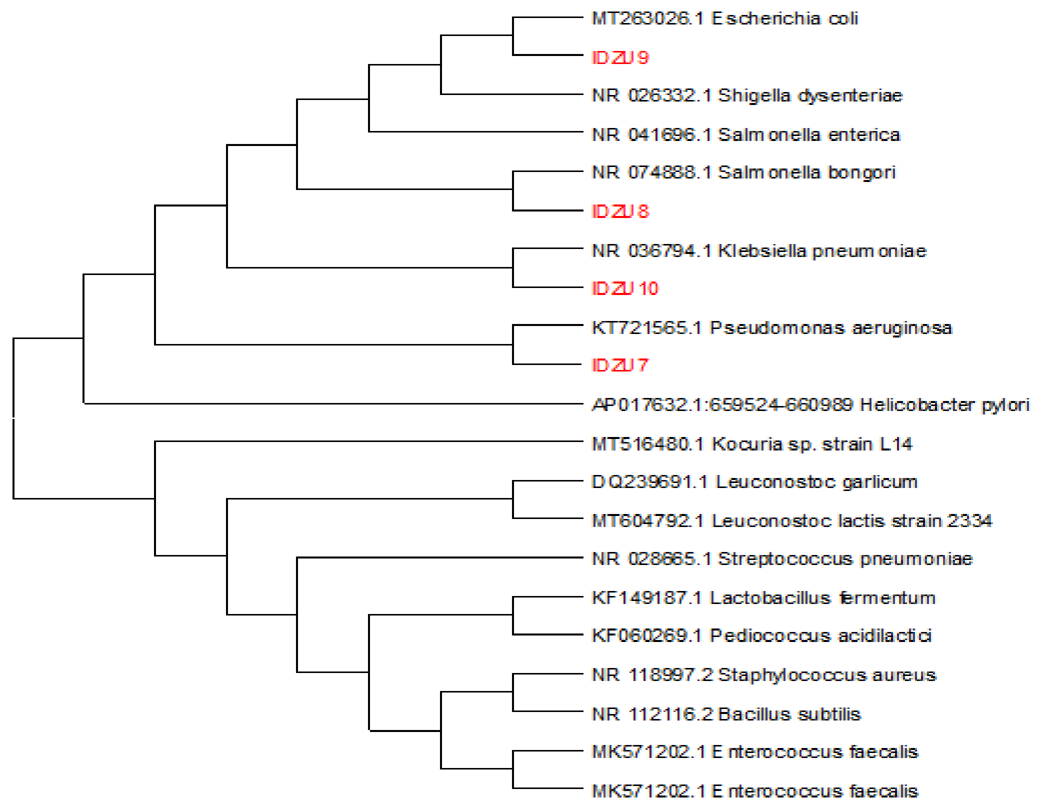

Figure 4.1: Phylogentic tree showing the relations among the bacteria isolates

Keys: IDZU7 = *Pseudomonas aeruginosa,* IDZU8 = *Salmonella bongori* and IDZU9 = *Eschrichia coli*

4.3 Percentage Yield of Plant Extract

The yield of the 500g weight of the pulverized plant material extracted exhaustively with methanol was measured and recorded. The percentage yields ranged from 5 to 9.8%. The highest yield was obtained from *Boswellia dalzielii* stem 48.8g (9.8%) followed by *Enantia chlorantha* stem with 29g (5.8%) and the lowest yield was *Abrus precatorius* seed 25g (5%) (Table 4.4).

The fractions of n-hexane, ethyl acetate and residual aqueous fraction of *Boswellia dalzielii* stem and *Enantia chlorantha* stem extract were obtained, but as of *Abrus precatorious* seed only the ethyl acetate and residual aqueous fractions were obtained, i.e. there was no yield for n-Hexane. The percentage yield of all recovered fractions was weighed and recorded (Table 4.5).

Table 4.4: Percentage yield of 500g Plant Extract of Aqueous solvent

Plant materials	Yield, g (%)
Boswellia dalzielii stem	48.8 (9.8)
Abrus precatorius seed	25 (5.0)
Enantia chlorantha stem	29 (5.8)

Table 4.5: percentage yield of partition fractions of crude extract

Plant materials	WDE (g)		% Weight of faction
	n-HX	EAT	RAQ
Boswella dalzielii stem	153.5 (30%)	6.2 (41%)	5.3 (70%)
Abrus precatorius seed	150 (0%)	4.5 (30%)	10.5 (35%)
Enantia chlorantha stem	15 0 (12%)	4 (27%)	11 (73%)

Key: n-HX = n-Hexane fraction, EAT = Ethyl acetate fraction and RAQ Residual aqueous, WDE = weight of dry Extract.

4.4 Antibacterial Evaluation of Crude Extracts and their Fractions

The crude methanol extract of three plants (*Boswellia dalzielii* stem, *Enantia chloranta* stem *and Abrus precatorius* seeds) were found to exhibit appreciable antibacterial effects on the bacteria isolates (*Escherichia coli, Pseudomonas aeruginosa* and *Salmonella bongori)* at concentration of 150 to 300 mg/mL. The crude methanol extract of *Boswellia dalzielii* stem shows more activity against *E. coli* and *S. bongori* with inhibition zones ranging from 5.50±0.0 to 18.55±1.12 and 1.50±1.5 to 15.05±0.05 mm respectively, however, the least activity was observed for *P. aeruginosa* (6.70±0.03 to 12.80±0.20 mm). The standard drugs (Amoxillicin and ciprofloxacin) used inhibited the growth of all the isolates with varying zones of inhibition ranging from 7.30±1.1 to 24.35±1.50 mm (Table 4.6). The crude methanol extracts of *Abrus precatorius* seed shows more activity against *E. coli* and *S. bongori* with zone of inhibition ranging from 8.75±0.25 to 14.00±1.00 and 7.50±0.50 to 13.10±0.90 mm respectively, More so, the least activity was observed against *P. aeruginosa,* with zones of inhibition ranging from 4.15±1.15 to 5.60±0.40 (Table 4.7). The crude methanol extracts of *Enantia chloranta* stem shows more activity against *E.coli* and *S. bongori* with zones of inhibition ranging from 5.35±0.65 to 15.00±0.10 mm and 4.50±0.50 to 12.50±0.00 mm respectiely, also, the least activity was observed for *P. aeruginosa* (09.80±0.50 to 11.90±0.60 mm) (Table 4.8). Furthermore, the antibacterial activities were all compared to the positive control (Amoxillicin 5mg/mL and ciprofloxacin 5mg/mL).

The n-hexane fraction shows a narrow zone of inhibition against all the bacterial isolates. The residual aqueous fractions show significant inhibition against *Pseudomonas aeruginosa, E.coli* and *S. bongori* at both 250 mg/mL and 300 mg/mL with inhibition zones ranging from 5.00±1.00 to 19.50±0.50 mm. The ethyl acetate fraction of the stem extract has significant activity against *Escherichia coli* at (9.00±1.00 to17.50±0.50 mm) (Table 4.9).

The residual aqueous fraction shows a significant inhibition across all the concentration (150-300 mg/ml against the test organisms (4.50±0.30 to 14.35±0.35 mm). The ethyl acetate fraction shows a significant activity at 250 and 300 mg/mL against *Escherichia coli* and *Pseudomonas aeruginosa* with the zones of inhibition ranging from 4.65±0.15 to 6.95±0.25 mm. More so, a minimal activity was observed at 300 mglmL against *Salmonella bongori* (3.55± 0.45) (Table 4.10)

The n-hexane fraction shows a narrow zone of inhibition against all the bacteria isolates (3.25±0.75 to 9.85±0.15 mm). The residual aqueous fraction shows a spectrum of activity in all the concentration against the bacteria isolate with zones of inhibition ranging from 5.55±0.45 to 15.50±5.50 mm. The ethyl acetate fraction inhibited the growth of *P. aeruginosa* and against *E. coli* at 250 and 300 mg/mL (4.40±0.40 to 21.90±0.10 mm) (Table 4.11)

Table 4.6: Mean zones of inhibition of methanol extract of *Boswellia dalzielii* stem against bacterial isolates

Organisms	100	150	200	250	300	Cipro 5 mg/mL	Amox 5 mg/mL
E. coli	7.50±0.00[b]	10.00±0.00[c]	13.05±0.05[d]	14.50±0.50[d]	18.45±0.25[e]	18.55±1.12[e]	3.10±0.2[a]
P. aeruginosa	-	-	6.70±0.30[a]	10.30±0.30[b]	12.80±0.20[c]	24.35±1.50[d]	12.75±0.22[c]
S. bongori	-	-	1.50±1.50[a]	13.85±0.15[c]	15.05±0.05 [d]	23.01±0.20[e]	7.30±1.11[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 *Abrus precatorius* seed against bacterial isolates

Organisms	150	200	250	300	Cipro 5 mg/ı	Amox 5 mg/ı
E. coli	8.75±0.25[b]	9.40±0.40[b]	8.75±0.25[b]	14.00±1.00[c]	18.55±1.12[d]	3.10±0.23[a]
P. aeruginos	_	_	4.15±1.15[a]	5.60±0.40[a]	24.35±1.50[c]	12.75±0.22[b]
S. bongori	7.50±0.50[a]	8.35±0.65[a]	10.50±0.50[b]	13.10±0.90 [b]	23.01±0.20[c]	7.30±1.11[a]

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 4.8: Mean zones of inhibition of methanol extract of *Enantia chlorantha* stem against bacterial isolates

Organisms	50	100	150	200	250	300	Cipro 5 mg/m	Amox 5 mg/n
E. coli	5.35±0.65[b]	10.50±0.50[c]	11.75±0.25[c]	14.80±0.20[d]	15.00±0.10[d]	15.90±0.10[d]	18.55±1.12[e]	3.10±0.23[a]
P. aeruginosa	_	_	_	9.80±0.50[a]	10.00±0.10[a]	11.90±0.60[a]	24.35±1.50[c]	12.75±0.22[b]
S. bongori	_	_	4.50±0.50[a]	6.00±1.00[b]	8.55±1.55[b]	12.50±0.00[c]	23.01±0.20[d]	7.30±1.11[e]

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 activities

Table 4.9: Mean zones of inhibition of n-hexane, ethyl acetate and aqueous fraction extract of *Boswellia dalzielii* stem bark against bacterial isolates

Organism	H250	H300	A150	A250	A300	E150	E250	E300
E. coli	6.50±0.50[a]	10.50±0.50[b]	11.50±1.50[b]	17.50±0.50[c]	19.50±0.50[e]	9.00±1.00[b]	15.50±0.50[d]	17.50±0.50[c]
p. aeruginosa	_	_	_	8.50±0.50[b]	11.50±0.50[c]	5.55±1.00[a]	6.50±0.50[a]	8.50±0.50[b]
S. bongori	_	_	_	5.00±1.00[a]	7.00±1.00[a]	6.90±0.0[a]	8.50±0.50[b]	11.50±0.50[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 extract of *Abrus precatorius* seed against bacterial isolates

Organism	A150	A250	A300	E150	E250	E300
E. coli	7.75 ± 1.25^{ab}	9.85 ± 1.35^{b}	12.60 ± 0.40^{c}	5.95 ± 0.50^{a}	5.30 ± 0.10^{a}	6.95 ± 0.25^{a}
P. aeruginosa	4.50 ± 0.30^{a}	5.60 ± 0.40^{a}	6.90 ± 0.10^{b}	_	4.65 ± 0.15^{a}	5.50 ± 0.50^{a}
S. bongori	11.10 ± 0.10^{c}	12.45 ± 0.45^{c}	14.35 ± 0.35^{d}	_	5.00 ± 0.00^{a}	7.55 ± 0.45^{b}

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 activities

Table 4.11: Mean zones of inhibition of n-hexane, ethyl acetate and aqueous fraction extract of *Enantia chlorantha stem bark* against bacterial isolates

Organism	H150	H250	H300	A150	A250	A300	E150	E250	E300
E. coli	3.25 ± 0.75^{a}	6.65 ± 1.05^{bc}	9.85 ± 0.15^{d}	5.55 ± 0.45^{b}	7.00 ± 0.00^{c}	15.50 ± 45.50^{e}	5.40 ± 1.05^{b}	6.40 ± 0.40^{bc}	10.05 ± 0.05^{d}
P. aeruginosa	_	_	_	_	8.55 ± 1.55^{a}	11.75 ± 0.75^{b}	9.50 ± 0.50^{a}	17.00 ± 1.00^{c}	21.90 ± 0.10^{d}
S. bongori	_	_	3.90 ± 0.90^{a}	9.10 ± 1.70^{c}	10.85 ± 1.85^{d}	12.50 ± 1.50^{e}	5.50 ± 0.00^{b}	6.05 ± 0.00^{b}	11.75 ± 0.25^{d}

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

4.5 Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of Methanol extracts of *Boswella dalzielii* stem, *Abrus precarious* seed and *Enantia chlorantha* stem extracts

The result of the antimicrobial activities of the extracts as determined by measuring the minimum inhibitory concentrations (MIC) of Methanol extracts of *Boswella dalzielii* stem, *Abrus precarious* seed and *Enantia chlorantha* stem are presented in figure 4.2 to 4.12 respectively.

In present study, MIC values ranged between 9.38-150 mg/mL and most of the cases were in correlation with the antibacterial activity determined by inhibition zone formation.

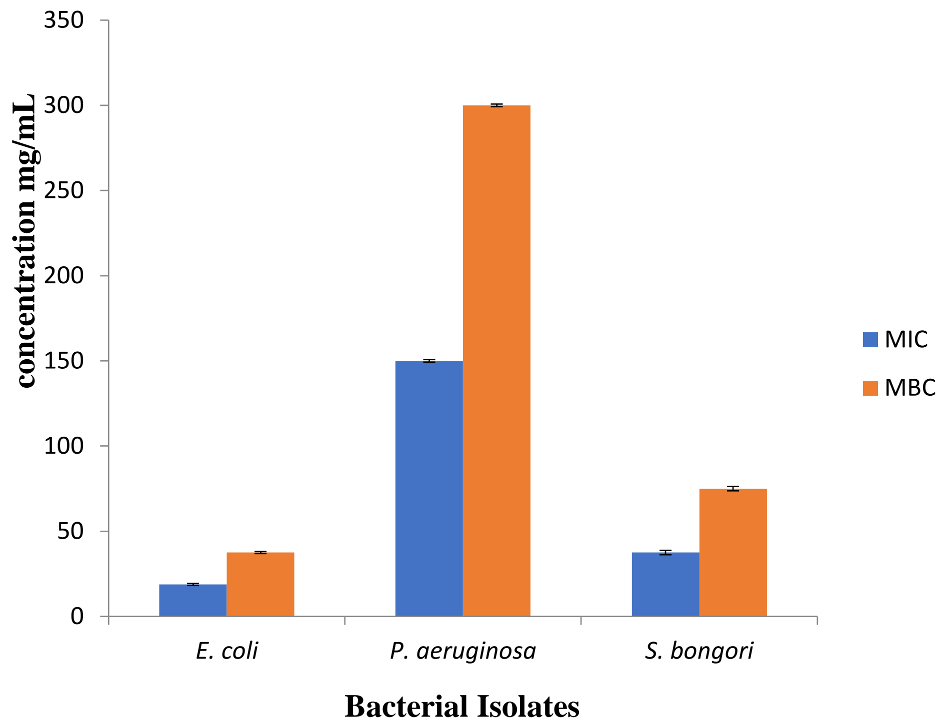

Figure 4.2: MIC and MBC of methanol crude fraction of *Boswella dalzielii* stem

Bar of same color with the same alphabet have no significant difference at $p < 0.05$

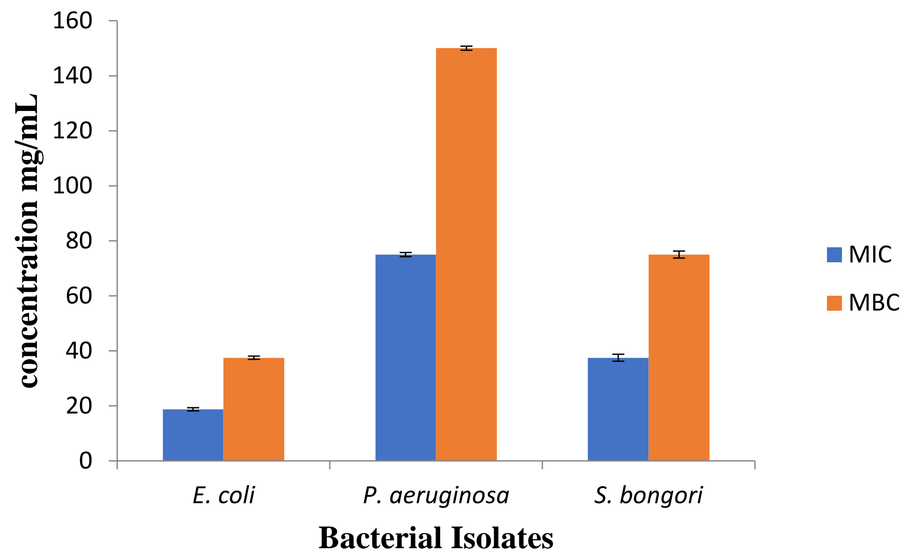

Figure 4.3: MIC and MBC of aqueous fraction of *Boswella dalzielii* methanol stem extract

Bar of same color with the same alphabet have no significant difference at $p < 0.05$

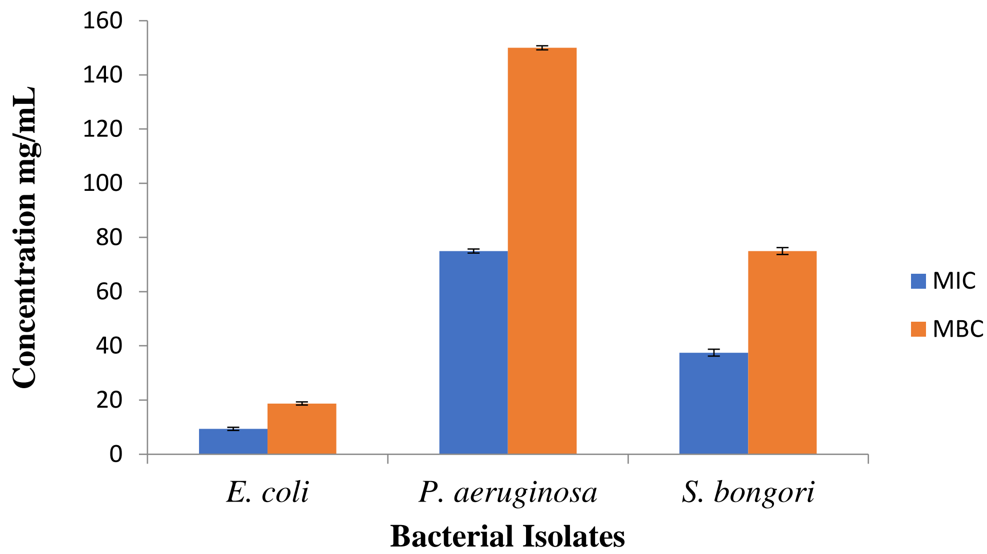

Figure 4.4: MIC and MBC of n-Hexane fraction of *Boswella dalzielii* methanol stem extract

Bar of same color with the same alphabet have no significant difference at $p < 0.05$

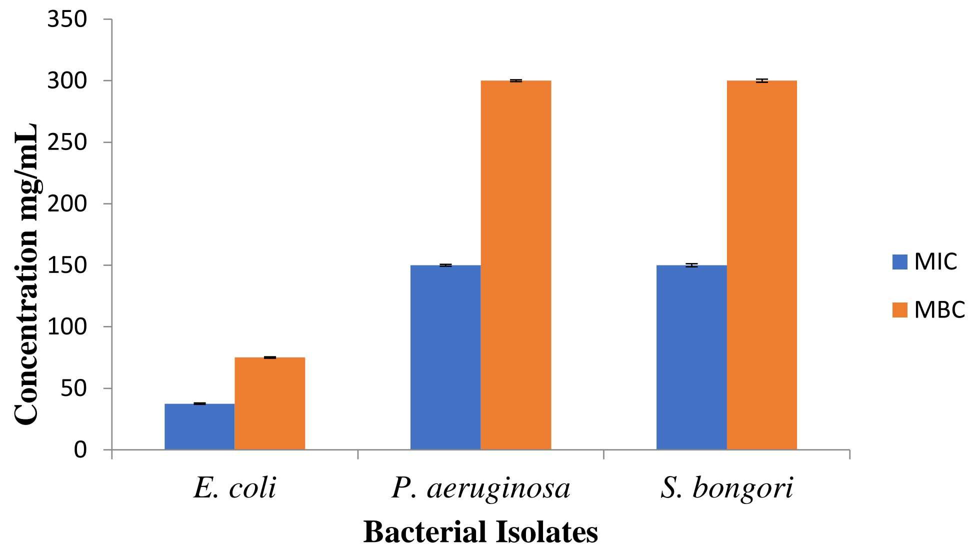

Figure 4.5: MIC and MBC of ethyl acetate fraction of *Boswella dalzielii* methanol stem extract

Bar of same color with the same alphabet have no significant difference at $p < 0.05$

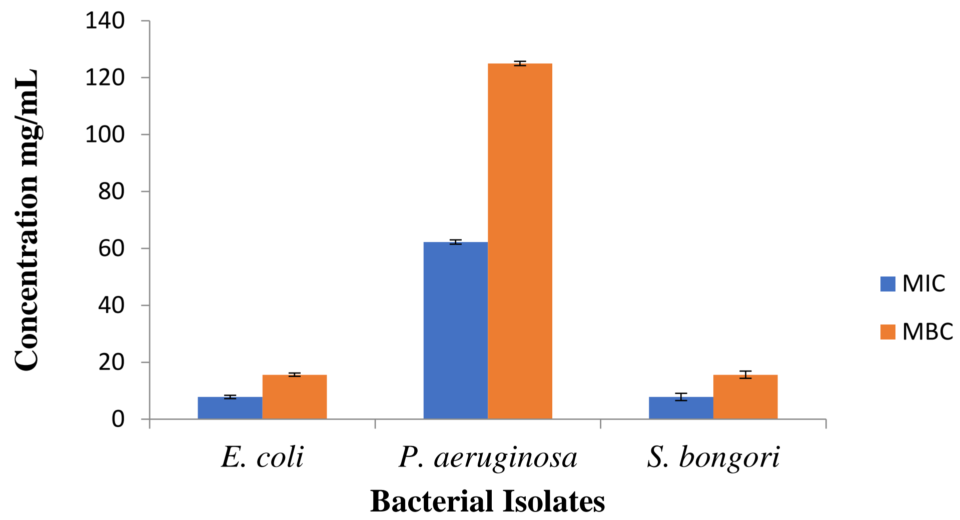

Figure 4.6: MIC and MBC of methanol crude extract of *Abrus precatorius* seed

Bar of same color with the same alphabet have no significant difference at $p < 0.05$

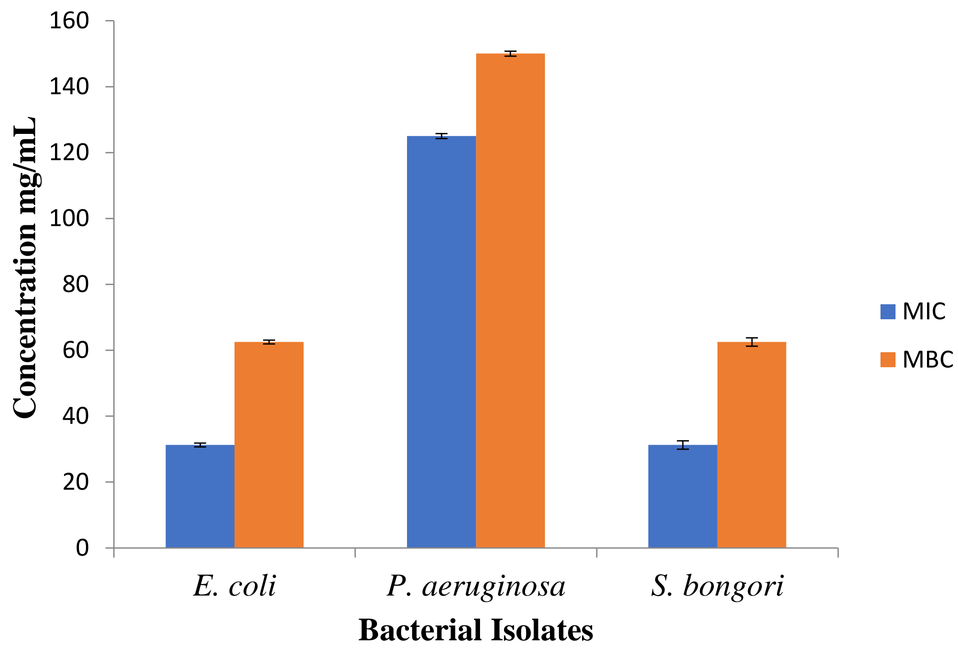

Figure 4.7: MIC and MBC of aqueous fraction of *Abrus precatorius* seed extract

Bar of same color with the same alphabet have no significant difference at $p < 0.05$

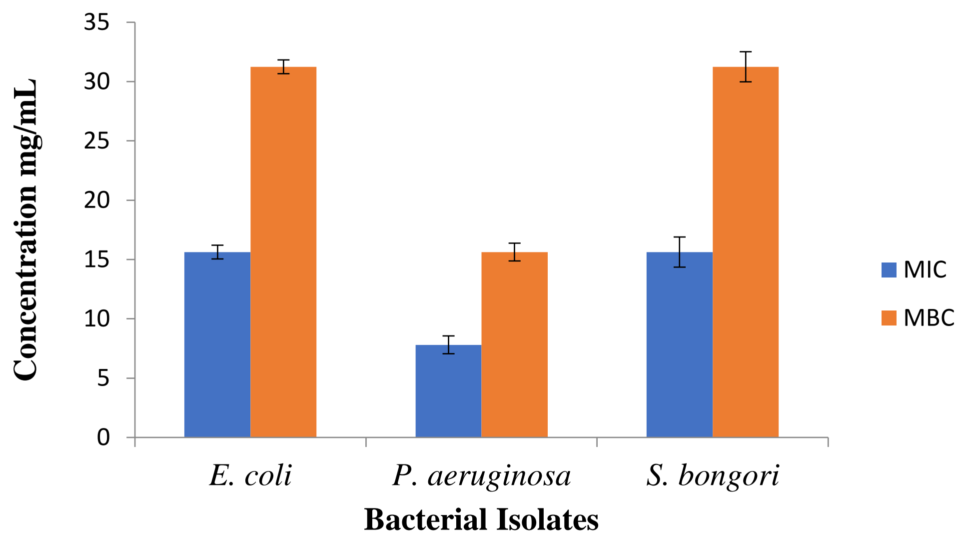

Figure 4.8: MIC and MBC of ethyl acetate fraction of *Abrus precatorius* seed extract

Bar of same color with the same alphabet have no significant difference at $p < 0.05$

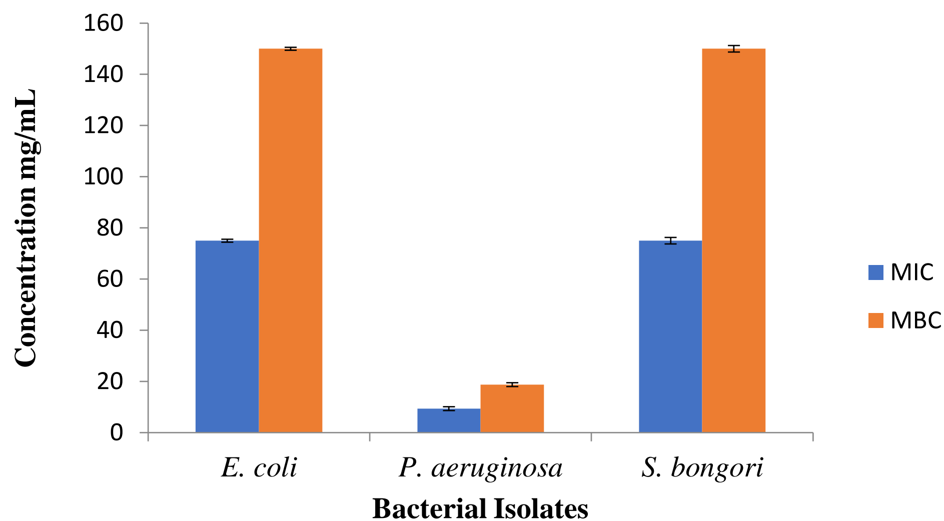

Figure 4.9: MIC and MBC of crude methanol extract of *Enantia chlorantha* stem

Bar of same color with the same alphabet have no significant difference at $p < 0.05$

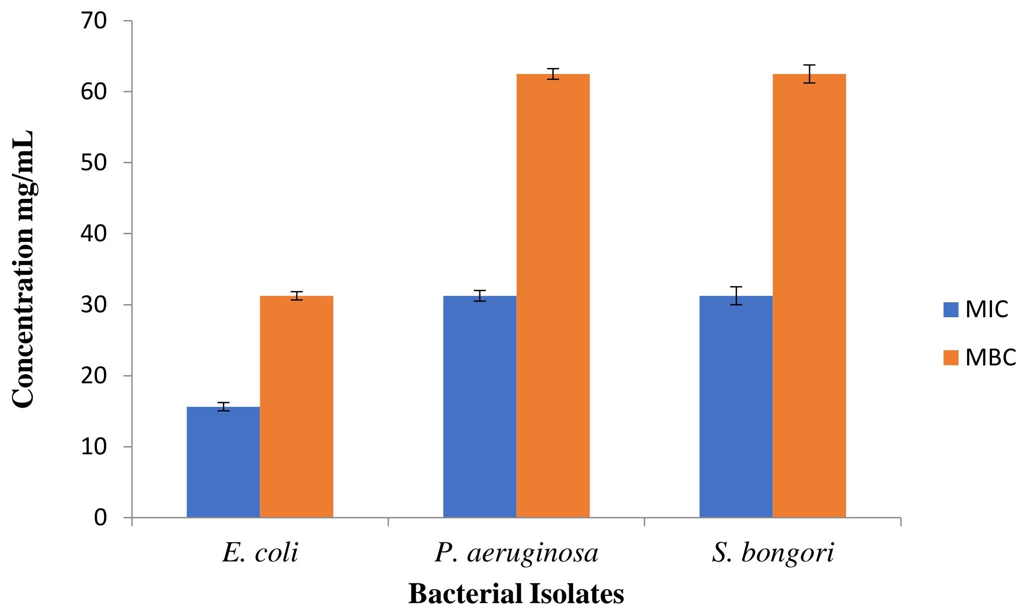

Figure 4.10: MIC and MBC of aqueous fraction of *Enantia chlorantha* stem extract

Bar of same color with the same alphabet have no significant difference at $p < 0.05$

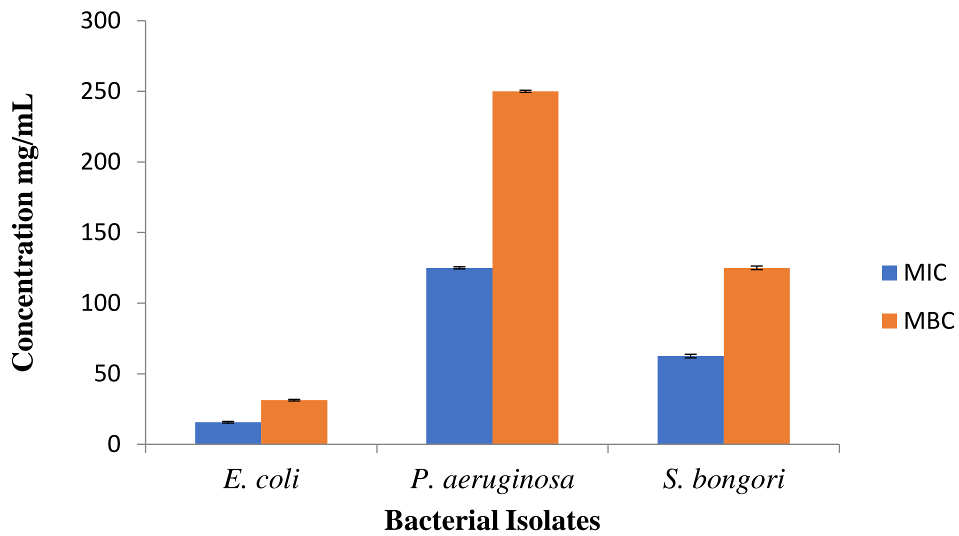

Figure 4.11: MIC and MBC of n-Hexane fraction of *Enantia chlorantha* stem extract

Bar of same color with the same alphabet have no significant difference at $p < 0.05$

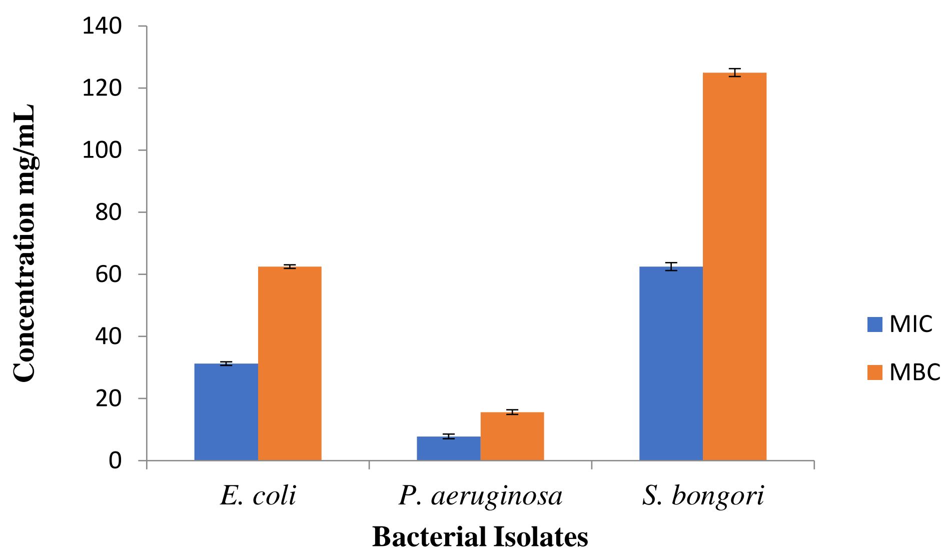

Figure 4.12: MIC and MBC of ethyl acetate fraction of *Enantia chlorantha* stem extract

Bar of same color with the same alphabet have no significant difference at $p < 0.05$

4.1.6 GC-MS Analysis of fractions from *B. dalzielii* stem, *E. chlorantha* stem and *A. precatorius* seed

The compounds present in the fraction of *Boswella dalzielii* stem bark were identified by GC-MS analysis. The active principles with their retention time (RT), molecular formula, molecular weight (MW) and peak area (%) are presented in Table 4.12 to 4.14. The major constituents identified in the fraction of all the three plants are 9-Octadecenal (53.78%), Hexadecanoic acid (22.82%), Octadecanioc acid or Stearic acid (10.68%), 2-methylnonane (3.42%) and 9, 12-Octadecadenoic acid or Linoleic acid (2.84%).

4.1.7 Quantitative Phytochemical Analysis of Plant Extracts

Quantitative analysis of the phytochemicals content revealed phenol to be in high concentration in all the three plants (*Boswellia dalzielii* stem*, Abrus precatorius* seed*, Enantia chlorantha* stem). The concentration of the phenol ranged from 416.07±0.07 to 989.04±0.60, saponin concentration ranged from 117.86±0.22 to 544.22±0.46, tannins concentration ranged from 26.45±0.17 to 239.30±15.85, flavonoids concentration ranged from 25.32±0.25 to 66.43±0.67. Alkaloids were found to be present in all the three plants extracts in trace amount (Table 4.15).

Table 4.12: GC-MS profiles of the fraction of *Boswella dalzielii* stem bark

Peak No	R. time	Compound names	M. Formula	M. weigl	% Conc.
		Aqueous residual fraction			
9	14.899	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	4.02
13	16.156	Tetradecanoic acid (Myristic acid)	$C_{14}H_{28}O_2$	228	23.94
14	16.188	9,12 Octadecadienoic acid (Z,Z)-	$C_{19}H_{34}O_2$	294	20.38
17	18.746	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl	$C_{19}H3_8O_4$	330	6.42
20	20.057	9,12-Octadecadienoic acid (Z,Z)-, 2,3-dihydroxypropyl	$C_{21}H_{38}O_4$	354	50.05
		Ethyl acetate fraction			
26	14.756	1,2-Benzenedicarboxylic acid, butyl octyl ester	$C_{20}H_{30}O_4$	334	5.90
27	15.019	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	12.02
29	16.217	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280	20.50
30	16.425	9-Octadecenamide, (Z)-	$C_{18}H_{35}NO$	281	6.31
34	19.035	Bis(2-ethylhexyl) phthalate	$C_{24}H_{38}O_4$	390	5.27
37	20.030	E,Z-1,3,12-Nonadecatriene	$C_{19}H_{34}$	262	12.85
		n-Hexane fraction			
15	14.940	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	[illegible].78
16	15.084	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284	5.11
21	16.139	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{36}O_2$	280	5.46
23	16.253	Cyclopropaneoctanoic acid	$C_{22}H_{38}O_2$	334	4.51

25	16.379	2,2-Dimethyl-1-(3-oxo-but-1-enyl) cyclopentanecarboxaldehyde	$C_{12}H_{18}O_2$	194	23.54

Table 4.13: GC-MS profiles of the fraction of *Abrus precatorius* seed

Peak No.	R. time	Compound names	M. Formula	M. weight	% Conc.
		Ethyl acetate fraction			
8	7.902	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy	$C_6H_8O_4$	144	5.07
14	10.676	1,2,3-Benzenetriol	$C_6H_6O_3$	126	30.83
15	14.05	[1,1'-Bicyclopropyl]-2-octanoic acid, 2'-hexyl	$C_{21}H_{38}O_2$	322	4.18
18	16.165	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280	14.53
26	20.003	9,12-Octadecadienoic acid (Z,Z)-, 2,3-dihydroxypropyl	$C_{18}H_{32}O_2$	280	8.11
		Aqueous residual fraction			
1	4.674	Hydrazine, ethyl-	$C_2H_8N_2$	60	4.72
14	10.601	1,2,3-Benzenetriol	$C_6H_6O_3$	126	36.80
15	14.348	1,5-Anhydro-d-mannitol	$C_6H_{12}O_5$	164	31.77
16	14.897	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	10.34

Table 4.14: GC-MS profiles of the fraction of *Enantia chlorantha* stem bark

Peak No.	R. time	Compound names	M. Formula	M. weight	% Conc.
		Ethyl acetate fraction			6.10
8	7.798	Erythritol	$C_4H_{10}O_4$	122	
14	12.819	1,5-Anhydro-d-mannitol	$C_6H_{12}O_5$	164	19.34
17	16.115	9,12-Octadecadienoic acid (Z	$C_{19}H_{34}O_2$	294	16.21
21	20.015	9,12-Octadecadienoic acid (Z , 2,3-dihydroxypropyl	$C_{18}H_{32}O_2$	280	28.05

Table 4.15: Quantitative phytochemical contents of methanolic extracts of *Boswella dalzielii* stem bark*,* *Abrus precatorius* seed*,* and *Enantia chlorantha* stem bark

Sample	Phenols	Flavonoids	Tannins (mg/100g)	Saponins	Alkaloids
BD Crude	989.04±0.60	25.32±0.25	141.58±0.50	544.22±0.46	32.08±0.80
AP Crude	680.05±0.02	66.43±0.67	239.30±15.85	346.43±0.56	57.04±0.08
EC crude	416.07±0.07	26.09±0.05	26.45±0.17	117.86±0.22	20.67±0.57

Keys: BD: ***Boswellia dalzieli*** **stem, AP:** ***Abrus precatorius*** **seed*,* EC:** ***Enantia chlorantha*** **stem**

4.2 DISCUSSION

4.2.1 Characterization and Identification of Bacterial Isolates

The isolates were characterized using morphological characteristics (Table 4.1 and 4.2). The bacterial isolate grew slowly on nutrient agar; visible growth was observed after two days. The isolate grew at optimum temperature of 30℃. the microbial world is characterized by an incredible metabolic and physiological versality that permits microorganisms to inhabit hostile ecological niches and to exploit, as carbon and energy sources, compounds unpalatable for higher organisms (Baylis *et al.*, 2013). Isolates in this study displayed the typical morphological characteristics for *Escherichia coli, pseudomonas aeruginosa and salmonella bongori.*

4.2.2 Molecular Characterization of Bacterial Isolates

New strains of *Escherichia coli, pseudomonas aeruginosa* and *Salmonella bongori* arise all the time from the natural biological process of genetic variability and hence monitoring the levels of these contamination is important (Gulcin *et al.,* 2016). The development of highly applicable nucleic acid based diagnostic analysis on the basis of culture media isolation and biochemical reactions enabled the detection of different bacteria. Among these, PCR is a commonly used molecular method that gives rapid and reliable detection of pathogenic bacteria strains that could show the prevalence and distribution of these pathogens (Gulcin *et al.,* 2016).

The PCR products revealed the following Accession Number: MK572634.1, MN623691.1 and CP074120.1 for *Pseudomonas aeruginosa, Salmonella bongori, and Escherichia coli*

respectively) (Table 4.3). The phylogenetic tree of the isolates shows the relationship among the organism and their origin using the NCBI data.

4.2.3 Percentage yield of plant Extract

Extraction yield is a measure of solvent and extraction method's efficiency to extract out specific components from plant matrix (Naraniwal *et al.,* 2015). The polarity of solvent used for extraction and the method of extraction play a vital role in efficiency (yield) and efficacy (magnitude of bioactivity) of prepared extracts (Fifa *et al*., 2019). With the key principle of solubility "like dissolves like", phyto constituents of varied polarity present in plant matrix can be extracted by suitable solvents (Neelam *et al*., 2016). In this study, the crude methanol extract of *Boswellia dalzielii* stem, *Enantia chlorantha* stem and *Abrus precatorius* seed in that order gave high yields of 9.8%, 5.8% and 5.0% respectively. Furthermore, the partition fraction of the three plants, indicate that the aqueous fraction has the highest yield ranging from 73% to 35% and n-hexane fraction has a poor yield ranging from 30% to 0%, indicating that the plant extract depends on the weight of the plant material used, the polarity of the bioactive material present and the solubility of the material in the solvents used for the extraction (Neelam *et al*., 2016).

4.2.4 Antibacterial Evaluation of Crude Extracts and their Fractions

Although the methanolic extract and fractions have potentials for antibacterial activity against *E. coli, P. aeruginosa* and *S. bongori*, the methanolic extract of the three plant and aqueous fraction seem to possess the most significant antibacterial activity than ethyl acetate fractions and n-Hexane fraction. The ethyl acetate extracts and n-Hexane extracts showed minimal antimicrobial activity against the isolates at 150, 250, 300mg/mL Concentrations while the methanolic crude and aqueous extracts gave wider zones of inhibition. The diameter of each zone of inhibition denotes the rate of extract diffusion and was used in this study to estimate an organism's sensitivity to a particular extract. *P.*

aeruginosa and *S. bongori* were resistant to n-Hexane fraction but sensitive to the aqueous fraction, ethyl acetate fraction and methanolic crude extracts of *B. dalzielii* stem, the methanolic extract of *B. dalzielii* stem bark showed broad spectrum activity as all the isolates were sensitive to it at 200, 250 and 300mg/mL concentration (4.6), with zones of inhibition comparable to the antibiotics used as standard. From the results of this study, it is clear that methanolic crude extract and residual aqueous extract of *B. dalzielii* stem bark possess promising antimicrobial activities against the test organisms.

The diameter zones of inhibition of bacterial growth at varying concentration revealed the antibacterial activity of methanolic crude extract of *E. chlorantha* barks (Table 4.8) and its various fractions (residual aqueous fraction and ethyl acetate fraction) (Table 4.11). The methanol extract of the *E. chlorantha* barks revealed significant antibacterial activities against *E. coli, P. aeruginosa* and *S. bongori* with increase in concentration of the extract. The same pattern was observed for each of its fractions, with the exception of *P. aeruginosa* and *S. bongori* which were resistant to n-Hexane fraction. Despite the fact that all the three test organism are Gram negative, thus, possess the presence of outer membrane that serves as an effective barrier in Gram negative species (Tawyabur *et al.,* 2020), the test organisms were susceptible to the extract. In addition, since the zone of inhibition is equal or greater than the standard, it shows that the test organisms are sensitive to the plant extracts. *E. coli* was the most susceptible bacterial, in contrast, *P. aeruginosa* was observed to have a minimal activity, which is attributed to the fact that *P. aeruginosa* has intrinsic resistance from a restrictive outer membrane barrier and transenvelope multidrug resistance pumps (MDRs) (Thuong *et al*., 2013). The extract has significant antimicrobial activity meaning that it could be used to effectively fight against bacterial infections. The activity could be due to the different classes of phytochemicals found, their quantities, and the possible interactions with other constituents of the extract.

The antibacterial activity of *A. precatorius* seed extracts was assayed in vitro by agar well diffusion method against three bacterial species. Table 4.7 and 4.10 summarizes the microbial growth inhibition of methanol extracts and the various fractions (residual aqueous fraction, and ethyl acetate fraction) of the screened bacterial species. Methanol extracts exhibited antibacterial activity towards almost all the bacterial microorganisms. The methanolic crude extracts and residual aqueous extracts showed maximum antibacterial activity on *Escherichia coli* followed by *Salmonella bongori* respectively while the extract showed minimal antibacterial activity against *Pseudomonas aeruginosa*. However, the ethyl acetate extracts showed minimal antibacterial activity on all the three bacterial isolate, On the other hand, the hexane extracts of three plants showed less or no antibacterial activity.

The studied plants were most active against all the bacteria tested. The significant antibacterial activity of the active plant extracts was comparable to the standard antibiotic Amoxicillin and Ciprofloxacin. The variation of susceptibility of the tested microorganisms could be attributed to their intrinsic properties that are related to the permeability of their cell surface to the extracts. Due to the emergence of antibiotic resistant pathogens in hospitals and homes, plants are being looked upon as an excellent alternate to combat the further spread of multidrug resistant microorganisms. The present results therefore offer a scientific basis for traditional use of solvent extracts of the three plants could be a possible source to obtain new and effective herbal medicines to treat infections caused by multi-drug resistant strains of microorganisms.

4.2.5 Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of Methanol extracts of *Boswella dalzielii* stem, *Abrus precarious* seed and *Enantia chlorantha* stem extracts

It was observed that the Minimum Inhibitory Concentration (MIC) at which the isolates were sensitive to the various extracts varied in most cases (Figure 4.2 to 4.12). The MIC values obtained for the entire test organisms ranged from 18.75 mg/mL to 150 mg/ml. The MBC in most cases varied from the MIC, indicating a different concentration needed to inhibit the growth of the microorganisms and an entirely different one to kill them. From the results of this study, it is clear that *B. dalzielii* stem bark, *E. chlorantha* stem bark and *A. precatorius* seed possess promising antimicrobial activities against the test organisms.

In microbiology, large diameters of zones of inhibition correlate with small minimum inhibitory concentrations (MIC). The MIC is usually described as the lowest concentration of an antimicrobial agent that results in inhibition of visible growth (that is colonies on an agar plate or turbidity in broth culture) under standard conditions (Atukpawu and Ozoh, 2014).

The basic quantitative measures of the In-vitro activity of antibiotics and plant extracts with antibacterial potentials are the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) (Atukpawu and Ozoh, 2014). The MIC values obtained for the entire test organisms were very high, ranging from 18.75 mg/ml to 150mg/ml, when compared to the values of 0.01-10 µg/mL usually recorded for typical antibiotics. This difference may be due to the fact that the extracts were in impure forms and would definitely contain substances which do not have antibacterial activities (Tan *et al.,* 2010).

It was observed that the Minimum Inhibitory Concentration (MIC) at which the isolates were sensitive to the various extracts (*B. dalzielii* stem, *E. chlorantha* stem and

A.precatorius seed) varied in most cases (Figure 4.2 to 4.12). The MIC values obtained for the entire test organisms ranged from 18.75 mg/mL to 300 mg/mL. The MBC (Figure 4.2 to 4.12) in most cases varied from the MIC, indicating a different concentration needed to inhibit the growth of the microorganisms and an entirely different one to kill them.

4.2.6 GC-MS Analysis of fractions from *B. dalzielii* stem, *E. chlorantha* stem and *A. precatorius* seed

The results from the above GC-MS analyses support the various ethanobotanical uses of the three plants and the observed antimicrobial activity in (Table 4.12 to 4.14) of this research as the major components identified were reported for their medicinal uses in many research works.

From the GC-MS analysis of aqueous fraction of *B. dalzielii* stem (Table 4.12), 7 compounds have been elucidated and effectively matched and identified. The major constituents with high percentage concentration include 9,12-Octadecadienoic acids (Z, Z)- 2,3-dihydroxypropyl (50.05%), 9-Hexadecenoic acid (23.38%), Tetradecanoic acid (23.94%). others with low concentration identified were n-Hexadecanoic acid (4.02%), 9,12 Octadecadienoic acid (Z, Z) (4.83%), hexadecadienoic acid, 2-hydroxy-1-(hydroxymethyl) (6.42%). In addition, 6 compounds were identified from the ethyl acetate fraction of *B. dalzielii* stem which include 9,12 Octadecadienoic acid (Z, Z) (20.50%), E, Z-1,3,12-Nonadecatriene (12.85%), n-Hexadecanoic acid (12.02%), 9-Octadecenamide, (Z)- (6.31%), 1,2-Benzenedicarboxylic acid, butyl octyl ester (5.90%) and Bis (2-ethylhexyl) phthalate (5.27%). Furthermore, 5 compounds were identified from the n-hexane fraction of *B. dalzielii* stem which include n-Hexadecanoic acid (5.78%), Hexadecanoic acid, ethyl ester (5.11%), 9,12 Octadecadienoic acid (Z, Z) (5.46%), and Cyclopropane octanoic acid (4.51%).

For instance, about 70% of the major components of residual aqueous fraction, ethyl acetate fraction and n-Hexane fraction of *B. dalzielii* stem bark include n-Hexadecanoic acid which is commonly known as Palmitic acid has been reported to have lubricant, flavor, hemolytic 5-alpha reductase inhibitor, nematicide, anti-androgenic, pesticide, antioxidant and hypo-cholesterolemic properties (Lucie *et al*., 2011); Hexadecenoic acid which is a fatty acid ester also have nematicide, pesticide, lubricant, anti-androgenic, flavor, and has hemolytic 5-alpha reductase inhibitor properties (Sermakkani and Thangapandian, 2012) and (z)-9-Octadecenoic commonly known as Oleic acid which is the major component of unsaturated fatty acid that is responsible for its antimicrobial activity, strong antioxidant and anticancer activity (Baha'uddeen *et al,* 2017). Furthermore, the extract has shown to contain 1,2-Benzenedicarboxylic acid, butyl octyl ester which possess antimicrobial and antifouling properties, Bis(2-ethylhexyl) phthalate has antimicrobial activity (Kavitha *et al*., 2009), Also, E, Z, 1,3,12 nonadecatriene posses anti inflammatory and antioxidant properties (Mohammed *et al.,* 2016). However, the varying degrees of the presence of this bioactive compound could be due to the ability of the solvents to extract some of the active ingredient or substances from the plant stem bark based on its polarity (Temitayo *et al*., 2020; Faeji *et al.*, 2017).

GC-MS analysis of the bioactive compositions of *E. chlorantha* stems bark from the residual aqueous fraction and ethyl acetate extracts (Tables 4.14). The major components of ethyl acetate extract of *E. chlorantha* stems bark include Erythritol (6.10%) which is known for its Anti-microbial activity (Temitayo *et al*., 2020). It also contains 9,12-Octadecadienoic acid (Z, Z)- (linoleic acid 16.21%), 9,12-Octadecadienoic acid (Z,Z)-, 2,3-dihydroxypropyl (28.05%) while1,5-Anhydro-d-mannitol (19.34%) is a useful anticarcinogenic agent. It also shows anti-inflammatory and anticancer effects (Alagammal *et al.,* 2012).

GC-MS analysis of the bioactive compositions of *A. precatorius* seed from the residual aqueous fraction (4 compounds) and ethyl acetate (5 compounds) extracts were highly complex compounds containing glycosides, ketone, saturated and unsaturated fatty acids, alcohols, and sterols (Tables 4.13). The major components of residual aqueous fraction and ethyl acetate extract of *A. precatorius* seed include 1,2,3 Benzenetriol (30.83%) which is known for its Anti-microbial activity (Israt *et al.,* 2020). The residual aqueous fraction of A. *precatorius* seed were shown to contain n-Hexadecanoic acid (10.34%), Ethyl hydrazine (4.72%) has anti neoplastic propertieswhile1,5-Anhydro-d-mannitol 1, 5–Anhydro-d-mannitol (31.77%), is a useful anti carcinogenic agent as it inhibits the growth of the oral pathogen Streptococcus mutans. It also shows anti-inflammatory and anticancer effects (Alagammal *et al.,* 2012).On the other hand, the ethyl acetate extracts contains 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy (5.07%) which possess antitumor properties (Lucie *et al*., 2011)**,** [1,1'-Bicyclopropyl]-2-octanoic acid 2'-hexyl (4.18%) and 9,12-Octadecadienoic acid (Z,Z) (14.53%) According to Israt *et al*., 2020, 2,3-dihydroxypropylwhich has several properties like anticancer, anti-inflammatory, anti androgenic, dermatitigenic and antileukotriene-D4.

4.2.7 Quantitative Phytochemical Analysis of Plant Extract

In the present study, phytochemical screening of methanol extract of *Boswella dalzielii* stem, *Abrus precatorius* seed and *Enantia chlorantha* stem showed the presence of alkaloids, flavonoids, tannins, phenols and saponins in all samples tested (Table 4.15).

Moses *et al*., (2019) reported that phenols, tannins, saponins flavonoids and alkaloids were known to show medicinal activity as well as exhibiting physiological activity. Phenolic compounds are commonly found in plants, including *Boswella dalzielii* stem, *Abrus precatorius* seed and *Enantia chlorantha* stem and have been reported to show a wide

range of biological activities including antibacterial activity (Moses *et al.,* 2019; Ismaila & Clement, 2017; Varaprasad and Varahalarao, 2009).

Alkaloids presence in significant amount is claimed to have powerful effects on humans and hence could be used as anti-malaria, anti-microbial and anti-oxidants (Lyczak *et al*., 2014; Sharif *et al.,* 2015).

Total flavonoids of methanol extract of *Boswella dalzielii* stem, *Abrus precatorius* seed and *Enantia chlorantha* stem are 25.32, 66.43 and 26.09 mg/100g respectively. Soad and Amani, (2016) stated that flavonoids are probably the most important natural phenol due to their broad spectrum of chemical and biological activities, including antioxidant and free radical scavenging properties. Flavonoids have been reported as antioxidants and as potential therapeutic agents against a wide variety of diseases. They show anti allergic, anti-microbial and anti-cancer activity by which it can be used for different diseases that are generally found in human body. It has been reported that the presence of phytoconstituents such as flavonoids, tannins and polyphenols prevent a number of diseases through their free radical scavenging activity (Kokilam and Vasuki, 2014) and these phenolic compounds, which include phenol, tannin and flavonoids, have been found in appreciable amounts in *Boswella dalzielii* stem, *Abrus precatorius* seed and *Enantia chlorantha* stem.

The presence of tannin is also important, as it forms irreversible complexes with prolin-rich protein, which results in protein synthesis inhibition (Kindo *et al.,* 2016). Akhlash and Sunil, (2019) also reported that tannins react with proteins to provide tanning effect that helps in the treatment of inflamed ulcerated tissues. Most herbs that contain tannin as a major constituent are claimed to be astringent in nature and useful in the treatment of intestinal disorders like diarrhea and dysentery, wounds, sprains, bruises and arresting

bleeding (Lyczak *et al*., 2014; Oghenejobo *et al*., 2014). Thus it can be suggested from the above that *Boswellia dalzielii*, *Abrus precatorius* seed and *Enantia chloranta* stem are source of bioactive compound that could have effect on the treatment of antimicrobial infections.

Saponins from plants have long been employed for their detergent properties. It is used as mild detergents and in intracellular histochemistry staining to allow antibody access to intracellular proteins (Moses *et al*., 2019).

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

The identities of Bacterial isolate were confirmed using standard procedure. The methanol crude extract of the three plants exhibited antibacterial activity against the bacterial isolates, However, *B. dalzielii* (stem bark) crude extracts exhibited the highest activity. The partitioned fractions of the aqueous fraction of the three plants were observed to have the highest antibacterial activity, while the least activity was observed for n-Hexane fraction.

GC-MS analysis of the fractions predicted therapeutically active compounds present in the residual aqueous and ethyl acetate fractions that may serve as lead compounds in drug formulation. The phytochemical composition revealed the presence of tannins, phenols, alkaloids and saponins.

5.2 Recommendations

From this study's reports, the following recommendations are suggested:

i. There is need for efficient conservation strategies of *B. dalzielii*, *A. precatorius* and *E. chlorantha* due to their high bioactivity and abundance in phytochemicals.

ii. Further studies should be carried out to determine the specific compounds that exhibit the antibacterial activity.

iii. Further purification and isolation of active compounds from these plants extract is also needed to determine their mode of action against bacterial agents.

iv. In addition, adequate toxicological data is needed to validate the safety of the plants of this plant for consumption purpose.

5.3 Contribution to Knowledge

This study established that the three plants (*Boswellia dalzielii*, *Abrus precatorius* and *Enantia chloranta*) exhibited antibacterial activity against the bacterial isolates.

The study also contributed that some important compounds and phytochemical constituents of *Boswella dalzielii, Enantia chlorantha* and *Abrus precatorius* such as 9,12-octadecadienoic acid, (z,z)-,2,3-dihydroxypropyl, 9,12-octadecadienoic acid (z,z)-1,2,3-Benzenetriol, 1,5-anhydro-d-mannitol, (z,z)-2,3-dihydroxypropyl, n-hexadecanoic acid, Erythritol and 1,5-Anhydro-d-mannitol, acetic acid are responsible for the plants biological activities.

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