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COMPARATIVE STUDIES AND SYNERGISTIC EFFECT OF *SENNA ALATA, RICINUS COMMUNIS* LEAVES AND *LANNEA BARTERI* STEM BARK EXTRACTS AND THEIR FORMULATION ON PATHOGENIC BACTERIA AND FUNGUS

RICHARD BOARE MOSOBIL

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COMPARATIVE STUDIES AND SYNERGISTIC EFFECT OF *SENNA ALATA, RICINUS COMMUNIS* LEAVES AND *LANNEA BARTERI* STEM BARK EXTRACTS AND THEIR FORMULATION ON PATHOGENIC BACTERIA AND FUNGUS

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(UDS/MCH/0006/13)

THESIS SUBMITTED TO THE DEPARTMENT OF APPLIED CHEMISTRY AND BIOCHEMISTRY, FACULTY OF APPLIED SCIENCES, UNIVERSITY FOR DEVELOPMENT STUDIES IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTER OF PHILOSOPHY DEGREE IN PHARMACEUTICAL CHEMISTRY

JUNE, 2018

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DECLARATION

I hereby declare that this thesis is the result of my own original work and that no part of it has been presented for a degree in this university or any other university

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ABSTRACT

Three medicinal plant parts, including the leaves of *Senna alata, Ricinus communis* and the stem bark of *Lannea barteri* were used for the current study. The aim was to investigate the antimicrobial effect of the crude extracts alone, their combinations and the crude extracts formulated individually with polyethylene glycol (PEG) and Shea butter, as well as phytochemical screening of the medicinal plants crude extracts. The microorganisms employed in this study were *E. coli, S. aureus P. aeruginosa, K. pneumonia* and *C. albicans* which are known to be associated with skin, wound and genitourinary infections.

The phytochemicals screened showed the presence of tannins, saponins, reducing sugars, polyuronoids, terpenoids, flavonoids, alkaloids, and anthraquinones. The antimicrobial assay indicated that, all the microorganisms were susceptible to the crude extracts used at various concentrations. The crude extracts inhibited the test microorganisms in vitro with lower MIC values. The MBC and MFC values evaluated gave a range between 200 – 400 mg/ml of the respective extracts against the test microorganisms. The polyethylene glycol (PEG) and shea butter formulated ointments showed significant antimicrobial activity against the test microorganisms, with PEG ointment indicating higher activity than the Shea butter ointment at variable concentrations. The MIC values of PEG ointment was relatively lower compared with the Shea butter ointment. The MBC and MFC of the formulated ointment revealed that PEG based ointment showed both bactericidal and fungicidal activity whilst shea butter ointment showed only bacteriostatic activity up to a concentration of 400 mg/g. The combination studies revealed both synergistic and additive effects with no antagonistic effect against the test microorganisms. The MBC/MFC of the combined extract for the test microorganisms was recorded at 150 mg/ml. The findings of the current research using the plant extracts and their formulations against the test microorganisms reveal that, the respective parts of the stated plants could be used as antimicrobial agents.

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DEDICATION

TO GOD BE THE GLORY AND PRAISE

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LIST OF ABBREVIATIONS

mm	millimeters
SD	Standard Deviation
n	Number of replications/repetitions
Amox	Amoxicillin
Fluco	Fluconazole
E. coli	*Escherichia coli*
S. aureus	*Staphylococcus aureus*
P. aeruginosa	*Pseudomonas aeruginosa*
K. pneumoniae	*Klebsiella pneumoniae*
C. albicans	*Candida albicans*
mg	milligram
ml	milliliter
DMSO	Dimethylsulphoxide
MIC	Minimum Inhibitory Concentration
UD	Undetected
aqLB	aqueous *Lannea barteri*
ELB	Ethanol *Lannea barteri*
aqSA	aqueous *Senna alata*
ESA	Ethanol *Senna alata*
aqRC	aqueous *Ricinus communis*
ERC	Ethanol *Ricinus communis*
FIC	Fractional Inhibitory Concentration

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PEG	Polyethylene glycol
A	Additive
S	Synergistic
$moldm^{-3}$	mole per deciliters cubic
$\%^{W}/_{V}$	percentage weight per volume
Cfu/ml	coliform forming units per milliliter
°C	degree Celsius
mg/g	milligram per gram
g/ml	milligram per milliliter
WHO	World Health Organization

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CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Human population expansion with its global effects on the environment over the past million years has ensued in the emergence of infectious diseases (McMichael *et al.,* 2004). Advancement in agriculture further contributed to this, since these infections could only be sustained in hefty and compact human populations (Wolfe *et al.,* 2007). The discovery of antibiotics during the twentieth century combined with momentous advances in antimicrobial drug development improved human health through improved treatment of infections (Tenover, 2006). Nonetheless, protracted use of antibiotics led to bacterial adaptation, resulting in the development of multidrug resistance in bacteria (Livermore, 2003). This has considerably restricted the efficacy of antibiotics, necessitating alternative strategies to combat microbial infections. The persistence of bacteria in the environment and their interaction with humans is central to most infections and illnesses (Upadhyay *et al.,* 2014**)**. Bacterial illnesses are orchestrated by means of an array of virulence factors that enable numerous aspects of their pathophysiology critical for disease in the host (Falkow, 1999) specifically, adhesins and membrane proteins that mediate bacterial attachment, colonization, and invasion of host cells. Additionally, microbial toxins cause host tissue damage and bacterial cell wall components including capsular polysaccharide which confer resistance against host immune system (Wu *et al.,* 2008). Since time in memorial, plants have played a crucial role in the development and well-being of human civilization. A large amount of plant products have been used as food preservatives,

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flavor enhancers, and dietary supplements to prevent food spoilage and retain human health. In addition, plant extracts have been extensively used in herbal medicine, both prophylactically and therapeutically for controlling diseases. The antimicrobial activity of several plant-derived compounds has been previously reported (Burt, 2004; Holley *and Patel,* 2005), and a wide range of active components have been identified (Dixon, 2001). The chief benefit of using plant-derived antimicrobials for therapeutic purposes is that they do not demonstrate the side effects often accompanying the use of synthetic chemicals (van Wyk, 2000). The discernable antimicrobial effect, nontoxic nature, and affordability of these compounds have formed the basis for their wide use as growth promoters in the livestock and poultry industry, effective antimicrobials and disinfectants in the food industry, components of herbal therapy in veterinary medicine, and source for development of novel antibiotics in pharmaceutics (Upadhyay *et al.,* 2014).

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1.2 Problem Statement and Justification

The problem of antibiotic resistance has led to the revival of interest in herbal products as sources of novel compounds to suppress the ever-increasing problems of re-emergence of newer diseases. Pharmaceutical companies all over the world have produced a number of antibacterial drugs to fight infectious diseases but, resistance to these drugs by bacteria has risen in recent times and thus raising global concern. In the broader perspective, bacteria have the ability to transmit and acquire resistance to drugs used as therapeutic agents (Nascimento *et al*., 2001). *Staphylococcus aureus* is noted to be one of the major causes of infections in humans. Methicillin-resistant and multi drug resistant

staphylococci are now a major nosocomial pathogen (NNIS system, 2004). The rising incidence of microbial diseases and non-infectious diseases in humans and associated therapeutic difficulties require the search for new drugs. Bacterial and fungal infections are widespread and antimicrobial resistance is now a global threat (Chatterjee and Fleck, 2011). It is not a new problem but one that is becoming more dangerous, particularly in West African countries where high prevalence of multi-drug resistant bacteria (MRSA, bacteria producing beta-lactamases) have been reported (Keasah *et al*., 1998; Okesola *et al*., 1999; Kacou-N'Douba *et al*., 2001; Akoua *et al*., 2004; Akinyemi *et al*., 2005). Medicinal plants produce number of bioactive compounds which are used mostly for medicinal purposes. These compounds either act on different systems of animals including man, and/or act through interfering in the metabolism of microbes infecting them. The microbes may be pathogenic or symbiotic. In either way, the bioactive constituents from medicinal plants play an important role in regulating host-microbe interaction in support of the host. Interest in medicinal plants as a re-emerging health aid has been necessitated by the increasing costs of prescription drugs in the maintenance of personal health and well-being and therefore bio prospecting of new plant-derived drugs is welcoming news (Lucy and Edgar, 1999). The on-going growing recognition of medicinal plants among other reasons, including escalating faith in herbal medicine (Kala, 2005), an increasing reliance on the use of medicinal plants in the industrialized societies and development of drugs and chemotherapeutics from these plants as well as from traditionally used herbal remedies (UNESCO, 1998). According to World Health Organization, medicinal plants would be the best source to obtain a variety of drugs (Nascimento *et al*., 2000). Therefore, properties, safety and efficacy of medicinal plants should be investigated (Nascimento *et al*., 2000). There is a general agreement among the

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scientific community that natural products have a dominant presence in discovering new leads for the development of drugs. In fact, of the 877 novel medicines that were developed in the period between 1981-2002, 6% were natural products, 27% were derivatives of natural products and 16% were synthetics developed based on a natural product (Newman, 2008). At least 80% of the world population is estimated to continue the use of such traditional medicines in primary health care, including 40,000-70,000 medicinal plants, approximately 20% of all higher-plant species (Verpoorte *et al*., 2006).

In rational drug therapy, the simultaneous administration of two or more drugs is very important and sometimes mandatory in order to achieve an acceptable therapeutic goal or to treat co-existing diseases (Hugo and Russel, 1993; Levinson and Jawetz, 2002). For instance, drugs combination has been used in treating the most dreadful diseases such as AIDS and cancer (Chou, 2006). Research indicates that, plant extracts in combination with two or more medicinal agents exhibit effective antimicrobial activity against a wide range of microorganisms including drug resistant bacteria (Prakash *et al*., 2006a; Karmegam *et al*., 2008).

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In spite of the existence of potent antibiotic and antifungal agents, resistant or multi-resistant strains are keep on appearing, imposing the need for a permanent search and development of new drugs. Thus the search for natural product that could hold the key to this rather challenging situation of our world today is understatement.

1.3 Aim

To determine potentiation and synergistic effect of parts of *Senna alata Ricinus communis and Lannea barteri* and their ointment formulations on selected pathogens.

1.4 Objectives

To prepare crude aqueous and ethanol extracts of parts of *Senna alata* and *Ricinus communis and ste*m bark of *Lannea barteri* and to:

(a)

- Determine the yield of the crude extrants
- Conduct phytochemical screening of the extracts.
- Examine *in vitro* antimicrobial activity of the plant crude extracts.
- Determine Minimum Inhibitory Concentration (MIC) of each extract.
- Determine the Minimum Bactericidal/ Fungicidal Concentration (MBC)/ (MFC) of the extracts.

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(b)

- Formulate the plants extracts separately with polyethylene glycol (PEG) and Shea butter
- Evaluate the antimicrobial effect of the formulated ointments.
- Determine MIC and MBC/MFC of each formulated ointment.

(c)

- Determine synergistic effect of the extracts used in combinations.
- Determine MIC of each combined extracts.
- Determine MBC/MFC of each combined extracts.
- Determine Fractional Inhibitory Concentration Index (FIC).

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1.5 Significance of the Study

This research is interested in discovery of an effective product with easy formulation and applicability for treatment of wound and skin diseases. The research also desired a final product which could be useful industrially for the production of ointment-based extract that would be highly potent against wound and skin disease pathogens and also easily handled by and attractive to users.

The importance of this research is also to add to existing knowledge. It may also contribute to the need for herbal drugs as alternative to be part of mainstay therapy for health delivery system due to numerous bioactive compounds.

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CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Medicinal Plants

Medicinal plants have to date been screened for various biological activities in both in vivo and in vitro models. The chemical investigation and purification of extracts from plants purported to have medicinal properties have yielded numerous purified compounds which have proven to be indispensable in the practice of modern medicine. Medicinal plants have been used by people since time immemorial without the knowledge of their active ingredients. There is however common practice of taking crude extracts with hazards as the extracts may contain some toxic constituents (Hoareau and DaSilva, 1999). Information on active ingredients and curative actions of the medicinal plants was obtained by the introduction of European scientific method (Bruneton, 1995). Information in the form of folklore practices showed that, the aborigines used many plant materials for curative purposes, long before the conquest by the Europeans (Bruneton, 1995). Plants are found to be sources of many chemical compounds, most of which account for their various uses by man. The most important of these compounds are alkaloids, terpenoids, steroids, phenols, glycosides and tannins (Bruneton, 1995; Rios and Recio, 2005).

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An impressive number of modern drugs have been isolated from natural sources. Many of these isolations were based on the uses of the agents in traditional medicine. The plant-based, traditional medicine systems continue to play an essential role in health care, with

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about 80 % of the world's inhabitants relying mainly on traditional medicines for their primary health care (Sofowora, 1982).

India has several traditional medical systems, such as Ayurveda and Unani, which have survived over 3000 years, mainly using plant-based drugs. The *materia medica* of these systems contains a rich heritage of indigenous herbal practices that have helped to sustain the health of most rural people of India. The ancient texts like Rig Veda (4500-1600 BC) and Atharva Veda mention the use of several plants as medicine. The books on ayurvedic medicine such as *Charaka Samhita* and *Susruta Samhita* also refer to the use of more than 700 herbs (Kirtikar & Basu, 1918; Kirtikar & Basu, 1935; Satyavati *et al.*, 1976).

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According to the World Health Organization (WHO, 1999) "a medicinal plant" is any plant, in which one or more of its organs contain substances that can be used for therapeutic purposes or which are precursors for the synthesis of useful drugs. This definition distinguishes those plants whose therapeutic properties and constituents have been established scientifically and plants that are regarded as medicinal but which have not yet been subjected to thorough investigation. Furthermore, WHO defines medicinal plant as herbal preparations produced by subjecting plant materials to extraction, fractionation, purification, concentration or other physical or biological processes which may be produced for immediate consumption or as a basis for herbal products (WHO, 1999, 2003).

The term "herbal drug" determines the part/parts of a plant (leaves, flowers, seeds, roots, barks, stems, etc.) used for preparing medicine (WHO, 1999, 2003)..

2.2 Uses of Medicinal Plants

Medicinal plants are plants containing inherent active ingredients used to cure disease or relieve pain (Sofowora, 1982; Rios and Recio, 2005). The use of traditional medicines and medicinal plants in most developing countries as therapeutic agents for the maintenance of good health has been widely observed. Modern pharmacopoeia still contains at least 25% drugs derived from plants and many others, which are synthetic analogues, built on prototype compounds isolated from plants (Sofowora, 1982; Rios and Recio, 2005).

The medicinal properties of plants could be based on the antioxidant, antimicrobial, antipyretic and anti-inflammatory effects of the phytochemicals in them (Kirtikar and Basu, 1918; Satyavati *et al.*, 1976; Sofowora, 1982; Farnsworth *et al.*, 1985; Bruneton, 1995). According to the World Health Organization, medicinal plants would be the best source to obtain a variety of drugs. Therefore, such plants should be investigated to better understand their properties, safety and efficacy (WHO, 2003).

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The instant rising demand of plant-based drugs is unfortunately creating heavy pressure on some selected high-value medicinal plant populations in the wild due to over-harvesting. Several of these medicinal plant species have slow growth rates, low population densities, and narrow geographic ranges, therefore they are more prone to extinction (Bruneton, 1995). Conversely, because information on the use of plant species for therapeutic purpose has been passed from one generation to the next through oral tradition, this knowledge of therapeutic plants has started to decline and become obsolete through the lack of recognition by younger generations as a result of a shift in attitude and

ongoing socioeconomic changes (Sofowora, 1982). Furthermore, the indigenous knowledge on the use of lesser-known medicinal plants is also rapidly declining.

2.3 *Ricinus Communis*

2.3.1 Description of *Ricinus communis*

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The plant *Ricinus communis* is a member of the family Euphorbiaceae. This plant is a member of the genus *Ricinus* which is traditionally known as castor bean. Castor seed is the source of castor oil, which has a number of uses. Castor oil is viscous, pale yellow, non-volatile and non-drying oil with a bland taste. It has good shelf life as compared to other vegetable oils. The seeds contain 40 to 60 % oil that is rich in triglycerides mainly ricinolein, a toxic alkaloid ricinine and very toxic albumen called ricin. The seed coat contains ricin, a poison which is present in lower concentrations throughout the plant. *Ricinus communis* has not only medicinal value but it also has great promises in the field of biodiesel production. It is inexpensive and environmentally friendly (Begum and Nath, 2000; Jena and Gupta, 2012; Rana *et al.*, 2012). There are different varieties of castor oil bean and on the average; they contain 46-55% oil by weight. Castor oil plant is a wooden tall plant that grows up to 3.5 to 13.5 m tall with simple hallowed stems. The leaves are long cured, cylindrical with purplish or green petioles. The flowers are monoecious, large and arranged on the thick rachis of an oblong panicle. The fruits of *R. communis* are greenish, deeply grooved, tricoccus capsules, dehiscing longitudinally and septicidally into six valves. The seeds are ovoid in shape, flattened and smooth shining (Jeyaseelan and Jashothan, 2012). The roots are light in weight almost straight with few rootlets, with

dull yellowish brown, outer surface and nearly smooth but marked with longitudinal wrinkels (Rana *et al.*, 2012). *R. communis* is found in South Africa, India, Brazil and Russia. The plant is non-toxic to most insects, even though small amounts of the toxic protein ricin and alkaloids tricinine are found in vegetative parts of this plant. *R. communis* stems contain Ricinine (1-methyl-3-cyano-4-methoxy-2-pyridine) amino acid. The stems also contain carbohydrates, saponins, flavonoids and tannins. *R. communis* has been traditional used for many purposes throughout the whole world (Singh *et al.*, 2010).

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Figure 2.1: *Ricinus communis* plant (Source: Chan et al., 2010)

2.3.2 Medicinal properties of *Ricinus communis*

Different parts of *R. communis* are used for medicinal purposes including the leaves, barks, seeds, roots and oil. *R. communis* is well known for its biological activities, most important of which are hepatoprotective, laxative, antidiabetic and antifertility activities. The uses of *R. communis* as medicines have been described in Ayurveda and these include the use of fresh leaves for headache, a decoction of the leaves as an emmenagogue and juice from leaves used against ringworms, warts, and dropsy. The root bark is purgative and it is also used for skin diseases. *R. communis* seeds contain a lot of oil prescribed against infestation of intestinal worms. The leaf, root and seed oil of castor oil plant have also been used for treatment of inflammation and liver disorders in India (Taur *et al.*, 2011).

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The plant has antioxidant activity, DPPH (1,1-diphenyl-2- picrylhydrazyl)-mediated in vitro study reveals that gallic acid, quercetin, gentisic acid, rutin, epicatechin and ellagic acid are the major phenolic compounds responsible for the antioxidant activity of the dry leaves of *Ricinus communis* Linn (Gupta *et al.*, 2007). It also has antitumour activity; Ricin A, a lectin isolated from *R. communis* possess antitumor activity that was more toxic to tumor cells than to non-transformed cells, judged from the ED50 of the lectin towards tumor cells and non-transformed cells. In addition, it has bone regeneration activity (Lin and Liu, 1986). *Ricinus communis* polyurethane (RCP) has been studied for its biocompatibility and its ability to stimulate bone regeneration. Results showed that RCP blended with calcium carbonate or calcium phosphate could promote matrix mineralization and are biocompatible materials. Incorporating alkaline phosphatase to RCP with subsequent incubation in synthetic body fluid could improve the biological

properties of RCP. The advantage seen in RCP as compared to demineralized bone is that, the former has a slower reabsorption process. Also it has anti-implantation activity; the ether soluble portion of the methanol extract of *Ricinus communis* var. minor possesses anti-implantation, anticonceptive and estrogenic activity in adult female rats and rabbits when administered subcutaneously (Desta, 1994; Makonnen *et al.*, 1999). Antiasthmatic activity of the plant revealed that the ethanol extract of *R. communis* roots possess antiasthmatic activity. It significantly decreases milk induced leucocytosis and eosinophilia and protects degranulations of mast cells in mice (Taur & Patil, 2011).

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2.3.3 Anti-microbial properties of *Ricinus communis*

The plant parts of *Ricinus communis* leaves, root, stem, and seed, showed good activity against dermatophytic and pathogenic bacteria. In earlier works, the methanol, ethanol extract (100/200 ml) of leaves of *R. communis* showed activity against *Staphylococcus aureus* and *Escherichia coli*. The stem extracts also, showed prominent activity against *Staphylococcus aureus* and *Escherichia coli whiles the* aqueous root extracts showed no antibacterial activity against these pathogens (Obumselu *et al.*, 2011).

R. communis has been studied by various researchers from different countries for antinociceptive activity of methanol extracts of the leaves. This activity was suggested to be due to the presence of saponins, steroids and alkaloids (Taur *et al.,* 2011) and presence of antioxidant activities due to methyl Ricinoleate and Ricinoleic acid from seeds extracts (Oloyede, 2012). Essential oils from *R. communis* have been reported to have potential antimicrobial and anticarcinogenic activities (Zarai *et al.,* 2012). The *Ricinus communis*

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also possess wound healing activity demonstrated by Donkor and his colleagues in 2016, which may be due to the active constituent of castor oil which produce antioxidant activity and inhibit lipid peroxidation. Those agents which inhibit lipid peroxidation are believed to increase the viability of collagen fibrils by increasing the strength of collagen fibres, increasing the circulation, preventing the cell damage and by promoting the DNA synthesis (Donkor *et al.*, 2016). The study of wound healing activity of castor oil was in terms of scar area, % closure of scar area and epithelization in excision wound model. The astringent and antimicrobial properties of tannins, flavonoids, triterpenoids and sesquiterpenes promote the wound healing process, which are responsible for wound contraction and increased rate of epithelialisation. The study revealed that, the castor oil showed wound healing activity by reducing the scar area and also the epithelization time in excision wound model. The comparison study of two different concentrations (5%w/w and 10%w/w) of castor oil revealed that, the 10 % w/w castor oil ointment possesses better wound-healing property (Prasad *et al.*, 2011a).

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2.4 *Senna alata*

2.4.1 Description of *Senna alata*

Senna alata is also known as *Cassia alata* and commonly known as Ringworm bush. It is from the bean family and originally found in the tropical regions of Africa, Southeast Asia, the Pacific Islands and America. It can reach a height of up to 30 feet in its native habitat, although 5 to 8 feet is more typical in a backyard garden, with a spread approximately half that width. An aggressive grower, especially in areas with a high-

water table, *Senna alata* often forms thickets through natural propagation. For this reason, the shrub is a good choice for borders. As a specimen plant, it could also be grown in outdoor containers or tubs (Bonnie, 2015**).** The thick, pithy stems are upright (i.e. erect or ascending) and occasionally branched. The compound, (i.e. pinnate) leaves are alternately arranged along the stems and very large (45-80 cm long and 12-25 cm wide). They are borne on stalks (i.e. petioles) 2-4 cm long and have 8-14 pairs of large leaflets. The individual leaflets (5-17 cm long and 2-5.5 cm wide) are either oblong, oval (i.e. elliptic) or egg-shaped in outline (i.e. ovate) and have entire margins. They are finely hairy (i.e. pubescent) and have rounded or slightly notched tips (i.e. obtuse, retuse or emarginate apices) (Navie, 2004). The golden yellow or orange flowers are borne in elongated clusters (15-60 cm long) at the tips of the stems or in the upper leaf forks (i.e. interminal or axillary racemes). These clusters are borne on hairy stalks (i.e. pubescent peduncles) 15-30 cm long and contain numerous (20-40) densely crowded flowers. The individual flowers (2-3 cm across) are borne on short stalks (i.e. pedicels) 5-8 mm long (Navie, 2004). The large and elongated pods (12-25 cm long and 8-20 mm in wide) turn dark brown to black in colour as they mature and contain numerous (about 50) seeds.

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Figure 2.2: ***Senna alata***

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2.4.2 Medicinal properties of *Senna alata*

This specie occasionally escapes from cultivation to become naturalized, but it does not readily spread. Ringworm bush is widely used as a traditional medicine, particularly valued for its laxative effect and also for treatment of several skin conditions, including ringworm and scabies. Research has tended to confirm the validity of these traditional treatments. A number of anthraquinone derivatives have been isolated from the leaves, such as aloe-emodin, chrysophanol, isochrysophanol and rhein, as well as the alkaloid tyramine and the common steroid beta-sitosterol. Crude leaf extracts have shown antibacterial activity against a range of bacteria (such as *Dermatophilus congolensis*, which causes a serious skin condition in cattle), (Akinyemi *et al*., 2000) antifungal properties (such as against *Pityriasis versicolor* in humans) and also antitumor activity.

The bark contains tannins and the petals contain anthraquinones, glycosides, steroids, tannins and volatile oil. Extracts of the petals have bactericidal activity against Gram-positive bacteria but not against gram-negative bacteria. The plant is laxative, antibacterial, anti-inflammatory, diuretic, analgesic, vulnerary, weakly antifungal, hypoglycaemic and antispasmodic (Makinde e*t al.,* 2007). The leaves are taken internally as a remedy for constipation and to purify the blood. The leaves are also decocted, with or without *Tripogandra serrulata* and *Persea americana,* as a treatment for biliousness and hypertension. They can be applied as a tincture; as a poultice; powdered, then mixed with oil as an ointment; or the sap can be spread over the affected area - they form an effective treatment for skin blemishes, scabies, ringworm and other fungal skin infections (Benjamin *et al.,* 1981). The bark is used to treat diarrhoea, worms, scabies and eczema. The root is laxative. An infusion is used in the treatment of diarrhea, tympanites, uterus problems and filaria worm expulsion. The root is applied externally to treat sores and skin fungi. The flowers are used as a laxative and vermifuge (Benjamin *et al.,* 1981). A decoction combined with *Zingiber officinale*, is used as a treatment for grippe and as an abortifacient. They are decocted with coconut milk for use as a laxative. The leaves, flowers and fruit are mixed in an infusion to treat stomach problems. The seed is laxative and anthelmintic. It is cooked and used as a remedy for intestinal worms. The leaf contains the purgative anthraquinone, and also shows some antimicrobial activity. The stem contains chrysophanol, emodin, rhein and aloe emodin. The leaf and fruit contain purgative anthracene derivatives of aloe emodin and rhein (Dalziel, 1937).

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2.4.3 Anti-microbial properties of *Senna alata*

Senna alata is often called the ringworm bush because it has effective fungicidal properties, for treating ringworm and other fungal infections of the skin. The leaves are ground in a mortar to obtain a kind of "green cotton wool". This is mixed with the same amount of vegetable oil and rubbed on the affected area two or three times a day (Afrin, 2015). A fresh preparation is made every day. Its active ingredients include the yellow chrysophanic acid. The methanol extracts of *Senna alata* exhibited very strong activity against some microbes with maximum activity in the fractions containing alkaloid salts and base, the results lend support to the traditional use of the plant (flower and leaf) for the treatment of fungal skin diseases (Makinde *et al.*, 2007). In a preliminary in vitro antimicrobial sensitivity screening, methanol extract of *Senna alata* showed excellent activity against *C. immitis, E. dermatitidis, A. fumigatus, C. albicans* and *S. aureus* (Kareru *et al.*, 2010; Alalor *et al.*, 2012; Majekodunmi & Essien, 2014). Most of these organisms are natural flora of the skin and the genitals, and also known as etiologic agents of several skin and mucous membrane infections of man. The plant extract did not show any activity against *C. albicans* at 25 and 50 mg/mL(Majekodunmi & Essien, 2014). The activity was concentration dependent as revealed by the zone of inhibition (Majekodunmi & Essien, 2014). Furthermore, investigation on *Senna alata* revealed that the water extract from *Senna alata* leaves contained potential antifungal agent against *Candidia albicans* and antibacterial agent against *Escherichia coli*. These results were comparable to commercial antifungal drug amphotericin B and antibiotic chloramphenicol (Somchit *et al.*, 2003).

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2.5 *Lannea barteri*

2.5.1 Description of *Lannea barteri*

Lannea barteri belongs to the family Anacardiacea. It is a deciduous tree with a spreading crown; it can grow from 5-18 metres tall. The bole is usually straight and clear of branches for several meters; it can grow up to 40 cm in diameter with a thick bark. The plant flowers are unisexual and usually regular with 8 stamens, 4 cell ovaries and 4 stigmas, terminal leaflet with long stalk, the fruit are cylindrical (Jansen *et al.*, 2005). It is an important source of a red-brown dye that is used in traditional dyeing in Africa and also supplies food, medicines and materials (Garba *et al.*, 2015). *Lannea barteri* is a tree of West and Central Africa and can be found in Ethiopia (Kokwaro and Gillett, 1980). In Uganda, it grows in wooded savannah grassland preferring rather hilly ground. It can also be seen at forest edges or near rivers. The bark is grey, spirally grooved, fairly smooth and branchlets hairy, red-brown. The leaves are compound, 5-9 leaflets on a stalk 10-25 cm which is brown and hairy, ovate-oblong 9-13 cm, the tip usually pointed, base one-sided and rounded. The flowers are clustered at the end of twigs for female trees. The fruit is oval-oblong, about 1 cm, dull purple when ripe and flattened (Kokwaro & Gillett, 1980).

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Figure 2.3: ***Lannea barteri***

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2.5.2 Medicinal properties of *Lannea barteri*

Phytochemical analysis of the roots and stem bark extracts (Kone *et al.*, 2011) reported the presence of steroids, triterpenoids, saponins, polyphenols, flavonoids, tannins, alkaloids and quinoine. Quantitative estimation proved that both extracts of roots and stem bark have considerably high constituents of phenolic compounds. Similar total phenolic contents were obtained from the stem bark and roots, 254.46 and 254.96 µg/g respectively (Kone *et al.*, 2011) . The bark is used externally to treat ulcers, sores and leprosy. A root decoction is taken to cure hernia. The root is ground, wrapped in the leaves of an unknown species, and applied as a poultice on wounds. A leaf decoction is

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taken to cure haemorrhoids (Garba *et al.*, 2015). Garba and his colleagues reported that, the stem bark of *L. barteri* is used in the treatment of epilepsy, gastritis, childhood convulsions among other uses in northern Nigeria for many years (Garba *et al.*, 2015). The popularity of its efficacy is well established among the Traditional Medical Practitioners. The bark is stomachic. A decoction is drunk as a treatment for gastric pains, diarrhoea, oedema, paralysis, epilepsy and madness (Kone *et al.*, 2011).

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2.5.3 Antimicrobial properties of *Lannea barteri*

Studies on the leaves of this plant revealed the presence of tannins, flavonoids and steroids with considerable antibacterial and antifungal activity (Donkor *et al.*, 2016). Similarly, the leaves had shown broad spectrum activity against Gram positive and Gram negative isolates (Adegoke *et al.*, 2013).

2.6 Phytomedicine

Herbs are natural products and their chemical make-up depends on several factors, such as botanical species, used chemo types, the anatomical part of the plant used (seed, flower, root, leaf, fruit rind, etc.), humidity, storage, type of ground, time of harvest, geographic area among others. The variation can result in significant differences in pharmacological activity in both pharmacodynamics and pharmacokinetics issues (Park 2008).

Guidelines for evaluating the quality, safety and efficacy of herbal medicines was published by World Health Organization (WHO) which aimed at assisting national

regulatory authorities, scientific organizations and manufacturers (Akerele, 1993). Plants produce a large number of different bioactive compounds with high concentrations of phytochemicals, which protect against free radical damage, accumulate in fruits and vegetables (Suffredini *et al.,* 2004). The table below shows biological activities of phytochemicals.

Table 2.1: Bioactivity Phytochemicals in Medicinal plants

Classification	main groups of compounds	Biological function
NSA (Non-starch polysaccharides)	Cellulose, hemicellulose, gums, mucilages, pectins, lignins	Water holding capacity delay in nutrient absorption, binding toxins and bile aids
Antibacterial and antifungal	Terpenoids, alkaloids, phenolics	Inhibitors of microorganisms, reduce the risk of fungal infection
Antioxidants	Polyphenoli compounds, flavonoids, carotenoids, tocopherols, ascorbic acids	Oxygen free radical quenching, inhibition of lipid peroxidation
Anticancer	Carotenoids, polyphenols, curcumine, flavonoids	Inhibitors of tumor, inhibited development of lung cancer, anti-metastatic activity
Detoxifying agents	Reduce acids, tocopherols ,phenols, indoles ,aromatic isothiocyanates, coumarins, flavones, carotenoids, retinoids, cynates, phytosterols	Inhibitors of procarcinogen activation inducers of drugs binding of carcinogens Inhibitors of tumourogenesis
Other	Alkaloids, terpenoids, volatile flavor compounds, biogenic amines	Neuropharmacological agents, antioxidants cancer chemoprevention

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2.6.1 Alkaloids

They are largest group of secondary chemical components made up of largely of ammonia compounds consist of basically of nitrogen bases synthesized from amino acid building blocks with various radicals replacing one or more of the hydrogen atoms in the peptide ring, most containing oxygen. In fact, one or more nitrogen atoms that are present

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in an alkaloid, typically as 1°, 2° or 3° amines, contribute to the basicity of the alkaloid. Below are some structures of alkaloids (Venketeshwer, 2012).

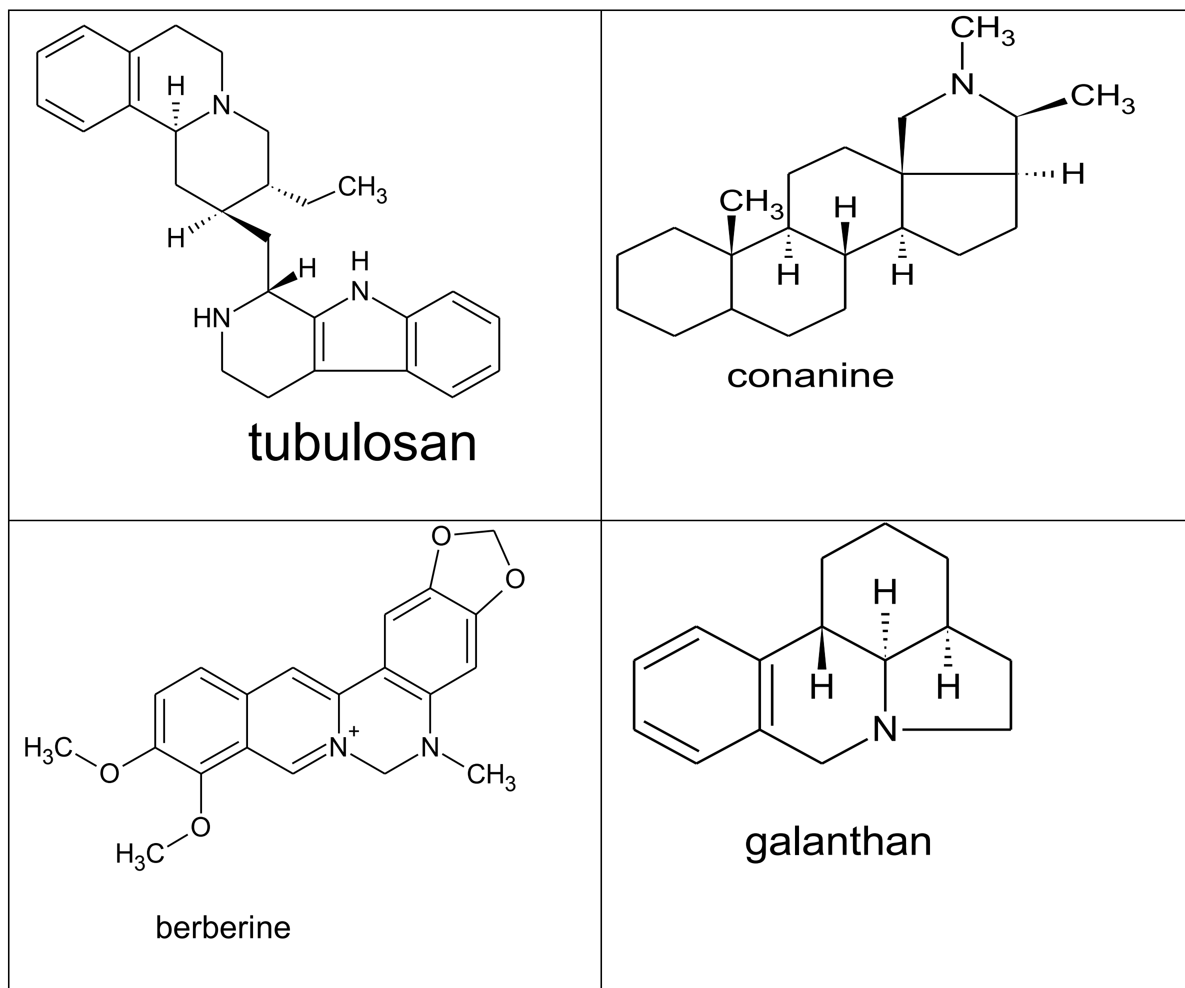

Figure 2.4: Examples of Alkaloids

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2.6.2 Flavonoids

Flavonoids are important group of polyphenols mostly found among the plant flora. They are made of more than one benzene ring in its structure (a range of C15 aromatic compounds) and are said to have antioxidants or free radical scavengers' properties (Kar, 2007; Venketeshwer, 2012). The compounds are derived from parent compounds known

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as flavans. Over four thousand flavonoids are known to exist and some of them are pigments in higher plants.

Genistein	Cyanidin-o-galactoside
Procyanidin B5	Flavone C-glycoside

Figure 2.5: Examples of Flavonoids

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2.6.3 Tannins

Tannins are polyphenols and are found in abundance in the tree bark, wood, fruit, fruit pod, leaves and roots and also in plant gall. Tannins can be categorized into two broad

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groups – hydrolysable tannins and condensed tannins. The tannin epigallo-catechin-3-gallate is said have anti-diabetic activity (Kang et al., 2011) .

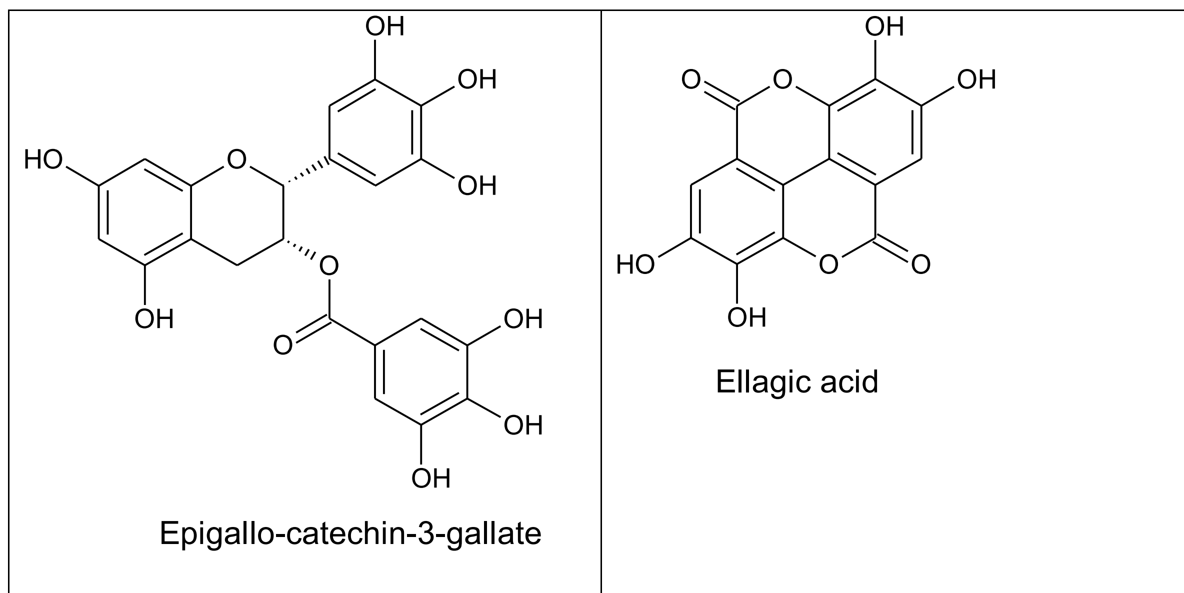

Figure 2.6: Examples of Tannins

2.6.4 Glycosides

Glycosides are condensate of sugars (including polysaccharides) with a host of different varieties of organic hydroxy (occasionally thiol) compounds (invariably monohydrate in character), in a way that the hemiacetal entity of the carbohydrate are involve in the condensation. Glycosides are colorless, crystalline carbon, hydrogen and oxygen-containing (some contain nitrogen and sulfur) water-soluble phytoconstituents, found in the cell sap. Glycosides are made up of carbohydrate (glucose) and a non-carbohydrate part (aglycone or genin) (Kar, 2007; Firn, 2010). Alcohols, glycerols or phenols are examples of aglycones. Glycosides are neutral in reaction and can easily hydrolyze into its components with ferments or mineral acids. Classifications of glycosides are based on the type of sugar component, chemical nature of aglycone or pharmacological action.

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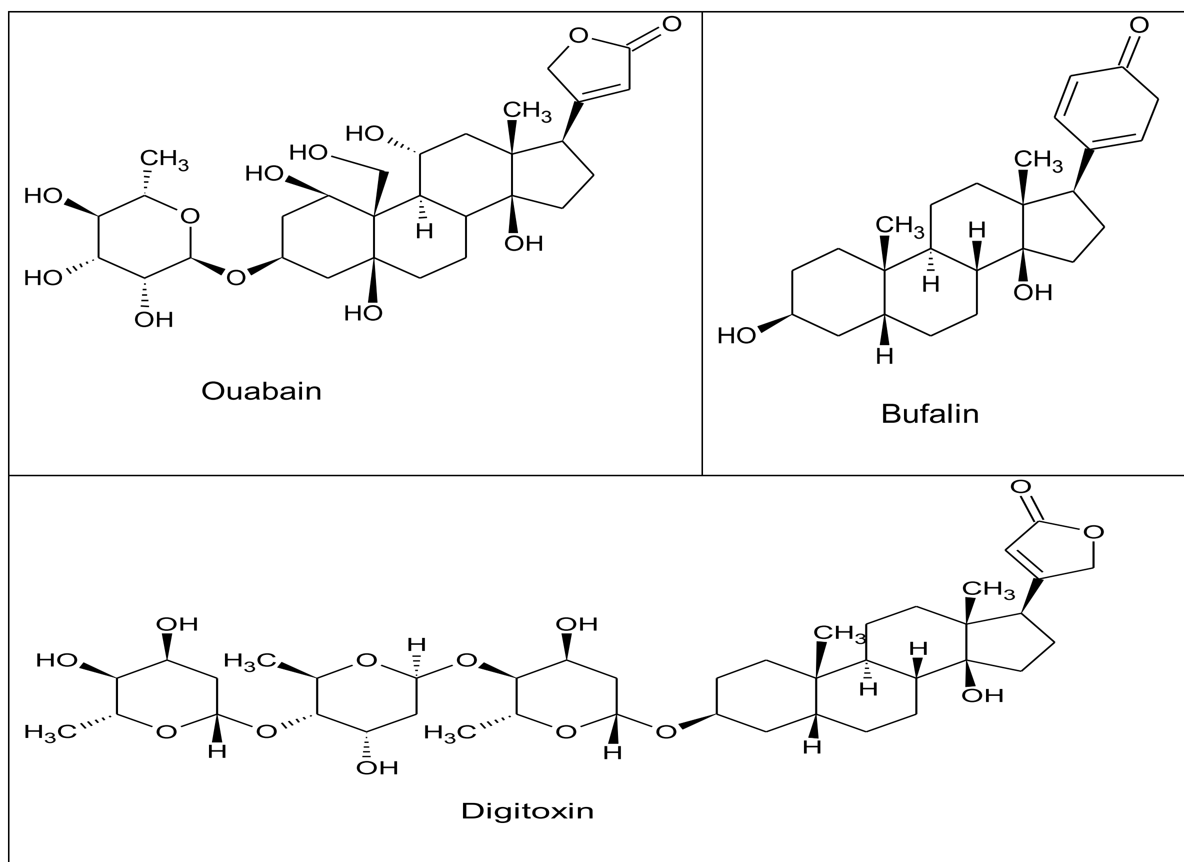

Figure 2.7: Examples of Glycosides

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2.6.5 Steroids

Plant steroids (or steroid glycosides) also known to as 'cardiac glycosides' are one of the most naturally occurring plant phytoconstituents and are found to have therapeutic applications (Firn, 2010).

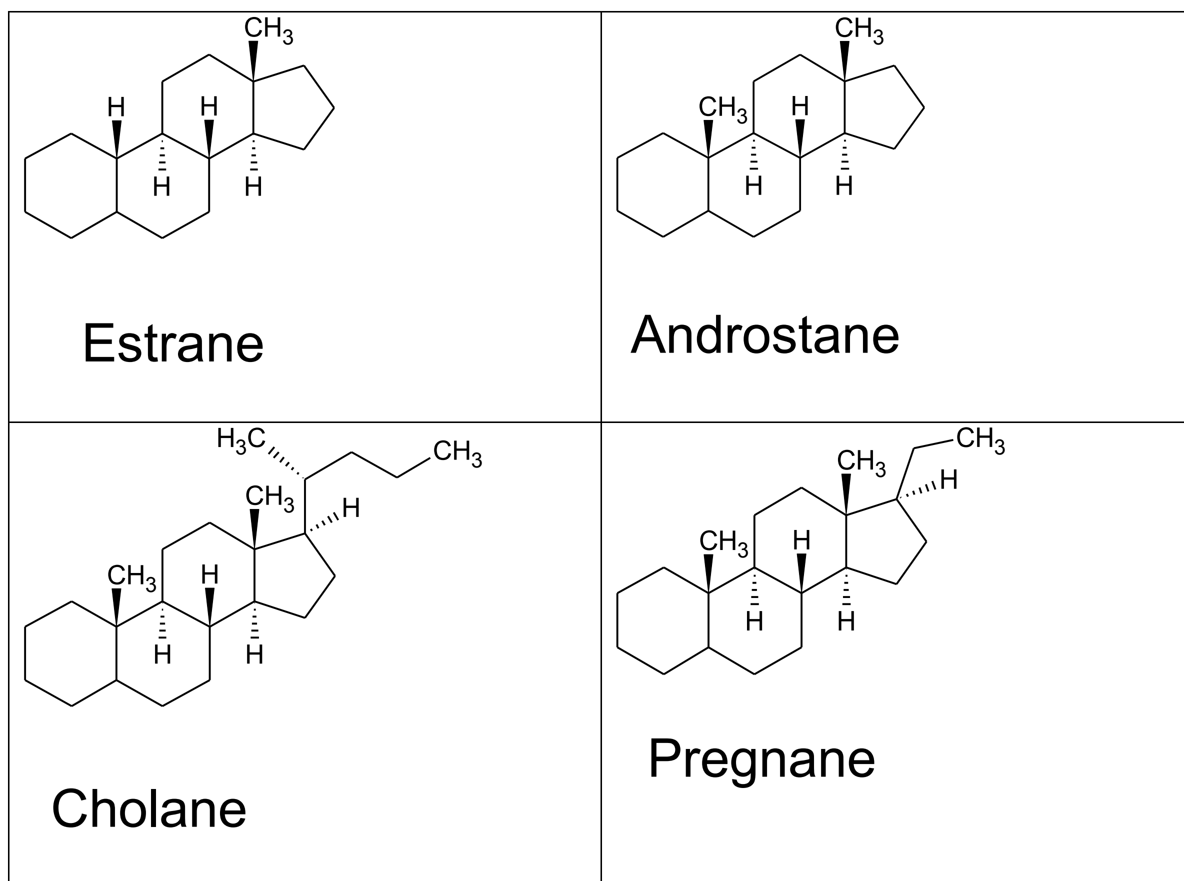

Figure 2.8: Example of Steroids

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2.6.6 Terpenes

Terpenes are well distributed and chemically diverse groups of natural products. They are flammable unsaturated hydrocarbons, which are known to exist in liquid form and are found in essential oils, resins or oleoresins (Firn, 2010). Terpenoids comprise hydrocarbons of plant origin of general formula $(C_5H_8)_n$ and are categorized into mono-, di-, tri- and sesquiterpenoids depending on the number of carbon atoms. Terpenoids are also classified based on number of isoprene units involved in the formation of a compound.

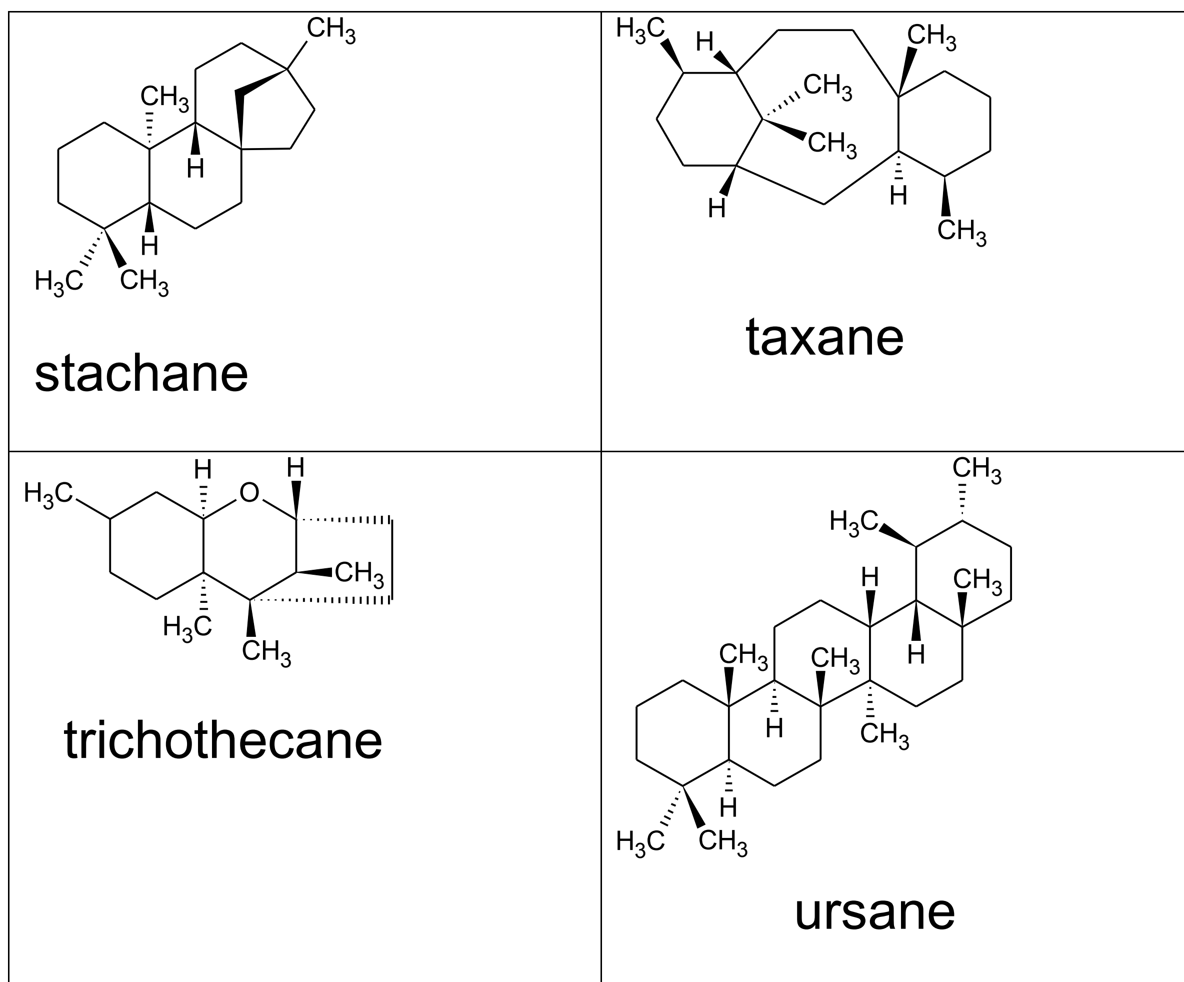

Figure 2.9: Examples of Terpenes

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2.6.7 Saponins

Saponins are plant compounds that occur either as steroid alkaloids, glycosides of triterpenoids or steroids. They have hypocholesterolaemic, immunostimulant, hypoglycemic effect and anticarcinogenic properties (Ros 2000). The hypoglycemic effect of saponins is said to due to stimulation of pancreatic β-cells, inhibition of glucose transport across the brush border cells of the small intestines and suppression of transfer of glucose from the stomach to the small intestines. Saponins are said to inhibit gastric emptying in a dose dependent manner (Tan and Vanitha, 2004). Saponins lower

cholesterol level by forming large micelles that are then excreted in bile. They also lower serum levels of low density lipoproteins-cholesterol and decrease absorption of cholesterol in the intestines (Chung, 2004)

2.7 Pathogens Responsible for Wound and Skin Infections

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There are thousands of kinds of bacteria, but only a few actually cause diseases in humans. Bacteria are frequently identified by their shape, the makeup of their cell walls, and their ability to grow in air. They can be round (such as *staphylococci or streptococci*), rod-shaped (such as bacillus or *E. coli*), or corkscrew-shaped (*Borrelia* species). In most cases, bacteria have cell walls that provide a target for many antibiotics (antibiotics easily identify bacteria) (Madigan *et al.*, 2008). They are also classified by their colour after a Gram stain is applied. Gram-positive bacteria stain blue, while Gram-negative bacteria stain pink. Gram-negative bacteria cell walls contain a substance known as lipopolysaccharide (LPS), a highly inflammatory chemical that provokes an immune response in the human body. LPS is responsible for triggering the overreaction of the host immune system, which results in the release of oxygen and nitrogen species, cytokines, and other pro-inflammatory mediators. Due to some antibacterial ineffectiveness, there is increase in population of patients that attend medical centres in developing countries (Madigan *et al.*, 2008). A large group of microbial pathogens are associated with various skin infections. The *streptococci* and *staphylococci* cause wound infections. These microbes cause opportunistic skin infections in immunosuppressed patients. The *E. coli* are part of physiological intestinal flora, however outside the intestine they may cause

wound infection and sepsis (Madigan *et al.*, 2008). *Pseudomonas aeruginosa* is a prevalent burn patient pathogen, this microbe is able to infect different parts of human body (Chanda and Baravalia, 2010). *Senna alata* has fungicidal properties for treating ringworm and other infections of the skin (Owoyale *et al.*, 2005). Abatan (1990), reported that leaf juice and decoction of *Senna alata* are used in the treatment of ringworm and other skin diseases. A phytopharmacological review by Jena and Gupta(2012), revealed that *Ricinus communis* has proven to possess antimicrobial activities as they were used against dermatophytic and pathogenic bacterial strains on *S. aureus*, *P. aeruginosa*, *K. pneumoniae* and *E. coli* (Jena and Gupta, 2012). Also, anti-fungal activity of the leaf was potent against *Candida albicans* (Khan and Yadav, 2011). *Ricinus communis* possess wound healing activity due to the active constituent of castor oil which produce antioxidant activity and inhibit lipid peroxidation. A study conducted by Prasad and colleagues in 2011 reported the potential of castor oil in wound healing activity by reducing the scar area and also the epithelization time in excision wound model (Prasad *et al.*, 2011b). *Lannea barteri* belongs to the family Anacardiaceae and is used as medicinal plant in most Africa countries. It is used for curing some infectious diseases (Mahesh and Satish, 2008). In this study, *Staphylococcus aureus*, *Pseudomonas aeruginosa, Klebsiella pneumoniae*, *Escherichia coli and Candida albicans* were the microbes on focus.

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2.8 Combination Effect

Plants constantly interact with the rapidly changing and potentially damaging external environmental factors. Being organisms devoid of mobility, plants have evolved elaborate alternative defense strategies, which involve an enormous variety of chemical metabolites

as tools to overcome stress conditions. The ability of plants to carry out combinatorial chemistry by mixing, matching and evolving the gene products required for secondary metabolite biosynthetic pathways, creates an unlimited pool of chemical compounds, which humans have exploited to their benefit. The use of plants by humans in both traditional and modern medicinal systems, therefore, largely exploits this principle. Combination therapies are practiced daily in many areas like cardiology, oncology or rheumatology. The use of combinations of potentially valuable drugs rather than their sequential use has indeed been proposed already in the 90s for rheumatoid arthritis (RA). The argument was that, the chronicity of RA reflects the failure to suppress multiple parallel pathologic pathways and therefore initial ''broad spectrum'' coverage might bear reasonable therapeutic approach (Klippel, 1990). The goals of combination therapy were formulated as an improved (clinical) efficacy, lower doses of drugs, and less drug toxicity (Klippel, 1990). The superiority of the use of combinations of second-line anti rheumatic drugs over the single agent treatment could not be demonstrated convincingly. Combination chemotherapy is also employed in clinical practice for the treatment of infectious diseases. Substantial research trials have been done to investigate the interaction effects of medicinal plant extracts with known clinical drugs in the treatment of various ailments, with several of them yielding positive interaction effects (Sato *et al.*, 2004; Filoche *et al.*, 2005; Prabhakar and Doble, 2011). Combinations of different medicinal plant components in the herbal formulation remedies are increasingly becoming a common phenomenon in most traditional medicinal systems. In an attempt to understand the antimicrobial efficacy of such herbal combinations, van Vuuren and Viljoen (2008) and Ndhlala *et al.* (2009) investigated this aspect with different plant parts and herbal preparations respectively, and reported both positive and negative interaction

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effects. While extract combinations may in some cases result in an enhanced efficacy, it must be pointed out that synergism should not always be assumed (van Vuuren and Viljoen, 2008; Ndhlala *et al.*, 2009).

2.9 Synergism Mechanism

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Synergy is 'working together' but a useful definition is 'an effect seen by a combination of substances that is greater than would have been expected from a consideration of individual contributions' (Heinrich *et al.*, 2012). More precise definitions, incorporating mathematical considerations, have been discussed by Berenbaum (1989), who favoured the use of isoboles in determining synergy, since these are concerned with the effect and not with the mechanisms involved. Synergy as applied to herbal products has been discussed well by Williamson (2001; 2002), who also included the attenuation of toxicity or adverse effects seen with a mixture compared with one of its constituents. An unexpected decrease in activity, sometimes called 'negative synergy' or 'antagonism' may also occur, particularly in some interactions between orthodox medication and some herbal products (Barnes *et al.*, 2003). In the case of the pharmacological or clinical effects of herbal material, two types of synergy are observed: In one case the activity of an active compound, or extract, is increased in the presence of another compound or extract which, on its own, has no effect in the system under test at the concentrations used. The more common situation is when all compounds concerned have activity but, in combination this is much greater than expected. This is exemplified by the reduction in platelet aggregation observed for the total mixture of ginkgolides from *Ginkgo biloba L.*, as

opposed to that given by individual compounds, because of their activity as platelet-activating factor antagonists (Williamson, 2002). In some traditional medicine systems, mixtures of plants are used rather than one species and so the situation is even more complex, although the same concepts of synergy apply, i.e. the mixture of the two (or more) species gives a better activity than either species on its own. This has been demonstrated for a mixture of extracts of *Salvia chamelaegnea Berg* and *Leonotis leonorus (L.)* against Gram positive bacteria (Kamatou *et al.*, 2006). This mixture was used traditionally in South Africa against infections.

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In other researches, synergy is described as the effect of the combination of drugs which is greater than the sum of the individual effects. In this way, a number of substances act at different receptor targets involved in the disease to enhance the overall therapeutic effect. Synergy between different constituents of extracts has been documented not only for anti-malarial activity, but also for many pharmacological activities. In pharmacokinetic synergy, substances with little or no activity on the causative agent assist the main active principle to reach the target by improving bioavailability, or by decreasing metabolism and excretion (Williamson, 2001; Houghton, 2009). The following mechanisms take place in synergism; synergistic multi-target effects, pharmacokinetic or physicochemical effects based on improved solubility, reabsorption rate and enhanced bioavailability and interactions of agents with resistance mechanisms of bacteria. The respective elimination or neutralization of adverse effects by agents contained in the extract, added to it, or achieved by heating, so that altogether a better effectiveness than without these additions or manipulations can be achieved (Wagner and Ulrich-Merzenich, 2009). The drug interaction may have different effects on the host as well as the infecting microorganism.

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The potential benefits of using combined antimicrobial therapy can be treatment of mixed infections, therapy of severe infections in which a specific causative organism is known, enhancement of antibacterial activity, reducing the time needed for long-term antimicrobial therapy and prevention of the emergence of resistant microorganisms (Hugo and Russel, 1993; Levinson and Jawetz, 2002).

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CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Materials

3.1.1 List of Materials/Equipment

Beakers, measuring cylinder, filter papers, petri dishes, calibrated ruler, test tubes, sterilized wire loop, transparent bottles, mortar and pestle, cotton, eppendorf tubes, conical flasks.

Water bath (p Selecta), oven, hot plate, incubator, vortex, rotary evaporator-vacuum pump (Heidolph 4001 efficient, Sartorius16695 respectively), fridge, deep freezer, Bunsen burner, desiccator, blender, electronic balance, cork borer, micropipettes.

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3.1.2 Media

Nutrient agar, Mueller–Hinton agar, Nutrient broth, (Biomark laboratories, Pune, India).

3.1.3 Chemicals/Solvents

Chemicals used were dimethylsulfoxide (DMSO-99.9%), ethanol, chloroform, ethyl acetate, acetone, methanol, distilled water, ammonia, hydrochloric acid, Mayer's reagent, normal saline (Oxoid limited, Basingstoke, Hampshire, England), sulphuric acid, ferric chloride, ammonium hydroxide, Fehling's solution A and B, magnesium metal.

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3.1.4 Microorganisms

The microorganisms used in the study were *Staphylococcus aureus* (Gram positive), *Pseudomonas aeruginosa* (Gram negative), *Klebsiella pneumoniae* (Gram negative), *Escherichia coli* (Gram negative) and *Candida albicans* (yeast, fungus).

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3.1.5 Sources of Microorganisms

They were clinically isolates obtained from the Microbiology Department of the Tamale Teaching Hospital in the Northern Region of Ghana; in the Month of March 2016.

3.1.6 Standard Drugs

Antibacterial: Amoxicillin 250 mg/ml; Antifungal: Fluconazole 150 mg/ml

3.2 Methods

3.2.1 Collection and Authentication of Plant Materials

Plant parts of *Senna alata*, *Ricinus communis* and *Lannea barteri* were collected from different areas in Navrongo, Upper East Region, Ghana in the months of October-November, 2015. The plants were identified and authenticated by a plant taxonomist at the herbarium of Ghana Herbaria, Northern Savanna Biodiversity; Savanna Herbarium. The voucher specimens were deposited with numbers of SH 710 (*Senna alata*), SH 720 (*Ricinus cummunis)* and SH 79 (*Lannea Barteri*) in the herbarium.

3.2.2 Preparation of Plant Material for Extraction

The leaves of both *S. alata* and *R. communis* and the bark of *L. barteri* were thoroughly washed with distilled water and air dried at room temperature for 2 weeks. The leaves were ground into a uniform powder using an electric blender and the bark was crushed using pestle and mortar to obtain coarse material. The ground plant materials were stored at room temperature in separate air–tight containers until further use.

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3.2.3 Extraction of Plant Material

Ethanol and water extracts were prepared by maceration of 100 g each of the plant materials in 1 L of the respective solvents at room temperature for 48 h. The extracts were filtered separately through a Whatman filter paper No 42. The extracts were concentrated using rotary evaporator connected to vacuum pump and further placed on water bath at 50˚C for ethanol and 70 ˚C for aqueous extracts respectively to produce semi solid and solid products respectively and the percentage yields of the extracts were determined.

3.2.4 Preliminary Phytochemical Screening of the extracts

3.2.4.1 Test for alkaloids (Mayer's reagent test)

About 0.2 g of the extract was dissolved with 8 ml of (1%) hydrochloric acid (HCl) then filtered. About 1 ml Mayer's reagent was added to 2 ml of the filtrate by the side walls of the test tube.

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Observation: A white or creamy precipitate indicated the presence of alkaloids (Ciulei, 1982).

3.2.4.2 Test for Saponins – (Froth test)

About 4 ml of distilled water was added to 0.4 g of the extract and shaken vigorously for 10-15 minutes.

Observation: Persistence of foamy layer above indicated saponins are present (Ciulei, 1982).

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3.2.4.3 Test for Tannins

About 4.0 ml of distilled water was added to 2.0 ml of the sample in a test tube. Few drops of 10 % Ferric chloride solution were added.

Observation: Blue-black or green to greenish black colour observation indicated, tannins were present (Sofowora, 2006).

3.2.4.4 Test for Polyuronoid

Few drops of acetone were added to 2 ml of the sample.

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Observation: Precipitate sticking along the walls of the test tube indicated presence of polyuronoids (Ciulei, 1982).

3.2.4.5 Test for reducing sugars (Fehling's test).

5 drops each of Fehling's solution A and B were added to one (1) ml of the sample and heated on water bath for about 15 minutes.

Observation: Brick-red precipitate indicated presence of reducing sugars (Ciulei, 1982).

3.2.4.6 Test for Anthraquinone (Borntrager's reaction test)

About 0.1 g of the extract was taken into dry test tube and 2.5 ml of chloroform was added and shaken for 5 minutes. The extract was filtered and the filtrate shaken with equal volumes of 10% ammonia solution.

Observation: A pink violet or red colour in the ammoniacal layer (lower layer) indicated the presence of anthraquinone (Ciulei, 1982).

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3.2.4.7 Test for Flavonoids (Shibata's reaction test)

The extract was evaporated to dryness and about 0.1 g of the residue was dissolved using 2 ml of 50% methanol and transferred into a test tube. A piece of magnesium metal was put into the test tube, followed by 1-3 drops of concentrated hydrochloric acid (HCl).

Observation: Cherish-red or orange colour indicated presence of flavonoids (Ciulei, 1982).

3.2.4.8 Test for Terpenoids

About 0.2 g of the extract was treated with 1 ml of acetic anhydride and 1 ml of chloroform. Then few drops of concentrated sulphuric acid were added slowly. **Observation:** Red violet colour indicated the presence of terpenoids (Ciulei, 1982).

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3.2.5 Preparation of Media

Mueller-Hinton agar was prepared by dissolving 38 g of the media in 1 L distilled water and boiled for complete dissolution. It was sterilized by autoclaving at 121 ˚C for 15 minutes.

Nutrient Agar, 28 g, was suspended in 1 L of distilled water. It was then heated to boil and autoclaved at 121 ˚C for 15 minutes.

Nutrient Broth: Broth powder, 13 g, was suspended in 1 L of distilled water, it was boiled to ensure uniformity and then autoclaved for 15 minutes at 121˚ C.

Normal Saline: One tablet of Normal saline was dissolved in 500 ml distilled water and sterilized by autoclaving for 15 minutes at 121 ˚C to prepare a concentration of 0.85%.

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3.2.6 Culturing of Microbes

The test microorganisms were inoculated in nutrient broth over night to revive them. The isolates were then streaked on nutrient agar and incubated at 37 °C for 24 hours. The microbes were periodically sub cultured and preserved at 4 °C prior to use to maintain them for easy use.

3.2.6.1 Preparation of McFarland Standard

To standardize the inoculum density for a susceptibility test, $BaSO_4$ turbidity standard equivalent to a 0.5 McFarland was used. A 0.5 McFarland standard was prepared as described in National Committee for Clinical Laboratory Standards (NCCLS, 1997). One per cent v/v solution of concentrated sulphuric acid was prepared. Additionally, a 1.175 % w/v solution of Barium chloride was prepared. To make the turbidity standard 0.5 McFarland and 0.5 ml of barium chloride was added to 1% 99.5 ml sulphuric acid and the two solutions were mixed thoroughly and stored in the dark at room temperature.

3.2.6.2 Inoculum Preparation

The inoculum suspension was prepared by direct method. Inocula were obtained from an overnight agar culture of the test organisms. Inoculum for the susceptibility test was prepared by taking samples at three well isolated colonies of the same morphology from agar plate culture. The top of each colony was touched with a sterile loop and the group was transferred into a tube containing 5 ml of normal saline and then vortexed. The broth culture was incubated at 37 ^{o}C and monitored until it achieved the turbidity of 0.5 McFarland standard (1.5x 10^8 cfu/ml) (McFarland,1907).

3.2.7 Preparation of Plant Extracts Concentrations.

Respective masses of 0.05, 0.1 and 0. 2 g of each of the plant extracts were dissolved in 1 ml of Dimethylsulfoxide (DMSO) and then vortexed to obtain concentrations of 50, 100 and 200 mg/ml uniform solutions respectively.

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3.2.8 Antimicrobial Susceptibility Assay

The antimicrobial activity of each of the individual extract was determined by the Agar-well diffusion method as described by Odoemena and Essien (1995). The plates containing prepared Mueller-Hinton agar were labeled according to the different concentrations of the plant extracts as well as the microorganisms involved. A 0.01 ml broth culture of each of the microorganism was inoculated onto a plate aseptically and was spread uniformly to cover the entire periphery using a glass spreader. A cork borer

size of 5 mm in diameter was used to create wells in the media and the prepared extract concentrations as well as both positive and negative controls were introduced into the wells and allowed to stand for about one hour for pre-diffusion before incubating at 37 °C for 24 h. The inhibition zones were measured in millimeters (mm). The tests were performed in duplicates and the results were expressed as mean ± standard deviation.

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3.2.9 Inoculum preparation for Minimum Inhibitory Concentration and Minimum Bactericidal Concentration

Inocula were obtained from an overnight agar culture of the test organisms. Inoculum for the MIC and MBC tests was prepared by taking at least three well isolated colonies of the same morphology from the agar plate culture. The top of each colony was touched with a sterile loop and the group was transferred into a tube containing 5 ml of normal saline and then vortexed. The broth culture was incubated at 37 ˚C until it achieved the turbidity of 0.5 McFarland standard (1.5x 10^8 cfu/ml) (McFarland,1907).

3.2.10 Determination of Minimum Inhibitory Concentration for Individual Extract

The broth culture obtained was uniformly spread to cover the entire periphery of already prepared Mueller-Hinton agar in a petri dish. Nine(9) wells were created in the innoculated agar and the respective concentrations of 50, 25, 12.5, 6.25, 3.125, 1.5625, 0.78125, 0.39 and 0.195 mg/ml were added to each well and then incubated at 37˚C for 24 hours. The MIC, that is the minimum concentration that showed inhibition was then determined (Odoemena and Essien, 1995).

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3.2.11 Determination of Minimum Bactericidal Concentration (MBC) and Minimum Fungicidal Concentration (MFC)

The tube diffusion method was employed as described by Lin *et al*., 1999 to determine the MBC and MFC. Sterilized transparent bottles were set up for the different microorganisms cultured. A 2 ml of normal saline solution containing the test microorganism (Broth culture) was dispensed into each the sterilized transparent bottles. A quantity of 0.1 ml extract of different concentrations was dropped into the transparent bottles containing the test microorganisms. The bottles were tightly corked, vortexed and were incubated anaerobically at 37 ^{o}C for 24 hours. Sterile nutrient agar plates were inoculated with samples from each of the transparent bottles after 24 hours. The plates were further incubated for 24 h at 37 ^{o}C and observed. The lowest concentration that killed 100% of the inoculum bacteria (MBC and FBC) was recorded as Minimum Bactericidal Concentration and Minimum Fungucidal Concentration for each bacterium and the fungus respectively.

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3.2.12 Determination of MBC/MFC for Combined Plant Extracts

Concentrations of 50, 100, 200 mg/ml of pairs of the different extracts were prepared. The respective solutions were combined into mixtures of 50:50, 50:100, 50:200, 100:50, 100:100, 100:200, 200:50, 200:100 and 200:200 mg/ml accordingly for the determination. The tube diffusion method was employed as described by Lin *et al.*(1999). Sterilized transparent bottles were set up for the different organisms cultured. A 2 ml of normal saline solution containing the test organisms (Broth culture) was each dispensed into the sterilized transparent bottles. A quantity of 0.1 ml of combined extracts

of different concentrations was dropped into the transparent bottles containing the test organisms. The bottles were tightly corked,vortexed and were incubated aerobically at 37 ^{o}C for 24 hours (Lin *et al.,*1999). Sterile nutrient agar plates were inoculated with samples from each of the transparent bottles . The plates were further incubated for 24 hours at 37 ^{o}C and observed. The lowest concentration that killed 100 % of the inoculum bacteria (no growth on plate) was recorded as Minimum Bactericidal Concentration for each bacterium and Minimum Fungicidal Concentration for the fungus.

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3.2.13 Preparation of Polyethylene glycol (PEG) Ointment Formulation

From samples of polyethylene glycol 4000 (PEG 4000) and polyethylene glycol 400 (PEG 400), 15 g each were weighed using an electronic balance into a beaker. The content was placed on a thermostatic water bath at 75˚C to completely liquefy the content. It was congealed under running water at room temperature whilst continuously stirring with stirring rod and stored in refrigerator for later use (Donkor *et al.,* 2014).

3.2.14 Preparation of Sterilized Shea Butter

Shea butter was purchased from the Navrongo market in Upper East Region of Ghana. The sample was heated on hotplate to about 120 ˚C for 2 hours. This was to enable all water in the sample to evaporate and to sterilize the oil. The oil was cooled at room temperature to form a solid material and then stored at room temperature.

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3.2.15 Preparation of Plant Crude Extract-PEG and Extract-Shea Butter Ointment

Masses of 0.4, 0.3, 0.2, 0.1, 0.050, 0.025, 0.0125, 0.00625, 0.003125, 0.0 015625, 0.00078125, 0.000390625 and 0.0001953125 mg of each extract was weighed into cleaned labeled beakers. The formulated PEG, 1.0 g each was added to each labeled beaker and warmed at 40 ˚C with continuous stirring to ensure uniformity. The mixture was then allowed to cool at room temperature to form crude extract-PEG formulation. The same procedure was used to prepare the crude extract-Shea butter formulations (Donkor *et al.,* 2014).

3.2.16 Antimicrobial Susceptibility Test for the Formulated Extracts

Antimicrobial test was carried out using a method described by Donkor *et al.,* 2014. The broth culture obtained was uniformily spread to cover the entire periphery of already prepared Mueller-Hinton agar in a petri dish. Wells were created and the respective extract formulations of concentrations: 200, 100 and 50 mg/g were added to each well. The test samples were allowed to stand for about one hour for pre-diffusion before incubating at 37 ˚C for 24 hours and zones of inhibitions were measured.

3.2.17 Minimum Inhibitory Concentration of the Formulated Plant Extracts

The broth culture obtained was uniformily spread to cover the entire periphery of already prepared Mueller-Hinton agar in a petri dish. Wells were created and the respective extract ointments concentrations of 50, 25, 12.5, 6.25, 3.125, 1.5625, 0.78125, 0.39 and

0.195 mg/g were added to different wells and then incubated at 37˚C for 24 hours. The MIC, that is the minimum concentration that showed inhibition was then determined.

3.2.18 Minimunm Batericidal/Fungicidal Concentration for the Formulated Extract

The tube diffusion method was employed as described by Lin *et al*., (1999). Sterilized transparent bottles were set up for the different organisms cultured. A 2 ml of normal saline solution containing each of the test organisms was each dispensed into the sterilized transparent bottles. A quantity of 0.1 ml of extract of different concentrations was dropped into the transparent bottles containing the test organisms. The bottles were tightly corked and incubated anaerobically at 37 ^{o}C for 24 hours. Sterile nutrient agar plates were inoculated with a sample from each of the transparent bottles . The plates were further incubated for 24 hours at 37 ^{o}C and observed. The lowest concentration that killed 100% of the inoculum bacteria (no growth on plate) was recorded as Minimum Bactericidal Concentration or Minimun Fungicidal Concentration.

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3.2.19 Preparation of Fractional Inhibitory Concentrations (FIC)

The fraction of each extract that showed minimum inhibition against the tested microorganisms were combined and then serially diluted, which were used for the susceptibility test to determine the minimum inhibitory concentrations.

3.2.20 Determination of Fractional Inhibitory Concentration (FIC) Index

The method by Isenberg (1992) and Noor (2016) research group for determining fractional inhibitory concentration index was slightly modified in current research. Fractional inhibitory concentrations (FIC) are the lowest concentrations of the extracts (Drug A) and (Drug B) in combination giving no detectable bacterial/fungus growth after incubation (Isenberg, 1992; Noor, 2016).

$$FICI = \frac{MIC\ of\ extract\ drug\ A\ in\ combination\ (Drug\ A\$B)}{MIC\ of\ extract\ A\ alone(Drug\ A)} + \frac{MIC\ of\ extract\ drug\ B\ in\ combination\ (Drug\ A\$B)}{MIC\ of\ extract\ B\ alone(Drug\ B)}$$

The following inequalities below were used to define the extracts combination effects

Synergy is defined if $\sum FIC \leq 0.5$,

Additive if $0.5 < \sum FIC \leq 1$

Indifference if $1 < \sum FIC \leq 4$

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Antagonism as $\sum FIC > 4$

(Isenberg, 1992; Noor, 2016)

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3.2.21 Statistical Analysis

The data obtained from the tests were subjected to statistical treatment using the following statistical analysis; GraphPad Prism, Version 5 for Mean, Standard Deviation and Two Way Analysis of Variance (ANOVA) at 95% confidence interval.

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CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Results

4.1.1 Percent yield of extracts

From the experimental study, the yield of *Lannea barteri*, *Senna alata and Ricinus comminus* plants were found to be 12.40 and 12.10, 7.4 and 7.2, 6.0, and 5.20% w/w for aqueous and ethanol respectively.

4.1.2 Preliminary Phytochemical Screening of the Crude Extracts

The preliminary study of the phytochemicals of the plant extracts revealed the presence of tannins, saponins, reducing sugars, polyuronoids, terpenoids, flavonoids, alkaloids, and anthraquinones. These secondary metabolites were uniformly distributed in all the respective extracts obtained (Table 4.1).

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Table 4.1: Phytochemical Profile of Plant Extracts

Plants/solvent	*PHYTOCHEMICALS* Tannins	Saponins	Polyuro-noids	Reducing Sugars	Terpenoids	Flavo-noids	Alkaloids	Anthra-quinones
Senna alata								
Aqueous	+	+	+	+	+	+	+	+
Ethanol	+	+	-	+	+	+	+	+
Lannea bateri								
ueous	+	+	+	+	+	+	+	+
anol	+	+	+	+	+	+	+	+
inus communis								
ueous	+	+	+	+	+	+	+	-
anol	+	+	+	-	-	+	+	-

+; Presence - ; not Detected

4.1.3 Zones of inhibition for *Senna alata* extracts at varying concentrations (mg/ml)

Table 4.2a: Aqueous Extract of Senna alata inhibition zone in mm

Test Microorganism	Diameter (mm) of Inhibition zone 50	100	200	*Control* Amox	DMSO
E. coli	13.5± 0.71	15.5± 2.12	17.0± 1.41	33.0± 1.41	0.00
S. aureus	15.0± 1.41	15.0± 0.00	16.0± 0.00	18.5± 2.12	0.00
P. aeruginosa	19.5± 0.71	22.5± 0.71	25.0± 1.41	15.5± 0.71	0.00
K. pneumoniae	24.0± 1.41	23.0± 1.41	28.5± 2.12	38.0± 1.41	0.00
Fungus				**Fluco**	
C. albicans	19.0± 1.41	19.5± 0.71	21.5± 2.12	14.0± 1.41	0.00

Amox=Amoxicillin (mg/ml), Fluco= Fluconazole (mg/ml), Data expressed as mean ± SD, n = 2

The data above (Table 4. 1a) indicates that, all the test microorganisms were suppressed by aqueous extract of *Senna alata* with zones of inhibition ranging from 13-28 mm

concentrations of 50, 100 and 200 mg/ml. *E. coli* was susceptible to the extract with diameter of zones of inhibition range of 13.5-17 mm at the varying concentrations. *S. aureus* was also susceptible to the extract with diameter zones of inhibition in the range of 15-16 mm at the varying concentrations. Similarly *P. aeruginosa* exhibited zones of inhibition in the range of 19.5-25 mm at the varying concentrations. *K. pneumoniae* was also susceptible to aqueous extract of *Senna alata* with zones of inhibition in the range of 24-28.5 mm at the varying concentrations while the extract exhibited inhibition against the fungus, C. *albicans* with range of 19-21.5 mm at the varying concentrations.

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Table 4.2b: Ethanol extract of *Senna alata* inhibition zone in mm

Test Microorganism	***Diameter (mm) of Inhibition zone***			***Control***	
	50	***100***	***200***	***Amox***	***DMSO***
E. coli	12.0 ±2.83	13.5 ± 0.71	16.0 ± 1.41	35.0 ± 0.00	0.00
S. aureus	14.5 ± 0.71	15.5 ± 0.71	24.0 ± 1.41	17.0 ± 0.00	0.00
P. aeruginosa	17.5 ± 0.71	20.5 ± 2.12	24.5 ± 0.71	16.5 ± 2.12	0.00
K. pneumoniae	12.5 ± 0.71	13.0 ± 1.41	19.5 ± 2.12	37.5 ± 2.12	0.00
Fungus				Fluco	
C. albicans	13.5 ± 0.71	15.5 ± 0.71	18.0 ± 1.41	16.5 ± 0.71	0.00

Amox=Amoxicillin (mg/ml), Fluco= Fluconazole (mg/ml), Data expressed as mean ± SD, n = 2

The above data (table 4. 2b) indicates that, all the test microorganisms were susceptible to ethanol extract of *Senna alata* with zones of inhibition ranging from 12 - 24.5 mm for all the selected microorganisms at varying concentrations of 50, 100 and 200 mg/ml. *E. coli* was susceptible to the extract with diameters of zones of inhibition range of 12 - 16 mm at the varying concentrations. *S. aureus* was susceptible to the extract with diameters of zones of inhibition range of 14.5 - 24 mm at the varying concentrations. Similarly, *P.*

aeruginosa had zones of inhibition range of 17 - 24.5 mm at the varying concentrations as indicated. Also *K. pneumoniae* was susceptible to ethanol extract of *Senna alata* with zones of inhibition range of 12.5 - 19.5 mm at the varying concentrations. The extract exhibited inhibition against *C. albicans*, a fungus with range 13.5 - 18 mm at the varying concentrations.

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4.1.4 Zone of Inhibition of aqueous *Lannea barteri* at varying concentrations (mg/ml)

Table 4.3a: Aqueous extracts of *Lannea barteri* inhibition zone

Test Microorganism	*Diameter (mm) of Inhibition zone*			*Control*	
	50	***100***	***200***	***Amox***	***DMSO***
E. coli	15.0±1.41	18.5±0.71	23.0±1.41	32.5±2.12	0.00
S. aureus	20.5±0.71	25.5±0.71	28.5±0.71	17.0±1.41	0.00
P. aeruginosa	25.5±2.12	26.0±1.41	30.5±2.12	16.5±0.71	0.00
K. pneumoniae	18.5±0.71	22.5±2.12	29.0±1.41	27.5±2.12	0.00
Fungus				**Fluco**	
C. albicans	17.5±2.12	28.0±2.83	32.0±1.41	16.0±1.41	0.00

Amox=Amoxicillin (mg/ml), Fluco = Fluconazole (mg/ml), Data expressed as mean ± SD, n = 2

From table 4.3a the results showed that the test microorganisms were susceptible to the aqueous extract of *Lannea barteri* with zones of inhibition ranging from 15-32 mm for all the selected microorganisms at concentrations of 50, 100 and 200 mg/ml. *E. coli* was also susceptible to aqueous extract with diameters of zones of inhibition range of 15-23 mm at the varying concentrations. *S. aureus* was susceptible to the extract with diameter zone of inhibition range of 20.5-28.5 mm at the stated concentrations. Similarly, *P. aeruginosa* had zone of inhibition range of 25.5-30.5 mm at the varying concentrations. Also *K.*

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pneumoniae was susceptible to the extract of *Lannea barteri* with zones of inhibition range of 18.5-29 mm at the varying concentrations. The extract also exhibited inhibition against the fungus, *C. albicans* with inhibition zones range of 17.5-32 mm at the varying concentrations.

Table 4.3b: Ethanol extract of *Lannea barteri* inhibition zone

Test Microorganism	Diameter (mm) of Inhibition zone				
				Control	
	50	100	200	Amox	DMSO
E. coli	17.0±1.41	18.5±0.71	23.0±1.41	33.0±2.83	0.00
S. aureus	17.5±2.12	24.5±2.12	32.5±2.12	16.5±0.71	0.00
P. aeruginosa	19.5±0.71	29.5±0.71	36.0±1.41	16.5±0.71	0.00
K. pneumoniae	16.5±0.71	19.5±2.12	19.5±0.71	34.0±2.83	0.00
Fungus				**Fluco**	
C. albicans	29.5±2.12	33.5±2.12	36.5±2.12	12.5±2.12	0.00

Amox=Amoxicillin (mg/ml), Fluco= Fluconazole (mg/ml), Data expressed as mean ± SD, n = 2

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The results indicate that ethanol extract of *Lannea barteri* showed inhibition against the microorganisms used in this experiment with zones of inhibition ranging from 16.5-36.5 mm at varying concentrations of 50, 100 and 200 mg/ml. *E. coli* was susceptible to ethanol extract with diameters of zones of inhibition range of 17-23 mm at stated concentrations. *S. aureus* was susceptible to the extract with diameters of zones of inhibition range of 17.5-32.5 mm at the stated concentrations. Similarly, *P. aeruginosa* had zone of inhibition range 19.5-36 mm at varying concentrations. Also *K. pneumoniae* showed susceptibility to ethanol extract of *Lannea barteri* with zone of inhibition range of 16.5-34 mm at the concentrations. The extract also exhibited inhibition against the only

fungus, *C. albicans* with inhibition zones range 29.5-36.5 mm at the varying concentrations (Table 4.3b).

4.1.5 Zone of Inhibition for *Ricinus communis* at varying concentrations (mg/ml)

Table 4.4a: Aqueous extract of *Ricinus communis* inhibition zone

	Diameter (mm) of Inhibition zone				
Test				***Control***	
Microorganism	***50***	***100***	***200***	***Amox***	***DMSO***
E. coli	18.0±2.83	18.5±0.71	20.0±1.14	28.0±1.41	0.00
S. aureus	16.5±0.71	17.0±1.41	20.5±2.12	15.5±0.71	0.00
P. aeruginosa	25.0±1.41	27.0±2.83	28.0±2.83	16.0±2.83	0.00
K. pneumoniae	21.0±2.83	24.0±1.41	29.5±0.71	15.0±0.00	0.00
Fungus				**Fluco**	
C. albicans	16.0±1.41	17.0±1.14	20.0±1.41	32.0±1.41	0.00

Amox=Amoxicillin (mg/ml), Fluco= Fluconazole (mg/ml), Data expressed as mean ± SD, n = 2

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The observed data above indicate that the extract exhibited inhibition activity against the test microorganisms with zones of inhibition ranging from 16.5-29.5 mm at stated concentrations of 50, 100, 200 mg/ml. *E. coli* was susceptible to aqueous extract with diameters of zones of inhibition range of 18-20 mm at the chosen concentrations. *S. aureus* growth was equally suppressed by the extract with diameters of zones of inhibition in the range of 16.5-20.5 mm by the selected concentrations. Similarly, *P. aeruginosa* had zones of inhibition range of 25-28 mm at the stated concentrations. Also, *K. pneumoniae* showed susceptibility to aqueous extract of *Ricinus communis* with zone of inhibition range of 21-29.5 mm at the varying concentrations. The extract exhibited

inhibition against the only fungus (*C. albicans*) with the zones of inhibition range 16-20 mm at the varying concentrations (Table 4.4a).

Table 4.4b: Ethanol extract of *Ricinus communis* Inhibition zone (mg/ml)

	Diameter (mm) of Inhibition zone				
				Control	
Test Microorganism	***50***	***100***	***200***	***Amox***	***DMSO***
E. coli	17.0 ±1.41	18.5± 0.71	23.0± 1.41	33.0±2.83	0.00
S. aureus	17.5±2.12	24.5± 2.12	32.5± 2.12	16.5±0.71	0.00
P. aeruginosa	19.5±0.71	29.5± 0.71	36.0± 1.41	16.5±0.71	0.00
K. pneumoniae	16.5±0.71	19.5± 2.12	19.5± 0.71	34.0±2.83	0.00
Fungus				**Fluco**	
C. albicans	29.5±2.12	33.5± 2.12	36.5± 2.12	12.5±2.12	0.00

Amox=Amoxicillin (mg/ml), Fluco= Fluconazole (mg/ml), Data expressed as mean ± SD, n = 2,

The results above indicate that, all the tested microorganisms were susceptible to ethanol extract of *Ricinus communis* with zones of inhibition ranging from 16.5-36.5 mm for all the selected microorganisms at stated concentrations of 50, 100, 200 mg/ml. *E. coli* was susceptible to ethanol extract with diameters of zones of inhibition in the range of 17-23 mm at the stated concentrations. *S. aureus* was susceptible to the extract with diameters of zones of inhibition in the range of 17.5-32.5 mm at stated concentrations. Similarly, *P. aeruginosa* had zones of inhibition in the range of 19.5-36 mm at stated concentrations. Also, *K. pneumoniae* was susceptible to ethanol extract of *Ricinus commmunis* with zone of inhibition in the range of 16.5-19.5 mm at the varying concentrations. The extract exhibited inhibition against the only fungus *C. albicans* with range 29.5-36.5 mm at the stated concentrations (Table 4.4b).

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4.1.6 MIC and MBC/MFC of the Plant Extract

Table 4.5a: Extract of *Senna alata* (mg/ml)

	Senna alata			
	Aqueous extract		*Ethanol extract*	
Test Microorganism	***MIC***	***MBC***	***MIC***	***MBC***
E. coli	6.25	200	6.25	200
S. aureus	12.5	300	12.5	300
P. aeruginosa	6.25	200	6.25	200
K. pneumoniae	12.5	200	12.5	300
Fungus		**MFC**		**MFC**
C. albicans	12.5	200	12.5	300

MIC= Minimum Inhibitory Concentration (mg/ml); MBC= Minimum Bactericidal Concentrations (mg/ml), MFC= Minimum Fungicidal Concentrations (mg/ml).

The observed MIC of aqueous fraction of *Senna alata* against the selected microorganisms were 6.25, 12.5, 6.25, 12.5 and 12.5 mg/ml for *E. coli, S. aureus, P. aeruginosa, K. pneumoniae and C. albicans* respectively. The ethanol fraction of *Senna alata* was similarly tested against the selected microorganisms and the MIC results indicates 6.25, 12.5, 6.25, 12.5 and 12.5 mg/ml for *E. coli, S. aureus, P. aeruginosa, K. pneumoniae* and *C. albicans* respectively. The MBC/MFC of aqueous *Senna alata* was at 200 mg/ml for *E. coli, P. aeruginosa, K. pneumoniae* and *300* mg/ml for *S. aureus* and *C. albicans.* Also for ethanol extract of *Senna alata* expressed MBC/MFC at 200 mg/ml for *E. coli, P. aeruginosa* and 300 mg/ml for *S. aureus, K. pneumoniae* and *C. albicans* (Table 4.5a).

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Table 4.5b: Extract *Lannea barteri* (mg/ml)

	Lannea barteri			
	Aqueous extract		***Ethanol extract***	
Test microorganism	***MIC***	***MBC***	***MIC***	***MBC***
E. coli	6.25	300	6.25	200
S. aureus	12.5	200	12.5	300
P. aeruginosa	6.25	200	6.25	200
K. pneumoniae	12.5	200	6.25	200
Fungus		**MFC**		**MFC**
C. albicans	12.5	200	12.5	300

MIC= Minimum Inhibitory Concentrations (mg/ml); MBC= Minimum Bactericidal Concentrations (mg/ml), MFC=Minimum Fungicidal Concentration.

The MIC of aqueous fraction of *Lannea barteri* was determined and the results found to be 6.25, 12.5, 6.25, 12.5 and 12.5 mg/ml for *E. coli, S. aureus, P. aeruginosa, K. pneumoniae, and C. albicans* respectively. The MBC/MFC of aqueous *Senna alata* was determined and was observed at 300, 200, 200, 200 and 200 mg/ml respectively for the selected microorganisms as indicated (Table 4.5b).

The MIC of ethanol fraction of Senna alata was 6.25, 12.5, 6.25, 6.25 and 12.5 mg/ml for *E. coli, S. aureus, P. aeruginosa, K. pneumoniae, and C. albicans* respectively. The observed MBC/MFC for ethanol extract was at 200, 300, 200, 200 and 300 mg/ml respectively for the selected microorganisms (Table 4.5b).

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Table 4.5c: Extract *Ricinus communis* (mg/ml).

	Ricinus communis			
	Aqueous extract		*Ethanol extract*	
Test microorganism	***MIC***	***MBC***	***MIC***	***MBC***
E. coli	6.25	UD	6.25	UD
S. aureus	3.13	300	25	400
P. aeruginosa	3.13	200	6.25	200
K. pneumoniae	12.5	400	6.25	200
Fungus		**MFC**		**MFC**
C. albicans	12.5	300	25.0	300

MIC= Minimum Inhibitory Concentrations (mg/ml); MBC= Minimum Bactericidal Concentration (mg/ml), UD= Undetected, MFC= Minimum fungicidal concentration.

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The MIC of aqueous fraction of *Ricinus communis* for the selected microorganisms were 6.25, 3.13, 3.13, 12.5 and 12.5 mg/ml for *E. coli, S. aureus, P. aeruginosa, K. pneumoniae and C. albicans* respectively. The plant extract was able to suppress the growth of *E. coli* but could not kill at concentration of 400 mg/ml. However, MBC/MFC was recorded for *S. aureus, P. aeruginosa, K. pneumoniae, and C. albicans* at 400, 200, 200 and 300 mg/ml respectively (Table 4.5c).

Similarly, ethanol extract of the plant was able to suppress the growth of *E.Coli,* but could not killed at concentration of 400 mg/ml, however, MBC/MFC was recorded for *S. aureus, P. aeruginosa, K. pneumoniae, C. albicans* at 400, 200, 200 and 300 mg/ml respectively (Table 4.5c).

4.1.7 Zones of inhibition for PEG ointment of plant extracts

Table 4.6a2: PEG - *Senna alata* Aqueous Extract ointment zone of inhibition (mm)

Test microorganism	*Ointment*		
	50 mg/g	*100 mg/g*	*200 mg/g*
E. coli	13.5±0.71	20.5±0.71	26.5±0.71
S. aureus	15.0±0.00	21.0±1.41	26.0±1.41
P. aeruginosa	12.5±0.71	19.5±0.71	29.5±0.71
K. pneumoniae	17.5±0.71	25.0±1.41	25.5±0.71
Fungus			
C. albicans	14.5±0.71	18.5±0.71	25.5±0.71

PEG= Polyethylene glycol, aqSA = aqueous *Senna alata,* Data expressed as mean ± SD, n = 2

The result above in table 4.1.7 shows that, plant extract ointment is a good delivery agent as all the tested microorganisms were susceptible to the ointment with zone of inhibition ranges from 13.5-29.5 mm for all the selected microorganisms at the concentrations of 50, 100 and 200 mg/g. *E. coli* showed susceptibility the to the plant extract ointment with diameters of zones of inhibition range of 13.5-26.5 mm at the varying concentrations. *S. aureus* showed susceptibility to the plant extract ointment with diameters of zones of inhibition range of 15-26 mm at the varying concentrations. *P. aeruginosa* was the most susceptible with the zones of inhibition range of 12.5-29.5 mm at the stated concentrations. Also *K. pneumoniae* showed susceptibility to ointment with zones of inhibition range of 17.5-25.5 mm at the stated concentrations. The ointment exhibited inhibition against fungus *C. albicans* with the zones of inhibition range 14.5-25.5 mm at the varying concentrations (Table 4.6a).

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Table 4.6b: PEG - *Senna alata* Ethanol Extract ointment zone of inhibition (mm)

Test microorganism	***Ointment***		
	50 mg/g	***100 mg/g***	***200 mg/g***
E. coli	27.0±0.00	28.5±0.71	29.5±0.71
S. aureus	19.5±0.71	21.0±0.71	24.5±0.71
P. aeruginosa	21.5±0.71	28.5±0.71	30.5±0.71
K. pneumoniae	29.0±1.41	28.5±0.71	30.5±0.71
Fungus			
C. albicans	21.5±0.71	22.5±0.71	26.5±0.71

PEG = Polyethylene glycol, ESA = Ethanol *Senna alata*, Data expressed as mean ± SD, n = 2

The ESA-PEG ointment demonstrated antimicrobial property by inhibiting the growth of the respective microorganisms with the inhibition range of 19.5-30.5 mm at the varying concentrations**.** *E. coli* showed susceptibility to the plant extract ointment with diameters of zones of inhibition range of 27-29.5 mm at the varying concentrations. *S. aureus* showed susceptibility to the plant extract ointment with diameters of zones of inhibition range of 19.5-24.5 mm at the varying concentrations. *P. aeruginosa* was susceptible with the zones of inhibition range of 21.5-30.5 mm at the stated concentrations. *K. pneumoniae* shows susceptibility to the ointment with zones of inhibition range of 28.5-30.5 mm at the varying concentrations. The plant extract ointment suppressed *C. albicans* with zones of inhibitions range of 21.5-26.5 mm at the stated concentrations (Table 4.6b).

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Table 4.7a: PEG - *Lannea barteri* aqueous Extract ointment zone of inhibition (mm)

Test microorganism	***Ointment***		
	50 mg/g	***100 mg/g***	***200 mg/g***
E. coli	23.5±0.71	23.0±1.41	26.0±0.00
S. aureus	19.0±1.41	22.5±0.71	24.5±0.71
P. aeruginosa	23.5±0.71	24.5±0.71	27.5±0.71
K. pneumoniae	26.5±0.71	31.0±1.41	31.0±1.41
Fungus			
C. albicans	21.5±0.71	23.5±0.71	28.5±0.71

PEG= Polyethylene glycol, Data expressed as mean ± SD, n = 2

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All the tested microorganisms were susceptible with zones of inhibition ranging from 19-31 mm for all the selected microorganisms at varying concentrations of 50, 100 and 200 mg/g. *E. coli* showed susceptibility to plant extract ointment with diameters of zones of inhibition range of 23-26 mm at the varying concentrations. *S. aureus* showed susceptibility to the plant extract ointment with diameters of zones of inhibition range of 19-24 mm at the varying concentrations. Similarly, *P. aeruginosa* was susceptible with the zone of inhibition range of 23.5-27.5 mm at varying concentrations. *K. pneumoniae* also showed susceptibility to pant extract ointment with zones of inhibition range of 26.5-31 mm at the varying concentrations. The plant extract ointment exhibited inhibition against the C. *albicans* with range 21.5-28.5 mm at the varying concentrations (Table 4.7a).

Table 4.7b: PEG - *Lannea barteri* Ethanol Extract ointment zone of inhibition (mm)

Test microorganism	*Ointment*		
	50 mg/g	***100 mg/g***	***200 mg/g***
E. coli	27.5±0.71	29.5±0.71	31.5±0.71
S. aureus	30.5±0.71	31.0±0.00	34.0±1.41
P. aeruginosa	26.5±0.71	32.0±1.41	39.5±0.71
K.pneumoniae	31.5±0.71	31.0±1.41	35.0±1.41
Fungus			
C. albicans	27.0±0.00	28.5±0.71	31.5±0.71

PEG = Polyethyleneglycol, ELB = Ethanol *Lannea barteri*, Data expressed as mean ± SD, n = 2,

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The data above (Table 4.7b) shows that all the tested microorganisms were susceptible to the extract-PEG formulation, with zones of inhibition ranging from 26.5-35 mm for all the selected microorganisms at varying concentrations. *E. coli* was susceptible to the plant extract ointment formulation with diameters zones of inhibition range of 27.5-31.5 mm at the stated concentrations. *S. aureus* was susceptible to the plant extract ointment with diameters zones of inhibition range of 30.5-34 mm at the varying concentrations. Similarly, *P. aeruginosa* was the most susceptible microorganism with zones of inhibition range of 26.5-39.5 mm at varying concentrations. *K. pneumoniae* was highly susceptible to the pant extract ointment with zones of inhibition range of 31-35 mm at the varying concentrations. The plant extract ointment formulation exhibited activity against the only fungus, *C. albicans* with zones range of 27-31.5 mm at the varying concentrations.

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Table 4.8a: PEG - *Ricinus communis* Aqueous Extract ointment zone of inhibition (mm)

Test microorganism	*Ointment*		
	50 mg/g	***100 mg/g***	***200 mg/g***
E. coli	16.5±0.71	20.5±0.71	26.0±1.41
S. aureus	23.5±0.71	29.0±1.41	32.5±0.71
P. aeruginosa	28.5±0.71	31.0±1.41	32.0±0.00
K. pneumoniae	24.5±0.71	27.0±1.41	31.0±1.41
Fungus			
C. albicans	16.5±0.71	21.0±1.41	27.0±1.41

PEG = Polyethylene glycol, aqRC = aqueous *Ricinus communis*, Data expressed as mean ± SD, n = 2

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The data above (Table 4.8a) shows that, all the tested microorganisms were susceptible to the extract-PEG formulation, with zones of inhibition ranging from 16.5-32.5 mm for all the test microorganisms at stated concentrations. *E. coli* was susceptible to the plant extract ointment formulation with diameters of zones of inhibition range of 16.5-26 mm at the varying concentrations. *S. aureus* was susceptible to the plant extract ointment with diameters of zones of inhibition range of 23.5-32.5 mm at the stated concentrations. Similarly, *P. aeruginosa* was the second most susceptible microorganism with zones of inhibition range of 28.5-32 mm at stated concentrations. Also, *K. pneumoniae* was susceptible to the plant extract ointment with zones of inhibition range of 24.5-31 mm at the stated concentrations. The ointment exhibited activity against the only fungus *C. albicans* with zones range 16.5-27 mm at the stated concentrations.

Table 4.8b: PEG - *Ricinus communis* Ethanol Extract ointment zone of inhibition (mm)

Test microorganism	***Ointment***		
	50 mg/g	***100 mg/g***	***200 mg/g***
E. coli	21.5±0.71	24.5±0.71	27.5±0.71
S. aureus	18.5±0.71	24.5±0.71	31.0±1.41
P. aeruginosa	19.0±1.41	25.5±0.71	34.5±0.71
K. pneumoniae	19.5±0.71	23.5±0.71	27.0±1.41
Fungus			
C. albicans	18.5±0.71	21.0±1.41	21.5±0.71

PEG= Polyethyleneglycol, Ethanol *Ricinus communis,* Data expressed as mean ± SD, n = 2

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The ERC- PEG ointment expressed zones of inhibition ranged of 18.5-34.5 mm against the selected microorganisms at stated concentrations. *E. coli* showed susceptibility against the ointment with diameters of zones of inhibition range of 21.5-27.5 mm at the varying concentrations. *S. aureus* showed susceptibility to the plant extract ointment with diameters of zones of inhibition range of 18.5-31 mm at the varying concentrations. *P. aeruginosa* was also susceptible with the zones of inhibition range of 19-25.5 mm at varying concentrations. *K. pneumoniae* showed susceptibility to plant extract ointment with zones of inhibition range of 19.5-27 mm at the varying concentrations. The plant extract ointment exhibited inhibition against the only fungus *C. albicans* with range 18.5-21.5 mm at the varying concentrations (Table 4.8b).

4.1.8 MIC and MBC/MFC of PEG - plant extract ointment (mg/g)

Table 4.9a: PEG - Senna *alata* aqueous leaf extract ointment (mg/g)

	Senna alata	
Test Microorganism	***MIC***	***MBC***
E. coli	3.13	200
S. aureus	6.25	200
P. aeruginosa	6.25	200
K. pneumoniae	6.25	200
Fungus		**MFC**
C. albicans	3.13	200

PEG = Polyethyleneglycol, MIC = Minimum Inhibitory Concentration, MBC = Minimum Bactericidal Concentration, MFC = Minimum Fungicidal Concentration

The MIC and MBC/MFC of the ointment against the test microorganisms were in the range of 3.13-6.25 mg/g and 200 mg/g respectively (Table 4.9a).

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Table 4.9b: PEG - Senna *alata* ethanol leaf extract ointment (mg/g)

	Senna alata	
Test Microorganism	***MIC***	***MBC***
E. coli	6.25	200
S. aureus	6.25	200
P. aeruginosa	3.13	200
K. pneumoniae	12.5	200
Fungus		**MFC**
C. albicans	6.25	300

PEG = Polyethylene glycol, MIC = Minimum Inhibitory Concentration, MBC = Minimum Bactericidal Concentration, MFC= Minimum Fungicidal Concentration

The ethanol extract-PEG ointment showed low MIC values against the tested microorganisms with MIC values range of 3.13-12.5 mg/ml and the MBC values ranged from 200-300 mg/g (Table 4.9b).

Table 4.10a: PEG - *Lannea barteri* aqueous leaf extract ointment (mg/g)

	Lannea barteri	
Test microorganism	***MIC***	***MBC***
E. coli	6.25	200
S. aureus	12.5	200
P. aeruginosa	6.25	200
K. pneumoniae	25	200
Fungus		**MFC**
C. albicans	3.13	400

MIC= Minimum Inhibitory Concentration, MBC=Minimum Bactericidal Concentration, MFC = Minimum Fungicidal Concentration aqLB = aqueous *Lannea barteri*

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The aqueous extract-PEG ointment showed low MIC values against the tested microorganisms with MIC values range of 3.13 -12.5 mg/ml and the MBC/MFC values range from 200- 400 mg/ml (Table 4.10a).

Table 4.10b: PEG - *Lannea barteri* ethanol leaf extract ointment (mg/g)

	Lannea barteri	
Test microorganism	**MIC**	**MBC**
E. coli	6.25	200
S. aureus	6.25	200
P.aeruginosa	3.13	200
K. pneumoniae	6.25	200
Fungus		MFC
C.albicans	6.25	300

PEG = Polyethylene glycol, MIC= Minimum Inhibitory Concentration, MBC = Minimum Bactericidal Concentration, MFC = Minimum Fungicidal Concentration

The observed result for MIC values ranged from 3.13-6.25 mg/ml and the MBC/MFC values ranged from 200 - 300 mg/ml for the respective microorganisms (Table 4.10b).

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Table 4.11a: PEG - *Ricinus communis* aqueous leaf extract ointment (mg/g)

	Ricinus communis	
Test microorganism	**MIC**	**MBC**
E. coli	6.25	200
S. aureus	12.5	200
P. aeruginosa	6.25	200
K. pneumoniae	6.25	200
Fungus		MFC
C. albicans	6.25	400

PEG = Polyethylene glycol, MIC= Minimum Inhibitory Concentration, MBC = Minimum Bactericidal Concentration, MFC = Minimum Fungicidal Concentration

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The aqueous extract-PEG ointment revealed low MIC values against the tested microorganisms with MIC values range of 6.25-12.5 mg/ml and the MBC/MFC values range from 200-400 mg/ml (Table 4.11a).

Table 4.11b: PEG - *Ricinus communis* ethanol leaf extract ointment (mg/g)

	Ricinus communis	
Test Microorganism	***MIC***	***MBC***
E. coli	6.25	300
S. aureus	6.25	200
P. aeruginosa	6.25	200
K. pneumoniae	6.25	200
Fungus		MFC
C. albicans	6.25	300

MIC= Minimum Inhibition Concentration, MBC= Minimum Bactericidal Concentration, PEG = Polyethylene glycol, ERC = Ethanol *Ricinus communis*

The ethanol Extract-PEG ointment showed low MIC values against the tested microorganisms with MIC values for all the tested microorganisms being 6.25 mg/ml and the MBC/MFC values range of 200-300 mg/ml (Table 4.11b).

4.1.9 Zones of inhibition for Shea butter ointment of plant extracts

Table 4.12a: Shea butter - *Senna alata* aqueous extract ointment zone of inhibition (mm)

Test microorganism	Ointment		
	50 mg/g	100 mg/g	200 mg/g
E. coli	7.5±0.71	9.5±0.71	10.5±0.71
S. aureus	7.5±0.71	8.5±0.71	10.5±0.71
P. aeruginosa	10.5±0.71	11.0±1.41	12.5±0.71
K. pneumoniae	8.5±0.71	10.5±0.71	12.5±0.71
Fungus			
C. albicans	8.5±0.71	9.0±1.41	10.0±1.41

aqSA = aqueous *Senna alata*, Data expressed as mean ± SD, n = 2

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The aqSA Shea butter ointment showed inhibition against the entire microorganisms tested with recorded range of 7.5-12.5 mm at the varying concentrations. *E. coli* showed susceptibility to the ointment with diameter of zones of inhibition range of 7.5-10.5 mm at the stated concentrations. *S. aureus* showed susceptibility to the plant aqueous extract ointment with diameters of zones of inhibition range of 7.5-10.5 mm at the varying concentrations. Similarly, *P. aeruginosa* was susceptible, with the zones of inhibition range of 10.5-12.5 mm at the varying concentrations. Also *K. pneumoniae* showed susceptibity to the plant extract ointment with zones of inhibition range of 8.5-12.5 mm at the varying concentrations. The plant extract ointment exhibited inhibition against the only fungus, *C. albicans*, with range of 8.5-10 mm at the varying concentrations (Table 4.12a).

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Table 4.12b: Shea butter - *Senna alata* ethanol extract ointment zone of inhibition (mm)

Test microorganism	*Ointment*		
	50 mg/g	***100 mg/g***	***200 mg/g***
E. coli	10.5±0.71	13.5±0.71	14.0±1.41
S. aureus	10.5±0.71	10.5±0.71	13.5±0.71
P. aeruginosa	11.5±0.71	13.0±1.41	14.0±0.00
K. pneumoniae	10.5±0.71	11.5±0.71	15.0±1.41
Fungus			
C. albicans	6.5±0.71	6.5±0.71	8.0±0.00

ESA= Ethanol *Senna alata*, mm= millimeter, Data expressed as mean ± SD, n = 2

The data above expresses plant extract-Shea butter ointment activity against the selected microorganisms with zone of inhibition ranges from 6.5-15 mm for all the selected microorganisms at stated concentrations of 50, 100 and200 mg/g. The ointment showed inhibition range of 10.5-14 mm against *E. coli.* Also, inhibition zones range of 10.5-13 mm was observed for *S. aureus*. Similarly, *P. aeruginosa* was susceptible with the zones of inhibition range of 11.5-14 mm at stated concentrations. Also, *K. pneumoniae* was susceptibity to plant extract ointment with zones of inhibition range of 10.5-15 mm at the varying concentrations. The plant extract ointment exhibited inhibition against *C. albicans*, with range 6.5-8 mm at the varying concentrations (Table 4.12b).

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Table 4.13a: Shea butter - *Lannea barteri* aqueous extract ointment zone of inhibition (mm)

Test microorganism	***Ointment***		
	50 mg/g	***100 mg/g***	***200 mg/g***
E. coli	9.5±0.71	10.5±0.71	14.5±0.71
S. aureus	13.5±0.71	13.0±1.41	14.0±0.00
P. aeruginosa	8.5±0.71	10.5±0.71	13.0±0.71
K. pneumoniae	6.5±0.71	7.0 ±1.41	14.5±0.71
Fungus			
C. albicans	10.5±0.71	12.5±0.71	13.5±0.71

aqLB= aqueous *Lannea barteri, mm=millimeter,*Data expressed as mean ± SD, n = 2

The aqLB-Shea butter ointment showed activity against the test microorganisms with zones of inhibition range of 6.5 -14.5 mm at stated concentrations of 50, 100 and 200 mg/g. The ointment expressed activity against *E.coli* given a range of 9.5-14.5 mm. The observed activity on *S. aureus* was in the range of 13-14 mm the ointment gave average range of 8.5-13 mm against *P. aeruginosa.* Also, *K. pneumoniae* showed a range of 6.5-14.5 mm when the ointment was used. The plant extract ointment exhibited inhibition against *C. albicans* with range 10.5-13.5 mm at the varying concentrations (Table 4.13a).

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Table 4.13b: Shea butter - *Lannea barteri* ethanol extract ointment zone of inhibition (mm)

Test microorganism	***Ointment***		
	50 mg/g	***100 mg/g***	***200 mg/g***
E. coli	7.5±0.71	10.5±0.71	13.5±0.71
S. aureus	8.5±0.71	10.5±0.71	11.0±1.41
P. aeruginosa	8.0±1.41	10.5±0.71	14.0±1.41
K. pneumoniae	8.5±0.71	10.5±0.71	16.5±0.71
Fungus			
C. albicans	7.5±0.71	10.5±0.71	14.5±0.71

ELB = Ethanol *Lannea barteri, mm = millimeter,* Data expressed as mean ± SD, n = 2

The data (Table 4.13b) shows an activity expressed by plant extract–Shea butter ointment against the test microorganisms with zones of inhibition ranging from 7.5 -16.5 mm at stated concentrations of 50, 100, 200 mg/g. *E. coli* was susceptible to the ointment with zones of inhibition range from 7.5-13.5 mm meanwhile *S. aureus* showed a range of 8.5-11 mm as a zone of inhibition expressed by the ointment. The ointment suppressed *P. aeruginosa* with zones of inhibition ranges 8-14 mm. *K. pneumoniae* was susceptible to pant extract ointment with zone of inhibition range of 8.5-16.5 mm at the varying concentrations. The ointment expressed activity against the *C. albicans,* with range 6.5-14 mm at the varying concentrations.

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Table 4.14a: Shea butter - *Ricinus communis* aqueous extract ointment zone of inhibition (mm)

Test microorganism	***Ointment***		
	50 mg/g	***100 mg/g***	***200 mg/g***
E. coli	7.5±0.71	7.5±0.71	9.5±0.71
S. aureus	11.5±0.71	12.5±0.71	13.0±1.41
P. aeruginosa	8.5±0.71	9.5±0.71	12.5±0.71
K. pneumoniae	7.0±0.00	7.5±0.71	11.5±0.71
Fungus			
C. albicans	8.5±0.71	10.5±0.71	13.5±0.71

aqRC= aqueous Ricinus *communis*, Data expressed as mean ± SD, n = 2

The aqRC-Shea butter ointment exhibited an activity against the test microorganisms with the diameters of inhibition ranged from 7-13.5 at the stated concentrations of 50, 100 and 200 mg/g. The ointment showed inhibition range of 7.5-9.5 mm against *E. coli.* Also, inhibition zones ranged of 11.5-13 mm was observed for *S. aureus*. Also, *P. aeruginosa* was susceptible with the zone of inhibition range of 8.5-12.5 mm at varying

concentrations. *K. pneumoniae* also showed susceptibility against the plant extract ointment with diameters of zones of inhibition range of 7-11.5 mm at the varying concentrations. The plant extract ointment exhibited inhibition against *C. albicans*, with range of 8.5-13.5 mm at the varying concentrations (Table 4.14a).

Table 4.14b: Shea butter - *Ricinus communis* ethanol extract ointment zone of inhibition (mm)

Test microorganism	Shea butter - Extract ointment of Ethanol *Ricinus communis* zone of inhibition (mm)		
	50 mg/g	**100 mg/g**	**200 mg/g**
E. coli	7.5±0.71	10.5±0.71	12.5±0.71
S. aureus	11.5±0.71	12.5±0.71	14.5±0.71
P. aeruginosa	12.0±1.41	12.5±0.71	16.5±0.71
K. pneumoniae	8.5±0.71	10.5±0.71	12.5±0.71
Fungus			
C. albicans	12.5±0.71	14.5±0.71	16.5±0.71

ERC= Ethanol *Ricinus communis,* Data expressed as mean ± SD, n = 2

The data above shows an activity exhibited by ERC-Shea butter ointment on selected the microorganisms with the expressed zones of inhibition range from 7.5-16.5 mm at the stated concentrations of 50, 100 and 200 mg/g. *E. coli* was susceptible to the ointment with zones of inhibition range from 7.5-13.5 mm meanwhile *S. aureus* showed a range of 11.5-14.5 mm as a zone of inhibition expressed by the ointment. The ointment successfully suppressed P. *aeruginosa* with zones of inhibition ranging from 12-16.5 mm. Also *K. pneumonia* was susceptible to plant extract ointment with zones of inhibition range of 8.5-12.5 mm at the varying concentrations. The ointment expressed activity against the *C. albicans*, with range from 12.5-16.5 mm at the varying concentrations (Table 4.14b).

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4.1.10 MIC and MBC/MFC of Plant Extract-Shea butter Ointment (mg/g)

Table 4.15a: Shea butter-*Senna alata* aqueous extract ointment (mg/g)

Test Microorganism	*Ointment*	
	MIC	*MBC*
E. coli	25	UD
S. aureus	25	UD
P. aeruginosa	25	UD
K. pneumoniae	12.5	UD
Fungus		MFC
C. albicans	12.5	UD

UD: MBC/MFC undetected up to 400 mg/g, MIC = Minimum Inhibitory Concentration, aqSA = aqueous *Senna alata*, MBC = Minimum Bactericidal Concentration, MFC = Minimum Fungicidal Concentration

The plant aqueous extract- Shea butter ointment showed low MIC values against the tested microorganisms with MIC values ranged from 12.5-25 mg/g and the MBC/MFC values were undetected up to 400 mg/g (Table 4.15a).

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Table 4.15b: Shea butter-*Senna alata* ethanol extract ointment (mg/g)

Test microorganism	*Ointment*	
	MIC	*MBC*
E. coli	25	UD
S. aureus	25	UD
P. aeruginosa	12.5	UD
K. pneumoniae	12.5	UD
Fungus		MFC
C. albicans	12.5	UD

UD: MBC undetected up to 400 mg/ml, MIC=Minimum Inhibition concentration, MBC=Minimum Bactericidal concentration, ESA= Ethanol *Senna alata*

The plant ethanol extract-Shea butter ointment showed low MIC values against the tested microorganisms with MIC values ranged from 12.5-25 mg/ml and MBC/MFC was undetected up to 400 mg/ml (Table 4.15b).

Table 4.16a: Shea butter- *Lannea barteri* aqueous extract ointment (mg/g)

	Ointment	
Test microorganism	***MIC***	***MBC***
E. coli	12.5	UD
S. aureus	12.5	UD
P. aeruginosa	12.5	UD
K. pneumoniae	6.25	UD
Fungus		MFC
C. albicans	6.25	UD

UD: MBC undetected up to 400 mg/mg, MIC= Minimum Inhibition Concentration, MBC=Minimum Bactericidal Concentration, MFC= Minimum Fungicidal Concentration aqLB= aqueous *Lannea barteri*

The plant aqueous extract-Shea butter ointment showed low MIC values against the tested microorganisms with MIC values ranged from 6.25 -12.5 mg/g and MBC/MFC was undetected at 400 mg/g (Table 4.16a).

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Table 4.16b: Shea butter- *Lannea barteri* ethanol extract ointment (mg/g)

	Ointment	
Test Microorganism	***MIC***	***MBC***
E. coli	25	UD
S. aureus	12.5	UD
P. aeruginosa	12.5	UD
K. pneumoniae	12.5	UD
Fungus		**MFC**
C. albicans	12.5	UD

UD: MBC undetected up to 400 mg/g, MIC= Minimum Inhibitory Concentration, MBC=Minimum Bactericidal Concentration, MCF=Minimum Fungicidal Concentration, ELB= Ethanol *Lannea barteri*

The plant ethanol extract-Shea butter ointment showed to be active with low MIC values against the tested microorganisms, MIC values ranged from 12.5-25 mg/g and the MBC was undetected up to 400 mg/g (Table 4.16b).

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Table 4.17a: Shea butter- *Ricinus communis* aqueous extract ointment (mg/g)

	Ointment	
Test Microorganism	**MIC**	**MBC**
E. coli	25	UD
S. aureus	25	UD
P. aeruginosa	25	UD
K. pneumoniae	12.5	UD
Fungus		**MFC**
C. albicans	12.5	UD

UD: MBC undetected up to 400 mg/ml, aqRC= aqueous *Ricinus communis*, MIC= Minimum Inhibitory Concentration, MBC= Minimum Bactericidal Concentration, MFC= Minimun Fungicidal Concentration.

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The plant aqueous extract-Shea butter ointment showed low MIC values against the tested microorganisms with MIC values ranged from 12.5-25 mg/g and MBC was undetected up to 400 mg/g (Table 4.17a).

Table 4.17b: Shea butter- *Ricinus communis* ethanol extract ointment (mg/g)

	Ointment	
Test Microorganism	**MIC**	**MBC**
E. coli	25	UD
S. aureus	25	UD
P. aeruginosa	25	UD
K. pneumoniae	12.5	UD
Fungus		**MFC**
C. albicans	12.5	UD

UD: MBC undetected up to 400 mg/ml, MIC= Minimum Inhibition Concentration, MBC = Minimum Bactericidal Concentration, MFC=Minimum fungicidal concentration, ERC= Ethanol *Ricinus communis*

The ERC extract- Shea butter ointment showed low MIC values when tested against the selected microorganisms with MIC values range from 12.5-25 mg/ml and MBC/MFC was undetected up to 400 mg/ml (Table 4.17b).

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4.1.11 Minimum Inhibitory Concentrations of Individual and combined extracts (mg/ml) on microorganisms

Table 4.18a: MIC of raw (individual) extract and combined extracts (mg/ml) of aqSA and aqLB

Test microorganism	aqSA	aqLB	combined aqSA + aqLB
E. coli.	6.25	3.13	1.17
S. aureus	12.5	12.5	3.12
P. aeruginosa	6.25	6.25	1.56
K. pneumoniae	12.5	6.25	2.34
Fungus			
C. albicans	12.5	12.5	3.12

MIC= Minimum inhibitory concentration, aqSA = aqueous *Senna alata*, aqLB = aqueous *Lannea barteri*

The data above (Table 4.18a) shows the individual extract MIC and their combined MIC values. The table clearly shows the reduction of MIC values for the respective combined extracts against the microorganisms by multiple of folds.

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Table 4.18b: MIC of raw (individual) extract and combined extracts (mg/ml) of ELB and aqSA

Test microorganism	ELB	aqSA	Combined ELB+aqSA
E.coli	6.25	3.13	1.17
S. aureus	12.5	12.5	3.12
P. aeruginosa	6.25	6.25	1.56
K. pneumoniae	6.25	6.25	1.56
Fungus			
C. albicans	12.5	12.5	3.12

MIC=Minimum Inhibitory Concentration, ELB = Ethanol *Lannea barteri,* aq*SA* = *aqueous Senna alata*

The data above (Table 4.18b) shows the individual extract MIC and their combined MIC values. The table shows a reduction of MIC values for the respective microorganisms by a multiple of folds.

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Table 4.18c: MIC of raw (individual) extract and combined extracts (mg/ml) of ELB and aqSA

MIC of raw (individual) extract and combined extracts (mg/ml)			
Test microorganism	**ELB**	**aqSA**	**Combined ELB+aqSA**
E. coli	6.25	3.13	1.17
S. aureus	12.5	12.5	3.12
P. aeruginosa	6.25	6.25	1.56
K. pneumoniae	6.25	6.25	1.56
Fungus			
C. albicans	12.5	12.5	3.12

MIC=Minimum Inhibitory Concentration, ELB = Ethanol *Lannea barteri,* aqSA = aqueous *Senna alata,*

The individual extract MIC and their combined MIC values are shown in table above. The activity of combined extract shows a reduction of MIC values for the respective microorganisms compared with the individual extracts by multiple of folds (Table 4.18c).

Table 4.18d: MIC of raw (individual) extract and combined extracts (mg/ml) of ELB and aqRC

MIC of raw (individual) extract and combined extracts			
Test microorganism	**ELB**	**aqRC**	**Combined MIC ELB+aqRC**
E. coli	6.25	6.25	1.56
S. aureus	12.5	3.13	1.95
P. aeruginosa	6.25	3.13	0.5
K. pneumoniae	6.25	12.5	1.17
Fungus			
C. albicans	12.5	12.5	3.12

MIC=Minimum Inhibitory Concentration, ELB = Ethanol *Lannea barteri*, aqRC = aqueous *Ricinus communis,*

The data above (Table 4.18d) shows the individual extracts MIC and their combined MIC values. The table clearly shows the reduction of MIC values for the respective microorganisms.

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Table 4.18e: MIC of raw (individual) extract and combined extracts (mg/ml) of aqLB and ESA

MIC of raw (individual) extract and combined extracts(mg/ml)			
Test microorganism	**aqLB**	**ESA**	**Combined MIC aqLB+ESA**
E. coli	6.25	6.25	1.56
S. aureus	12.5	6.25	1.96
P. aeruginosa	6.25	6.25	1.56
K. pneumoniae	12.5	12.5	3.12
Fungus			
C. albicans	12.5	12.5	3.12

MIC = Minimum Inhibitory Concentration, aqLB = aqueous *Lannea barteri*, ESA = Ethanol *Senna alata*

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The data in table above (Table 4.18e) indicates the individual extract MIC and their combination MIC values. The table shows significant reduction of MIC values for the respective microorganisms.

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Table 4.18f: MIC of raw (individual) extract and combined extracts (mg/ml) of ELB and ESA

MIC of crude (individual) extract and combined extracts(mg/ml)			
Test microorganism	**ELB**	**ESA**	**Combined MIC ELB+ ESA**
E. coli	6.25	6.25	1.56
S. aureus	12.5	12.5	3.13
P. aeruginosa	6.25	6.25	1.56
K. pneumoniae	6.25	12.5	2.34
Fungus			
C. albicans	12.5	12.5	3.12

MIC = Minimum Inhibitory Concentration, ELB = Ethanol *Lannea barteri*, ESA = *Ethanol Senna alata*

The MIC of each and the combined extracts clearly show a reduction against the respective microorganisms (Table 4.18f).

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Table 4.18g: MIC of raw (individual) extract and combined extracts (mg/ml) of aqRC and aqSA

MIC of raw (individual) extract and combined extracts (mg/ml)			
Test microorganism	**aqRC**	**aqSA**	**Combined aqRC+aqSA**
E. coli	6.25	3.13	0.975
S. aureus	3.13	12.5	0.975
P. aeruginosa	3.13	6.25	1.17
K. pneumoniae	12.5	6.25	1.17
Fungus			
C. albicans	12.5	12.5	3.12

MIC=Minimum Inhibitory Concentration, aqRC=aqueous *Ricinus communis*, aqSA=aqueous *Senna alata*

The data in table above (Table 4.18g) indicates the individual extracts MIC and their combination MIC values. The table depicts a reduction of MIC values for the respective combined extracts against the microorganisms by a number of folds.

Table 4.18h: MIC of raw (individual) extract and combined extracts (mg/ml) of ERC and aqSA

MIC of raw (individual) extract and combined extracts(mg/ml)			
Test microorganism	**ERC**	**aqSA**	**Combined ERC+aqSA**
E. coli	6.25	3.13	0.975
S. aureus	25	12.5	2.34
P. aeruginosa	6.25	6.25	1.56
K. pneumoniae	25	6.25	1.955
Fungus			
C. albicans	25	12.5	3.12

MIC = Minimum Inhibitory Concentration, ERC = Ethanol *Ricinus communis*, aqSA = aqueous *Senna alata*

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The MIC results for both each and combination of extracts show a reduction for the respective microorganisms by a number of folds as shown above (Table 4.18h).

Table 4.18i: MIC of raw (individual) extract and combined extracts (mg/ml) of aqLB and ERC

MIC of raw (individual) extract and combined extracts(mg/ml)			
Test microorganism	***aqLB***	***ERC***	***Combined aq LB+ERC***
E. coli	6.25	6.25	1.56
S. aureus	12.5	25	4.69
P. aeruginosa	6.25	6.25	1.56
K. pneumoniae	12.5	6.25	1.95
Fungus			
C. albicans	12.5	25	2.44

MIC=Minimum Inhibitory Concentration, aqLB=aqueous *Lannea barteri*, ERC= Ethanol *Ricinus communis*

The data as indicated in Table 4.18i above shows a reduction in MIC values for both individual and the combined extracts against the test microorganisms.

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Table 4.18j: MIC of raw (individual) extract and combined extracts (mg/ml) of ELB and ERC

MIC of raw (individual) extract and combined extracts(mg/ml)			
Test microorganism	***ELB***	***ERC***	***COMBINED ELB+ERC***
E. coli	6.25	6.25	1.56
S. aureus	12.5	12.5	3.12
P. aeruginosa	6.25	6.25	1.56
K. pneumoniae	6.25	6.25	1.56
Fungus			
C. albicans	12.5	25	4.69

MIC=Minimum Inhibitory Concentration, ELB=Ethanol *Lannea barteri*, ERC= Ethanol *Ricinus communis*

The data above (Table 4.18j) indicates the individual extracts MIC and their combination MIC values. The table depicts a reduction of MIC values for the respective combined extracts against the microorganisms by a number of folds.

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Table 4.18k: MIC of raw (individual) extract and combined extracts (mg/ml) of aqRC and ESA

MIC of raw (individual) extract and combined extracts (mg/ml)			
Test microorganism	***aqRC***	***ESA***	***COMBINED aqRC+ESA***
E. coli	6.25	6.25	1.56
S. aureus	3.13	12.5	1.95
P. aeruginosa	3.13	6.25	1.17
K. pneumoniae	12.5	12.5	3.12
Fungus			
C. albicans	12.5	12.5	3.12

MIC=Minimum Inhibitory Concentration, aqRC= aqueous *Ricinus communis*, ESA = Ethanol *Senna alata*

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The data as indicated above (Table 4.18k) shows a reduction in MIC values for both individual and the combined extracts against the test microorganisms.

Table 4.18l: MIC of raw (individual) extract and combined extracts (mg/ml) of ERC and ESA

MIC of raw (individual) extract and combined extracts			
Test Microorganism	***ERC***	***ESA***	***COMBINED ERC+ESA***
E .coli	6.25	6.25	1.56
S. aureus	25	12.5	2.34
P. aeruginosa	6.25	6.25	1.56
K. pneumoniae	6.25	12.5	2.34
Fungus			
C. albicans	25	12.5	2.34

MIC=Minimum Inhibitory Concentration, ERC = Ethanol *Ricinus communis*, ESA = Ethanol *Senna alata*

The data above (Table 4.18l) indicates the individual extracts MIC and their combination MIC values. The table depicts a reduction of MIC values for the respective combined extracts against the microorganisms by a number of folds.

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Table 4.19a: Minimum Inhibitory Concentration of Combined Extracts (mg/ml) on microorganisms

	MIC of Combined Plant Extracts (mg /ml)					
Test Microorganism	***aqLB + aqSA***	***ELB + aqSA***	***aqLB + aqRC***	***ELB + aqRC***	***aqLB + ESA***	***ELB + ESA***
E. coli	1.17	1.17	1..56	1.56	1.56	1.56
S. aureus	3.12	3.12	1.95	1.95	1.955	3.13
p. aeruginosa	1.56	1.56	1.17	0.50	1.56	1.56
K. pneumoniae	2.34	1.56	3.12	1.17	3.12	2.34
Fungus						
C. albicans	3.12	3.12	3.12	3.12	3.12	3.12

aqLB =aqueous *Lannea barteri,* aqSA=aqueous *Senna alata*, ELB=Ethanol *Lannea barteri*, aqRC=aqueous *Ricinus communis,* ESA=Ethanol *Senna alata,* MIC= Minimum inhibitory concentration

The data shows MIC values for the respective combined plant extracts. The observed data showed a reduction in MIC values of the respective microorganisms which implies the plant extract mixture was potent (Table 4.19a).

Table 4.19b: Minimum Inhibitory Concentration of Combined Extracts (mg/ml)

	MIC of Combined Plant Extracts mg /ml					
Test Microorganism	***aqRC + aqSA***	***ERC + aqSA***	***aqLB + ERC***	***ELB + ERC***	***aqRC + ESA***	***ERC + ESA***
E. coli	0.975	0.975	1.56	1.56	1.56	1.56
S. aureus	0.975	2.34	4.69	3.12	1.95	2.34
p. aeruginosa	1.17	1.56	1.56	1.56	1.17	1.56
K. pneumoniae	1.17	1.96	1.95	1.56	3.12	2.34
Fungus						
C. albicans	3.12	3.12	2.44	4.69	3.12	2.34

aqRC = aqueous *Ricinus communis,* aqSA= aqueous *Senna alata*, ERC = Ethanol *Ricinus communis*, aqLB = aqueous *Lannea barteri* ELB = Ethanol *Lannea barteri*, aqRC = aqueous *Ricinus communis,* ESA=Ethanol *Senna alata,* MIC = Minimum Inhibitory Concentration

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The data above (Table 4.19b) shows MIC values for the respective combined plant extracts. The data revealed significant reduction in MIC values indicating that the plant extract mixture was effective.

4.1.12 Minimum Inhibitory Concentration and the Fractional Inhibitory index of combined extracts against microorganisms

Table 4.20a: Fractional Inhibitory Index and the effect of combined extracts against microorganisms

Test Microorganism	*MIC* aqSA	aqLB	*Combined MIC aqSA + aqLB*	*FIC INDEX*	*EFFECT*
E. coli	6.25	3.13	1.17	0.5610	A
S. aureus	12.5	12.5	3.12	0.4992	S
P. aeruginosa	6.25	6.25	1.56	0.4992	S
K. pneumoniae	12.5	6.25	2.34	0.5616	S
Fungus					
C. albicans	12.5	12.5	3.12	0.4992	S

S = Synergism, A = Additive, MIC = Minimum Inhibitory Concentration in mg/ml, aqSA = aqueous *Senna alata*, aqLB = aqueous *Lannea barteri*, S = Synergism, A = Additive, FIC = Fractional Inhibitory Concentration.

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The data above (Table 4.20a) shows the FIC Index as well as the effect of those values. The FIC Index signifies the effect of combination of different extracts on microorganisms tested with the aid of the formula of Isenberg, (1992) and Noor, (2016). The data further showed that, the antimicrobial actions of the plant extract mixture was effective against all the test microorganisms which significantly lowered the MICs.

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Table 4.20b: Fractional Inhibitory Index and the effect of combined extracts against microorganisms

Test microorganism	*MIC*		*COMBIND*	*FIC INDEX*	*EFFECT*
	ELB	*aqSA*	*ELB+aqSA*		
E. coli	6.25	3.13	1.17	0.561002	A
S. aureus	12.5	12.5	3.12	0.4992	S
P. aeruginosa	6.25	6.25	1.56	0.4992	S
K. pneumoniae	6.25	6.25	1.56	0.4992	S
Fungus					
C. albicans	12.5	12.5	3.12	0.4992	S

S = Synergism, A= Additive, MIC = Minimum inhibitory concentration in mg/ml, FIC = Fractional inhibitory concentration, ELB = Ethanol *Lannea barteri*, aqSA = aqueous *Senna alata*

The data (Table 4.20b) shows the FIC Index as well as the effect of those values. The FIC Index signifies the effect of combination of different extracts on microorganisms tested with the aid of the formula of Isenberg, (1992) and Noor, (2016). The results show that, the mixture of the plant extract resulted in synergistic effect against the test microorganisms with a reduction in MIC.

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Table 4.20c: Fractional Inhibitory Index and the effect of combined extracts against microorganisms

Test microorganism	*MIC*		*COMBINED*		
	aqLB	*aqRC*	*aqLB + aqRC*	*FIC INDEX*	*EFFECT*
E. coli	6.25	6.25	1.56	0.4992	S
S. aureus	12.5	3.13	1.95	0.779003	A
P. aeruginosa	6.25	3.13	1.17	0.561002	A
K. pneumoniae	12.5	12.5	3.12	0.4992	S
Fungus					
C. albicans	12.5	12.5	3.12	0.4992	S

S = Synergism, A = Additive, MIC=Minimum inhibitory concentration in mg/ml. FIC = Fractional inhibitory concentration, aqLB = aqueous *Lannea barteri*, aqRC = aqueous *Ricinus communis*

The data above (Table 4.20c) shows the FIC Index as well as the effect of those values. The FIC Index signifies the effect of combination of two different extracts on tested microorganisms with the aid of the formula of Isenberg, (1992) and Noor, (2016).

Table 4.20d: Fractional Inhibitory Index and the effect of combined extracts against microorganisms

Test Microorganism	*MIC*		*COMBINED*		
	ELB	*aqRC*	*ELB+aqRC*	*FIC INDEX*	*EFFECT*
E. coli	6.25	6.25	1.56	0.4992	S
S. aureus	12.5	3.13	1.95	0.779003	A
P. aeruginosa	6.25	3.13	0.5	0.239744	S
K. pneumoniae	6.25	12.5	1.17	0.2808	S
Fungus					
C. albicans	12.5	12.5	3.12	0.4992	S

S = Synergism, A = Additive, MIC = Minimum Inhibitory Concentration in mg/ml, FIC = Fractional Inhibitory Concentration, ELB = Ethanol *Lannea barteri*, aqRC = aqueous *Ricinus communis*

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The data above (Table 4.20d) shows the FIC Index as well as the effect of those values. The FIC Index signifies the effect of combination of different extracts on microorganisms tested with the aid of the formula by Isenberg, (1992) and Noor, (2016).

Table 4.20e: Fractional Inhibitory Index and the effect of combined extracts against microorganisms

Test microorganism	*MIC*		*COMBINED*	*FIC INDEX*	*EFFECT*
	aqLB	*ESA*	*aqLB+ESA*		
E. coli	6.25	6.25	1.56	0.4992	S
S. aureus	12.5	6.25	1.955	0.4692	S
P. a.eruginosa	6.25	6.25	1.56	0.4992	S
K. pneumoniae	12.5	12.5	3.12	0.4992	S
Fungus					
C. albicans	12.5	12.5	3.12	0.4992	S

S = Synergism, A = Additive, MIC = Minimum Inhibitory Concentration in mg/ml, FIC = Fractional Inhibitory Concentration, aqLB = aqueous *Lannea barteri*, ESA = Ethanol *Senna alata*

The data above (Table 4.20e) shows the FIC Index as well as the effect of those values. The FIC Index signifies the effect of combination of different extracts on microorganisms tested with the aid of the formula by Isenberg, (1992) and Noor, (2016).

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Table 4.20f: Fractional Inhibitory Index and the effect of combined extracts against microorganisms

	MIC		COMBINED		
Test microorganism	**ELB**	**ESA**	**ELB+ ESA**	**FIC INDEX**	**EFFECT**
E. coli	6.25	6.25	1.56	0.4992	S
S. aureus	12.5	12.5	3.13	0.5008	A
P. aeruginosa	6.25	6.25	1.56	0.4992	S
K. pneumoniae	6.25	12.5	2.34	0.5616	A
Fungus					
C. albicans.	12.5	12.5	3.12	0.4992	S

S = Synergism, A = Additive, MIC = Minimum Inhibitory Concentration in mg/ml, FIC = Fractional Inhibitory Concentration.

The data above (Table 4.20f) shows the FIC Index as well as the effect of those values. The FIC Index signifies the effect of combination of different extracts on microorganisms tested with the aid of the formula by Isenberg, (1992) and Noor, (2016).

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Table 4.20g: Fractional Inhibitory Index and the effect of combined extracts against microorganisms

Test microorganism	***MIC***				
	aqRC	***aqSA***	***Combined aqRC+aqSA***	***FIC INDEX***	***EFFECT***
E. coli	6.25	3.13	0.975	0.4675	S
S. aureus	3.13	12.5	0.975	0.3895	S
P. aeruginosa	3.13	6.25	1.17	0.5610	A
K. pneumoniae	12.5	6.25	1.17	0.2808	S
Fungus					
C. albicans	12.5	12.5	3.12	0.4992	S

S = Synergism, A = Additive, MIC = Minimum Inhibitory Concentration in mg/ml, FIC = Fractional Inhibitory Concentration

The data above shows (Table 4.20g) the FIC Index as well as the effect of those values. The FIC Index signifies the effect of combination of different extracts on microorganisms tested with the aid of the formula by Isenberg, (1992) and Noor, (2016).

Table 4.20h: Fractional Inhibitory Index and the effect of combined extracts against microorganisms

Test Microorganism	*MIC*		*COMBINED*	*FIC*	
	ERC	*aqSA*	*ERC+aqSA*	*INDEX*	*EFFECT*
E .coli	6.25	3.13	0.975	0.468	S
S. aureus	25	12.5	2.34	0.281	S
P. aeruginosa	6.25	6.25	1.56	0.499	S
K. pnuemoniae	25.0	6.25	1.96	0.391	S
Fungus					
C. albicans	25.0	12.5	3.12	0.374	S

S = Synergism, A = Additive, MIC= Minimum Inhibitory Concentration in mg/ml, FIC = Fractional Inhibitory Concentration.

The data above (Table 4.20h) shows the FIC Index as well as the effect of those values. The FIC Index signifies the effect of combination of different extracts on microorganisms tested with the aid of the formula by Isenberg, (1992) and Noor, (2016).

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Table 4.20i: Fractional Inhibitory Index and the effect of combined extracts against microorganisms

Test Microorganism	MIC aqLB	MIC ERC	COMBINED aqLB+ERC	FIC INDEX	EFFECT
E. coli	6.25	6.25	1.56	0.4992	S
S. aureus	12.5	25	4.69	0.5628	A
P. aeruginosa	6.25	6.25	1.56	0.4992	S
K. pneumoniae	12.5	6.25	1.95	0.4680	S
Fungus					
C. albicans	12.5	25	2.44	0.2928	S

S= Synergism, A= Additive, MIC=Minimum inhibitory concentration in mg/ml, FIC= Fractional Inhibitory Concentration.

The data above (Table 4.20i) shows the FIC Index as well as the effect of those values. The FIC Index signifies the effect of combination of different extracts on microorganisms tested with the aid of the formula by Isenberg, (1992) and Noor, (2016).

Table 4.20j: Fractional Inhibitory Index and the effect of combined extracts against microorganisms

Test Microorganism	*MIC ELB*	*MIC ERC*	*COMBINED MIC ELB+ERC*	*FIC INDEX*	*EFFECT*
E. coli	6.25	6.25	1.56	0.4992	S
S. aureus	12.5	12.5	3.12	0.4992	S
P. aeruginosa	6.25	6.25	1.56	0.4992	S
K. pneumoniae	6.25	6.25	1.56	0.4992	S
Fungus					
C. albicans	12.5	25	4.69	0.5628	A

S= Synergism, A= Additive, MIC=Minimum Inhibitory Concentration in mg/ml FIC= Fractional Inhibitory Concentration

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The data above (Table 4.20j) shows the FIC Index as well as the effect of those values. The FIC Index signifies the effect of combination of different extracts on microorganisms tested with the aid of the formula by Isenberg, (1992) and Noor, (2016).

Table 4.20k: Fractional Inhibitory Index and the effect of combined extracts against microorganisms

Test Microorganism	*MIC aqRC*	*MIC ESA*	*COMBINED MIC aqRC+ESA*	*FIC INDEX*	*EFFECT*
E. coli	6.25	6.25	1.56	0.4992	S
S. aureus	3.13	12.5	1.95	0.7790	A
P. aeruginosa	3.13	6.25	1.17	0.5610	A
K. pneumoniae	12.5	12.5	3.12	0.4992	S
Fungus					
C. albicans	12.5	12.5	3.12	0.4992	S

S = Synergism, A = Additive, MIC=Minimum Inhibitory Concentration in mg/ml FIC = Fractional Inhibitory Concentration.

The data above (Table 4.20k) shows the FIC Index as well as the effect of those values. The FIC Index signifies the effect of combination of different extracts on microorganisms tested with the aid of the formula by Isenberg, (1992) and Noor, (2016).

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Table 4.20l: Fractional Inhibitory Index and the effect of combined extracts against microorganisms

Test microorganisms	*MIC*		*COMBINED*	*FIC*	
	ERC	*ESA*	*ERC+ESA*	*INDEX*	*EFFECT*
E. coli	6.25	6.25	1.56	0.4992	S
S. aureus	25	12.5	2.34	0.2808	S
P. aeruginosa	6.25	6.25	1.56	0.4992	S
K. pneumoniae	6.25	12.5	2.34	0.5616	A
Fungus					
C.albicans	25	12.5	2.34	0.2808	S

S = Synergism, A = Additive, MIC=Minimum inhibitory concentration in mg/ml FIC = Fractional Inhibitory Concentration

The data above (Table 4.20l) shows the FIC Index as well as the effect of those values. The FIC Index signifies the effect of combination of different extracts on microorganisms tested with the aid of the formula by Isenberg, (1992) and Noor, (2016).

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Table 4.21a: Minimum Bactericidal/Fungicidal Concentration of Combined Extracts (mg/ml) on microorganisms

	MBC/MFC of Combined Plant Extracts (mg /ml)					
Test Microorganism	**aqLB + aqSA**	**ELB + aqSA**	**aqLB. + aqRC**	**ELB + aqRC**	**aqLB + ESA**	**ELB + ESA**
Bacteria	MBC	MBC	MBC	MBC	MBC	MBC
E. coli	150	150	150	150	150	150
S. aureus	150	150	150	150	150	150
P. aeruginosa	150	150	150	150	150	150
K. pneumoniae	150	150	150	150	150	150
Fungus	MFC	MFC	MFC	MFC	MFC	MFC
C. albicans	150	150	150	150	150	150

aqLB =aqueous *Lannea barteri,* aqSA=aqueous *Senna alata*, ELB=Ethanol *Lannea barteri*, aqRC=aqueous *Ricinus communis,* ESA=Ethanol *Senna alata,* MIC= Minimum inhibitory concentration

The data (Table 4.21a) shows MBC/MFC values for the respective combined plant extracts. The combined plant extracts showed a remarkable reduction in MBC/MFC values being 150 mg/ml.

Table 4.22b: Minimum Bactericidal/Fungicidal Concentration of Combined Extracts (mg/ml)

	MBC/MFC of Combined Plant Extracts mg /ml					
Test Microorganism	***AqRC + AqSA***	***ERC + aqSA***	***aqLB + ERC***	***ELB + ERC***	***aqRC + ESA***	***ERC + ESA***
Bacteria	MBC	MBC	MBC	MBC	MBC	MBC
E. coli	150	150	150	150	150	150
S. aureus	150	150	150	150	150	150
P. aeruginosa	150	150	150	150	150	150
K. pneumoniae	150	150	150	150	150	150
Fungus	MFC	MFC	MFC	MFC	MFC	MFC
C. albicans	150	150	150	150	150	150

aqRC = aqueous *Ricinus communis,* aqSA= aqueous *Senna alata*, ERC = Ethanol *Ricinus communis*, aqLB = aqueous *Lannea barteri* ELB = Ethanol *Lannea barteri*, aqRC = aqueous *Ricinus communis,* ESA = Ethanol *Senna alata,* MIC = Minimum Inhibitory concentration

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The data above (Table 4.22b) shows MIC values for the respective combined plant extracts. The combined plant extracts significantly reduced the activity of test microorganisms resulting in MBC/MFC values of 150 mg/ml.

4.2 Discussion

4.2.1 Percent yield of extracts

In general, for 100 g each of plant material the percentage yields of the extracts were found to be between 5.20 -12.40 % w/w. *Lannea barteri* was found to be the highest, 12.40 and 12.10 % for aqueous and ethanol respectively. *Senna alata* followed with 7.4 and 7.2 % for aqueous and ethanol extractants respectively whilst *Ricinus comminus* yielded 6.00 %, and 5.20 %, for aqueous and ethanol extractants respectively. Both aqueous and ethanol extracts of *Lannea barteri* were crystalline solid. Those of *Senna alata were* solid (pasty) for aqueous and ethanol and Semi solid for both aqueous and ethanol extracts of *Ricinus communis.*

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4.2.2 Phytochemical Screening

Secondary plant metabolites (phytochemicals) with antibacterial potency have been given serious attention as an alternative source of therapeutic treatments (Sato *et al*, 1995; Leu and Donnell, 2001). Primary Health Care in most developing continents such as Africa has depended on traditional herbal medicine for their wellbeing (Sheldon *et al.,* 1997). The Preliminary Phytochemical Screening of the crude extracts of three (3) medicinal plants was analyzed.

The results for the preliminary phytochemical screening (Table 4.1) showed the presence of tannins, saponins, polyuronoids, reducing sugars, terpenoids, flavonoids and alkaloids for both aqueous and ethanol extracts of *Senna alata,* but in the ethanol extract, polyuronoids was absent. This observation is in line with the report by Manivannan *et al.,*

2016, for ethanol extract of *Senna alata* with exception of flavonoids which was found to be absent. The result for the phytochemical screening of the ethanol extract of *Senna alata* is in concordance with the results obtained by Ehiowemwenguan *et al.,* 2014. The phytochemicals revealed confirms the work of Doughari and Okafor, 2007 with some exceptions and the differences may be due to extraction process, geographical location among others.

For *Lannea barteri* extract*,* the following bioactive compounds were found: saponins, polyuronoids, reducing sugars, terpenoids, flavonoids and alkaloids in both aqueous and ethanol extracts. The results obtained confirm Kone *et al*. (2011) with the exception of alkaloids in the aqueous extract. The differences in the results of phytochemical screening could be due to geographical location, genetic factors, and age of the plant among others (Figueiredo *et al.,* 2008).

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Ricinus communis, the secondary metabolites found include tannins, saponins, polyuronoids, flavonoids and alkaloids in both aqueous and ethanol extracts. However, in the ethanol extract, reducing sugars, terpenoids, and anthraquinones were absent whilst anthraquinones and terpenoids were present in the aqueous extract. Similar observations were made by Das *et al*. (2015) with some exception in both aqueous and ethanol extracts. The difference as stated by Figueiredo *et al*. (2008) could be attributed to differences in geographical location, genetic factors, and age of the plant among other reasons. Alkaloids are one of the most important classes of phytochemicals and have been known for their bactericidal effects (Okwu and Okwu, 2004) and are said to be pharmacologically active and their activity is felt in blood vessels, uterus, malignant diseases, gastrointestinal tract, respiratory system (Trease and Evans, 1989). Alkaloids are

also reported to have cytotoxicity properties (Nobori *et al.,* 1994). Alkaloids, terpenoids and phenolic compounds are also known to inhibit bacterial as well as reducing fungal infections (Saxena *et al*., 2013). Terpenoids which contain essential oil derivatives are said to have antibacterial properties (Aureli *et al*., 1992). Anticancer agents, antioxidants and nero pharmaceutical agents are attributed to alkaloids, flavonoids and terpenoids (Saxena *et al*., 2013). Flavonoids have broad biological and pharmacological activities for they exert multiple biological properties such as anti-tumor, inhibiting lung cancer, anti-metastatic, antimicrobial, cytotoxicity, anti-inflammatory and antioxidant (Saxena *et al.,* 2013). Chung *et al.,* (1998) and Karou *et al.,* (2005) reported antimicrobial effect of flavonoids.

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Saponins were reported to have numerous pharmacological properties and were present in all the plant extracts fraction that were used (Estrada *et al*., 2000). Saponins are also known to have hemolytic activities, cough suppressant and use as expectorant (Okwu, 2005; Sofowora 2006). Saponin interferes with cell replication, including cancer cells (Oliveire, 2015). Asquith and Butler (1986), Okwu and Okwu (2004) reported that tannins are involved in the treatment of sore throat and have antidiarrheal and wound healing agents.

4.2.3 Antimicrobial Studies of the crude extracts

4.2.3.1 Antimicrobial Studies of Senna alata

The antimicrobial susceptibility tests of the three plants were employed against the selected microorganisms. The antimicrobial activity of the ethanol extract of the leaf of

Senna alata against five (5) selected clinical isolates revealed that the extract was active against all the microorganisms used in this research (Table 4.2a). The aqueous extract of *Senna alata* suppressed the growth of the respective microorganisms at varying concentrations (Table 4.2a). The antimicrobial activity of the aqueous extract on the Gram positive and negative bacteria as well as fungus showed strong antimicrobial activity with the highest activity recorded against *K. pneumoniae* with zone of inhibition of 28.5 mm at 200 mg/ml and the least activity was recorded against *E. coli* and *S. aureus* with zones of inhibition 17 and 16 mm respectively. The activity of the extract against the test microorganisms was not dose dependent, as when concentration of drug doubled from 50 to 100 mg/ml, there was no significant difference in terms of activity on the test microorganisms ($p > 0.05$). However, when the concentration was quadrupled from 50 to 200 mg/ml, it showed dose dependence against some of the test microorganisms: *E. coli, P. aeruginosa, K. pneumoniae* ($p < 0.05$), whilst activities against *S. aureus* and *C. albicans* were insignificant ($P > 0.05$). The activity of plant crude extract against tested microorganisms is greatly influence by increased concentration of plant extracts (Oluduro and Omoboye, 2010) and most natural substances of plant origin are reported to be biologically active and enriched with antimicrobial properties (Gupta *et al.,* 2004). The results further revealed that, there was significant effect of the extract at 50 mg/ml among the microorganisms tested ($p < 0.05$) and similarly, the concentrations of 100 and 200 mg/ml indicated that, the aqueous extract had significant effect on *S. aureus*, *P. aeruginosa, K. pneumoniae* and *C. albicans* but it was insignificant against *E. coli*. Again, the extract expressed higher activity than the standard drugs against *P. aeruginosa* and *C. albicans* ($p < 0.05$) at varying concentrations, whilst the standard drug showed higher antimicrobial activity than the crude extract against *E. coli, S. aureus, K. pneumoniae* (p <

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0.05). The negative control used (DMSO) showed no activity against all the test microorganisms.

The activity of the ethanol extract showed dose independence as the concentration of the drug was increased from 50 to 100 mg/ml against the microorganisms ($P > 0.05$). However, when the concentration was quadrupled to 200 mg/ml, the extract showed significant activity against the entire microorganisms ($P < 0.05$) (Table 4.2b). The extract expressed higher antimicrobial activity than the standard drugs used (amoxicillin/fluconazole) against *S. aureus* at 200 mg/ml and *P. aeruginosa* at both 100 and 200 mg/ml ($p < 0.05$). However, the standard drug showed a pronounced activity against *E. coli* and *K. pneumoniae* than that of the extract ($p < 0.05$). Doughari and Okafor (2007) reported of antimicrobial activity exhibited by leaves and root extracts of *Senna alata* against Gram positive and Gram-negative bacteria as well as fungi and this confirms the current work. The antimicrobial activity exhibited by the ethanol extract of *Senna alata* could be attributed to extracted potent phytochemicals from the plant (Table 4.2b).

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4.2.3.2 Antimicrobial Studies of Lannea barteri

The results of aqueous extract of *Lannea barteri* bark (Table 4.3a) indicated that all the test microorganisms were susceptible to the extract at the different concentrations. All the test microorganisms showed high sensitivity towards the extract with maximum zone of inhibition of 32 mm at a concentration of 200 mg/ml. The observed results further revealed that the activity of the crude extract was dose dependent against each. The observed results revealed that the activity of the crude extract was dose dependent against

each microorganism when the concentration was increased from 50 to 100 mg/ml ($p < 0.05$) and when the concentration was quadrupled from 50 to 200 mg/ml, a similar trend of dose dependence was observed ($p<0.05$). This result confirms findings by Azu and Onyeagba (2007), that efficacy of most plant extract concentration is dose dependent. The suppression activity of the crude extract was more significantly ($p < 0.05$) pronounced than the standard drug used against *S. aureus, P. aeruginosa, C. albicans* whilst it was insignificant ($p > 0.05$) against *K. pneumoniae* and *E. coli* when compared to the standard drug used. Also, the extract activity results were statistically significant ($p < 0.05$) on the microorganisms studied at varying concentrations used at 50, 100 and 200 mg/ml however it was insignificant ($p > 0.05$) against *C. albicans* at 50 mg/ml.

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Equally the ethanol extract of *Lannea barteri* bark exhibited antimicrobial activity against the test microorganism with zone of inhibition ranging from 17-36.5 mm (Table 4.3b). The activity exhibited by the crude ethanol extract demonstrated dose dependency, as increasing concentration of the extract greatly influenced growth of the microorganisms. Increasing concentration from 50 to 100 mg/ml significantly ($P < 0.05$) affected the growth of *S. aureus, P. aeruginosa* and *C. albicans* but was statistically insignificant ($P > 0.05$) against *E. coli* and *K. pneumoniae*. When concentration was raised to 200 mg/ml, S. *aureus, E. coli, P. aeruginosa* and *C. albicans* were highly ($p > 0.05$) susceptible to the extract ($P < 0.05$), however, there was no significant effect on *K. pneumoniae*. The antimicrobial activity exhibited by the extract could be attributed to various secondary metabolites present such as tannins and alkaloids which may be toxic to have caused suppression or inhibition of the microorganisms (Ngaloy and Andrada, 2005).

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4.2.3.3 Antimicrobial Studies of Ricinus communis

The antimicrobial activity of aqueous extracts of *Ricinus communis* against the test microorganisms showed sensitivity with diameter of inhibition ≥ 20 mm at 200 mg/ml against the entire test microorganisms (Table 4.4a). The highest inhibition of the crude extract was against *P. aeruginosa* and *K. pneumoniae* with zone of inhibition ≥ 28 mm. The extract showed no dose effect against the entire microorganisms when the concentration was raised from 50 to 100 mg/ml. However, it showed significant effect against *S. aureus, K. pneumonia and C. albicans* when the concentration was raised to 200 mg/m*l* but was insignificant *($P > 0.05$)* against *E. coli,* and *K. pneumoniae*. Generally, the crude extract exhibited higher antimicrobial activity than the standard drugs used against *S. aureus, P. aerugonosa* and *K. pneumoniae* ($P < 0.05$) but showed insignificant ($P > 0.05$) activity against *E. coli and C. albicans* when compared with the standard drug used. The photochemistry of the selected plants contains enormous number of bioactive-compounds that could be used as antimicrobial agents especially against skin and wound causing microorganisms.

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The ethanol extract of *Ricinus communis* exhibited antimicrobial activity against the test microorganisms with maximum zone of inhibition of 36.5 mm at concentration of 200 mg/ml. The highest diameter of zone of inhibition was against *P. aeruginosa*, and *C. albicans* at 200 mg/ml (Table 4.4b). The plant crude extract showed dose dependence (Yankanchi, 2014) against *S. aureus, P. aeruginosa, and C. albicans* when the concentration was raised from 50 to 100 mg/ml, however, it was insignificant *($P > 0.05$)* against *E. coli* and *K. pneumoniae.* Also when the concentration was again quadrupled to 200 mg/ml, it manifested an increase in antimicrobial activity indicating dose dependency

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against E. coli, S. aureus, P. aeruginosa, and C. albicans ($P < 0.05$), but it was not significant against *K. pneumoniae* as activity remain constant even though there was significant increase in the concentration of the crude extract. The activity exhibited by the crude extract is in line with the report by Porohit *et al*., (1999) that plants are rich in secondary metabolites which contain different structures with high density and perform different functions such as bactericidal, bacteriostatic, chemotherapy and antimicrobial functions.

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4.2.4 Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal/Fungicidal Concentration (MBC/MFC) of the Crude Extracts

Agar well diffusion method was used to determine the minimum inhibitory concentration (MIC). The MIC values of aqueous *Senna alata* extract was found to be 6.25, 6,25, 12.5, 6.25, 12.5 mg/ml against *E. coli, S. aureus, P. aeruginosa, K. pneumoniae* and *C. albicans* respectively (Table 4.5a). Ethanol extract of *Senna alata* produced MIC values of 6.25, 12.5, 6.25, 12.5 and 12.5 mg/ml with respect to *E. coli, S. aureus, P. aeruginosa, K. pneumoniae* and *C. albicans*. Some research reports have indicated that, low MIC values are an indication of high potency of extracts whilst high MIC values are indicative of low efficacy (Majorie, 1999; Doughari and Okafor, 2007). The low MIC values obtained from both the aqueous and ethanol extracts of *Senna alata* suggested that the highly active bioactive compounds were extracted by these solvents. The MIC values for the stem bark of *Lannea barteri* aqueous extract was evaluated and was found to be exhibiting low MIC values of 6.25, 12.5, 6.25, 12.5 and 12.5 mg/ml for E. *coli, S. aureus, P. aeruginosa, K. pneumoniae* and *C. albicans* respectively. The ethanol extract of

Lannea barteri bark was similarly investigated and the following MIC values were observed 6.25, 12.5, 6.25, 6.25 and 12.5 mg/ml for *E. coli, S. aureus, P. aeruginosa, K. pneumoniae and C. albicans* respectively. Validating the more bioactive components from the plants being extracted, the susceptibility of aqueous extract of *Ricinus communis* leaf to the test microorganisms' revealed high activity indicating high potency against the test microorganisms. The observed MIC results of aqueous *Ricinus communis* was found to be 6.25, 3.13, 3.13, 12.5 and 12.5 mg/ml for *E. coli, S. aureus, P. aeruginosa, K. pneumoniae* and *C. albicans* respectively and the ethanol extract of *Ricinus communis* leaf showed MIC values of 6.25, 25, 6.25, 6.25 and 12.5 mg/ml for *E. coli, S. aureus, P. aeruginosa, K. pneumoniae and C. albicans* respectively. The aqueous extract of *Ricinus communis* was found to be the most active exhibiting the highest potency with the lowest MIC value of 3.13 mg/ml against *S. aureus* and *P. aeruginosa* which are Gram positive bacteria. The plants crude extracts of the respective solvents exhibited antimicrobial activity with low MIC values indicating the plant extracts were highly potent with aqueous *Ricinus communis* extract recording the most effective potency.

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The MIC and MBCMFC values of aqueous *Senna alata* leave*s* extract obtained in this current research ranged from 6.25-12.5 mg/ml and 200-300 mg/ml respectively (Table 4.5a). The activity of the plant crude extract against the test microorganisms could be attributed to secondary metabolites in the crude extract such as alkaloids, flavonoids and terpenoids which are known to contribute to disruption of the cell wall and cell membrane as well as interfere with protein and folic acid syntheses. These compounds have significant effect on Gram positive and Gram-negative bacteria as well as fungi, as

expressed in the bactericidal activity which occurred within the concentration range of 200-300 mg/ml.

The ethanol extract of Se*nna alata* investigated against the test microorganisms showed MIC values between the range of 6.25-12.5 mg/ml and MBC/MFC ranged between 200-300 mg/ml (Table 4.5a). The low MIC values observed against the test microorganisms indicate high efficacy of the extracts deduced to be as a result of high bioactive compounds extracted by the solvents. High MIC values may mean low efficacy of extracts against microorganisms as an indication of potential resistance to bioactive compounds (Doughari *et al.,* 2007).

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The stem bark of *Lannea barteri* aqueous extract exhibited low MIC values which ranged between 6.25-12.5 mg/ml and MBC/MFC values ranged between 200-300 mg/ml. The ethanol extract of *Lannea barteri* bark was equally found to have determined MIC values ranging from 6.25-12.5 mg/ml and MBC/MFC values were found in the range of 200-300 mg/ml (Table 4.5b). The activities expressed by these crude extracts of *Lannea barteri* against the respective microorganisms may be due the high potency of the phytochemicals present (Table 4.5b).

The crude aqueous extract of *Ricinus communis* exhibited low MIC values in the range of 3.13-12.5 against the respective microorganisms (Table 4.5c). The MBC and MFC values ranged from 200-400 mg/ml. However, the crude extract exhibited bacteriostatic effect against *E. coli* as it could not effectively kill the microorganism at the concentration of 400 mg/ml. The aqueous *Ricinus communis* had a significant effect in the MIC values against *S. aureus and P. aeruginosa* with lowest MIC value observed at 3.13 mg/ml. The

ethanol crude extract of *Ricinus communis* had MIC activity in the range of 6.25 - 25 mg/ml for the respective microorganisms as shown in table 4. 5c. The observed MBC and MFC were found to be in the range of 200-400 mg/ml of the extracts against the test microorganisms but bacteriostatic activity was observed against *E. coli* at the concentration up to 400 mg/ml. Nishteswar *et al.* (2013), reported the effectiveness of *Ricinus communis* leaf extract as it exhibited antibacterial and antifungal activity in different solvents and further indicated that the aqueous solvent was the most effective. The current research confirms his findings as aqueous extract gave the lowest MIC values.

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4.2.5 Antimicrobial Studies of Formulated Extracts

4.2.5.1 Antimicrobial Studies of Extract - PEG Ointment Formulation

4.2.5.1.1 Senna alata extract– PEG Ointment

The antimicrobial susceptibility test of Extract–PEG Ointment on Gram positive bacterium: *S. aureus,* and Gram-negative bacteria: *E. coli, K. pneumoniae, P. aeruginosa* as well as a fungus, *C. albicans* which are associated with various form of diseases such as skin, wound, genitourinary track among others. Various plants - base ointments were formulated to test for their antimicrobial effect on these microorganisms. The antimicrobial effect of aqueous extracts of *Senna alata* (aqSA) - PEG Ointment on the selected microorganisms showed excellent antimicrobial activity against all the microorganisms with zones of inhibition >25 mm at 200 mg/g. The effectiveness of aqSA-PEG ointment was noticed at the various concentrations tested (Table 4.6a). The

ointment exhibited dose dependence against the entire organisms as the concentration of aqSA-PEG ointment was raised from 50 to 100 mg/g ($p < 0.05$) saw an increase in diameter of zone of inhibition. Similarly, when the concentration was further increase to 200 mg/g, the antimicrobial activity recorded significant ($p > 0.05$) increase against the entire microorganisms tested.

The results of Ethanol extract of *Senna alata* (ESA) -PEG Ointment antimicrobial susceptibility test indicated that, the formulated ointment was potent against all selected microorganisms with zone of inhibition > 24 mm at 200 mg/g. The activity exhibited at varying concentrations showed an excellent antimicrobial effect which could be used as broad-spectrum antimicrobial as found in (Table 4.6a). The ointment expressed dose dependency against *S. aureus*, *P. aeruginosa* ($p < 0.05$) but exhibited dose independency against *E. coli, K. pneumoniae and the fungus, C. albicans,* when the concentration was raised from 50 to 100 mg/g. This could be due to inability of the ointment to diffuse into the media and get in contact with the microorganisms. When the concentration was further increased to 200 mg/g, it significantly increased the activity against *S. aureus, P. aeruginosa* and insignificant against *E. coli, K. pneumoniae*. The trend of activity exhibited here showed that the lower the concentration of the ointment formulation, the higher the antimicrobial activity.

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4.2.5.1.2 Lannea barteri extract– PEG Ointment

When aqueous extract of *Lannea barteri* (aqLB) - PEG Ointment was evaluated against the test microorganisms, it revealed that aqLB - PEG ointment exhibited sensitivity

towards the microorganisms at the various concentrations tested (Table 4.7a). The activity recorded at 200 mg/g showed that the ointment was potent against the entire microorganisms with average inhibition zone >25.5 mm. This indicates that the ointment base could release the active components in the plant extract for smooth contact with the microorganisms. The results further revealed that, aqLB - PEG ointment showed dose dependency as the concentration was raised from 50 to 100 mg/g with a significant increase in the antibacterial activity but recorded no statistically significant ($p > 0.05$) difference against the fungus (*C. albicans*). When the concentration was further increased to 200 mg/g, there was a significant ($p < 0.05$) increase in diameter zone of inhibition against the entire microorganisms indicating dose dependent.

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The ethanol extract of *Lannea barteri* (ELB)-PEG ointment exhibited antimicrobial effect as it inhibited the activity against the microorganisms at varying concentrations studied (Table 4.7b). The ointment expressed a very strong antimicrobial activity against the selected microorganisms with diameters of zones of inhibition greater than 30 mm at the concentration of 200 mg/g. However, the most sensitive formulation, ELB-PEG ointment was active against *K. pneumoniae* with diameter of 35 mm at a concentration of 200 mg/g. The ointment indicated no significant increase in effect on the microorganisms when the concentration was increased from 50 to 100 mg/g. This implies there was no dose dependency. Meanwhile, when the concentration was further increased to 200 mg/g, it recorded significant effect against the test microorganisms indicating that the activity of the ointment increases with an increased concentration. The activity expressed by the ointment could be attributed to enhanced delivery of the bioactive compounds to the test

microorganisms by the delivery agent, PEG. The choice of ointment base is crucial as it influences the release and bioactivity of drugs.

4.2.5.1.3 Ricinis communis – PEG Ointment

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The assessment of antimicrobial sensitivity test against the selected microorganisms revealed that, the entire microorganisms were susceptible to the aqRC–PEG formulation, resulting in zone of inhibition greater than 26 mm at 200 mg/g (Table 4.8a). The activity exhibited by the formulated ointment could be due to the ability of the ointment base to effectively release the bioactive substance for easy contact with the microorganisms and hence high activity. The ointment expressed higher antimicrobial activity than the standard drug used against some of the test microorganisms (Table 4.4a). The results further revealed dose independence against *E. coli, K. pneumoniae* and the fungus*, C. albicans,* whilst it showed statistically ($p < 0.05$) significant difference against *P. aeruginosa* and *S. aureus* when the concentration was increased from 50 to 100 mg/g. Again, when the concentration was raised to 200 mg/g, there was significant effect against the entire microorganisms with the exception of *C. albicans*.

Increasing concentration of the formulated ointment of the extract resulted in increasing zone of inhibition against *E. coli, S. aureus, P. aeruginosa,* and *C. albicans,* but was insignificant ($p > 0.05$) against *K. pneumoniae*. When concentration was raised to 200 mg/g, there was corresponding appreciable increase in the zone of inhibition against the entire microorganisms.

The antimicrobial activity of ERC-PEG Ointment exhibited high potency against test microorganisms. The ointment showed a diameter of zone of inhibition >27 mm at 200 mg/g. It exhibited dose dependency against *S. aureus, P. aeruginosa and K. pneumoniae* as the concentration was raised from 50 to 100 mg/g and insignificant ($p > 0.05$) against *E. coli and C. albicans* (Table4.8b). Also, when the concentration was raised to 200 mg/g, there was significant ($p < 0.05$) effect against *E. coli, S. aureus, P. aeruginosa and K. pneumoniae* and insignificant ($p > 0.05$) against *C. albicans*. The PEG could release bioactive substances for effective activity.

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4.2.6 MIC and MBC of Formulated PEG Ointment

The activity of aqSA-PEG-ointment against the test microorganisms proved to be effective resulting in low MIC values of 3.13, 6.25, 6.25, 6.25 and 3.13 mg/g for *E. coli*, *S. aureus, P. aeruginosa, K. pneumoniae and C. albican*s respectively. The formulated extract also showed bactericidal property against the test microorganisms (Table4.9a). The low MIC values expressed by aqSA-PEG ointment could be attributed to the phytochemicals present in the crude extract as well as the delivery agent capable of releasing the bioactive substances. Antimicrobial effect of ESA-PEG Ointment against the test microorganisms resulted in low MIC values of 6.25, 6.25, 3.13, 12.5 and 6.25 mg/g for *E. coli*, *S. aureus, P. aeruginosa, K. pneumoniae* and *C. albican*s respectively (Table 4.9b). The ointment also expressed both bactericidal and fungicidal effects. In addition, ESA-PEG ointment exhibited a broad-spectrum effect as it was active against both gram positive and gram-negative bacteria as well the fungus tested.

The aqLB–PEG ointment recorded low MICs against the test microorganisms at concentrations of 6.25, 12.5, 6.25, 6.25 and 3.13 mg/g for *E. coli*, *S. aureus, P. aeruginosa, K. pneumoniae, and C. albicans* respectively (Table 4.10a). Both bactericidal and fungicidal activity of the formulated ointment was recorded against the test microorganisms in the range of 200-400 mg/g (Table 4.10a). This report is confirmed by a similar research work by Briskin (2000). The resulting activities of *Lannea barteri* may be attributed to various constituents found in the extract which was able to suppress or inhibit the growth of the microorganisms tested. The phytochemicals present in the plant may be responsible for cytotoxicity towards the test microorganisms (Briskin, 2000).

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The results in table 4.10b indicate that ELB-PEG ointment inhibited the pathogenic microorganisms with MIC values of 6.25, 6.25, 3.13, 6.25 and 6.25 mg/g for *E. coli*, *S. aureus, P. aeruginosa, K. pneumoniae and C. albicans* respectively. This implied the ointment effectively suppressed the growth of both Gram positive and Gram-negative bacteria and fungus at the lowest concentrations tested. The ELB-PEG ointment exhibited both bactericidal and fungicidal activity the against test microorganisms indicating that the formulated extract ointment was potent in killing the pathogenic microorganisms within concentration range of 200-300 mg/g.

The aqRC-PEG ointment exhibited low MIC values against the test microorganisms. This is an indication of how effectively the formulated extract ointment could be used for treatment, especially for topical diseases. The observed antibacterial and antifungal activity of aqRC-PEG ointment could be attributed to bioactive compounds such as flavonoids, alkaloids and terpenoids (Saxena *et al.,* 2013) as well as the ability of the

ointment base to effectively release the secondary metabolites for an easier contact with the microorganisms and hence the activity. The ointment effectively killed the test microorganisms as it exhibited both bactericidal and fungicidal potency at concentration range between 200 - 400 mg/g (Table 4.11a). The presence of secondary metabolites such as: saponins which are said to have anti-yeast, antifungal and antibacterial activities. It is also involved in protecting the body against potential attack by pathogens (Sparg *et al.,* 2004). Antimicrobial and antihypertensive properties of plants are associated with flavonoids which are involved in a wide range of therapeutic applications (Veerachari and Bopaiah, 2011). *Ricinus communis* is reported to be used specially for targeting and destroying cancer cells (Lord *et al.,* 2003).

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The study revealed that ERC-PEG ointment was active against all the test microorganisms. The ointment exhibited high antimicrobial activity with low MIC value of 6.25 mg/g for *E. coli*, *S. aureus, P. aeruginosa, K. pneumoniae, and C. albicans*. Bactericidal and fungicidal activity of the ointment was observed at a concentration range of 200-300 mg/g (Table 4.11b). The demonstrated activity exhibited by the formulated extract may be attributed to potential bioactive components in the ointment as well as the ease with which the delivery agent helped in releasing the bioactive compounds.

4.2.7 Antimicrobial Studies of Plant Extract - Shea Butter Ointment Formulation

4.2.7.1 **Senna alata** ***Extract– Shea butter Ointment***

Plant Extract- Shea butter ointment was formulated and using well diffusion method, the effectiveness of the ointment was tested against the selected microorganisms.

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The aqSA-shea butter ointment revealed antimicrobial activity with zones of inhibition ranging from 10.5 to 12.5 mm at a concentration of 200 mg/g (Table 4.12a). The inhibition activity expressed by the ointment was statistically insignificant ($p > 0.05$) with respect to increasing concentration from 50 to 100 mg/g. Similarly, when concentration was increased to 200 mg/ml the inhibition activity of the ointment against *E. coli, S. aureus, P. aeruginosa and C. albicans* were statistically insignificant but expressed significance against *K. pneumoniae*. The inability of the ointment to give better inhibition zone as compared to the crude extract form and PEG ointment may be attributed to the inability of the Shea butter to effectively release the bound bioactive compounds and hence no contact with the test microorganisms.

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The antimicrobial effect of the ESA-Shea butter ointment formulation showed antimicrobial activity with some inhibition at range of 8 to 24.5 mm at 200 mg/g concentration (Table 4.12b). The ointment showed pronounced activity against *E. coli* at 200 mg/g with zone of inhibition 24.5 mm which moderately expressed increase in activity against *S. aureus, P. aeruginosa, K. pneumoniae and C. albicans*. The results further revealed that, there was no statistically significant difference with regards to increasing concentration of the ointment exhibiting low antimicrobial activity at 100 and 200 mg/g, whilst the Extract-PEG formulation and the crude extract form performed excellently by suppressing the growth of microorganisms at the same concentrations.

4.2.7.2 Lannea barteri–Shea butter Ointment

The antimicrobial effect of aqLB-Shea butter ointment was examined and the results in table 4.13a indicates that, it was able to suppress the growth of the respective microorganisms with zones of inhibition at a concentration of 200 mg/g ranging from 10.5 to14.5 mm. However, the aqLB–Shea butter ointment activity increased as the concentration of the ointment increased from 50 to 100 mg/g against the entire microorganisms. When the concentration was further increased to 200 mg/g there was appreciable increase in diameters of zones of inhibition against *E. coli and K. pneumoniae* which was statistically different ($p < 0.05$) but exhibited no statistical difference against *S. aureus, P. aeruginosa* and *C. albicans* ($p > 0.05$). Here, the activity expressed by the extract-shea butter ointment was insignificant compared with the crude extract alone and that of the extract-PEG formulation which showed higher antimicrobial activity. The inability of aqLB-Shea butter ointment could be the inability of the Shea butter to release the active compounds for antimicrobial effect.

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The ELB–Shea butter ointment showed inhibition against the entire microorganisms with the diameter range between 10.5 to 16.5 mm at concentration of 200 mg/g (Table 4.26). The expressed antimicrobial activity exhibited by ELB–Shea butter ointment was lower than that of the crude extract alone and that of the extract-PEG formulation. The inability of the ointment base to effectively deliver the potent bioactive constituents might have contributed to the low inhibition zone recorded (Table 4.13b). The difference in ointment activity against the microorganisms compared with the standard drug used in this research was insignificant. Increasing activity was significant when the concentration was raised from 50 to100 mg/g except the fungus, *C. albicans,* which did not show any appreciable

activity with respect to increasing concentration. When the concentration was raised to 200 mg/g, activity against some of the microorganisms increased appreciably against *E. coli, S. aureus, P. aeruginosa and C. albicans*. However, increasing concentration showed no statistical difference on *K. pneumoniae* with respect to activity (P > 0.05). Generally, the aqLB-Shea butter ointment formulation performed better in terms of activity against the test microorganism than that of ELB-Shea butter ointment. The difference in activity between the two extract formulations may be due to difference in polarity of the solvents resulting in the different bioactive compounds extracted in the respective solvent.

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4.2.7.3 Ricinus communis–Shea butter Ointment

The effect of aqRC-Shea butter ointment suppressed the growth of the test microorganisms at varying concentrations (Table 4.14a). The aqRC ointment showed high potency against the microorganisms at a concentration of 200 mg/g with zones of inhibition range of 9.5 to 13.5 mm. There was no significant increase in zones of inhibition when the initial concentration was raised from 50 to 100 mg/g against the entire microorganisms. However, there was significant increase in zones of inhibition when the concentration was raised to 200 mg/g and that was applicable to *P. aeruginosa, K. pneumoniae* and *C. albicans,* whilst no such trend was manifested against *E. coli and S. aureus*. Similarly, the activity expressed by aqRC- Shea butter ointment was lower than those expressed by the crude extract alone and that of the extract-PEG formulation.

The expressed antimicrobial effect of ERC-Shea butter ointment can be found in Table 4.14b. The activity exhibited by the ointment at the varying concentrations was more pronounced at the 200 mg/g where the most susceptibility was against *P. aeruginosa* with inhibition zone of 16 mm. The ointment effectively exhibited no dose dependence when the concentration was increased from 50 to 100 mg/g against the entire microorganisms. On the other hand, increasing concentration to 200 mg/g saw a remarkable increase in zones of inhibition against *E. coli and P. aeruginosa* but less pronounced against *S. aureus, K. pneumoniae* and *C. albicans*.

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4.2.8 MIC and MBC of Formulated Shea Butter Ointment

The MIC values of aqSA-Shea butter ointment on the test microorganisms were 25, 25, 25, 12.5 and 12.5 mg/g for *E. coli, S. aureus, P. aeruginosa, K. pneumoniae* and *C. albicans* respectively and showed only bacteriostatic property against the microorganisms tested (Table 4.15a). However, the crude extract alone and the extract-PEG ointment expressed higher activities indicating lower MIC values and exhibiting both bactericidal and fungicidal activity (Table 4.15a).

The ESA–Shea butter ointment produced MIC values of 25, 25, 12.5, 12.5 and 12.5 mg/g for *E. coli, S. aureus, P. aeruginosa, K.* pneumoniae and *C. albicans* respectively. However, it showed only bacteriostatic effect against the selected microorganisms. Similarly, the crude extract alone and the extract-PEG ointment showed higher antimicrobial activities with lower MIC values and exhibited bactericidal properties (Table 4.15b).

The sensitivity of both aqueous and ethanol extract-Shea butter ointment on the test microorganisms revealed that AqLB produced MIC values of 12.5, 12.5, 12.5 and 6.25, 6.25 mg/g for *E. coli, S. aureus, P. aeruginosa, K. pneumoniae* and *C. albicans* respectively (Table 4.16a) whilst ELB gave MICs of 25, 12.5, 12.5, 12.5 and12.5 mg/g for *E. coli, S. aureus, P. aeruginosa, K. pneumoniae* and *C. albicans* respectively (Table 4.16b). Both ointments showed neither bactericidal nor fungicidal effect up to a maximum concentration of 400 mg/g, but exhibited bacteriostatic properties against the test microorganisms. However, the crude extracts alone and in combination with PEG rather showed higher sensitivity with lower MIC values and also showed bactericidal properties against the test microorganisms.

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The aqRC-Shea butter ointment investigated against the selected microorganisms produced MICs of 25, 25, 25, 12.5 and 12.5 mg/g for *E. coli, S. aureus, P. aeruginosa, K. pneumoniae* and *C. albicans* respectively (Table 4.17a). The aqRC-Shea butter ointment indicated that the ointment could rather suppress the activity of the test microorganisms but could not effectively eliminate them as it only exhibited bacteriostatic properties against the entire microorganisms studied (Table 4.17a). The crude extracts alone and that of the extract-PEG ointment showed lower MICs values and bactericidal properties against the test microorganisms (Table 4.8a). The ERC-Shea butter ointment showed activity towards the entire microorganisms as it recorded MIC values of 25, 25, 25, 12.5, and 12.5 mg/g for *E. coli, S. aureus, P. aeruginosa, K. pneumoniae* and *C. albicans* respectively (Table 4.17b). The ointment also expressed bacteriostatic properties as concentrations up to 400 mg/g could only suppress the growth but could not eliminate the microorganisms completely. The crude extract and extract-PEG ointment however,

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expressed lower MIC values and also exhibited both bactericidal and fungicidal activity against the microorganisms.

The activity of ERC-Shea butter ointment against test microorganisms gave MIC values of 25, 25, 25, 12.5 and 12.5 mg/g for *E. coli, S. aureus, P. aeruginosa, K. pneumoniae* and *C. albicans* respectively (Table 4.17b). However, ERC-Shea Butter Ointment could only suppress growth of the microorganisms but could not effectively kill any of the microorganism at a maximum concentration of 400 mg/g used in this research, even though, the formulation exhibited both bacteriostatic and fungistatic property. The crude extract and PEG ointment of ERC on the other hand exhibited bactericidal properties against *S. aureus, P. aeruginosa, K. pneumoniae* and *C. albicans* at a concentration range of 200-400 mg/g but indicated bacteriostatic activity against *E. coli.* Seemingly, the delivery agent used could not sufficiently release the active components for effective antimicrobial activity and thus higher MIC values.

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4.2.9 Synergistic Studies

Drug interaction between known antibiotics and plant extract could be beneficial (that is synergistic or additive effect), deleterious (that is antagonistic) or toxic (Chou and Talalay, 1984). Plants are abundant in nature and have been known to have therapeutic properties, however, no plant derived chemicals have successfully been used clinically as antibiotic (Gibbons, 2004). The in vitro interactions between plant extracts against the selected microorganisms using agar well diffusion method was studied and it was observed that the combined plant extracts showed significantly lower MIC values against the entire microorganisms as compared to the individual extracts (Tables 4.18a to 4.19b).

The lower MIC values which were recorded against the test microorganisms could be attributed to various bioactive compounds present in each extract. The combination of extracts may have resulted in different mechanisms of interaction with the microorganisms that enhance the suppression of the growth of the respective microorganisms. Combination therapy has been around since time immemorial but current drug resistance against infectious microorganism is a great concern and thus the need for alternative drugs.

The Fractional Inhibitory Concentration (FIC) index was applied in the current research, which indicates the lowest concentration of the extracts (Drug A) and (Drug B) in combination that gives no detectable microbial growth after incubation.

FIC indices for the combination of the extracts in the current study were determined and according to Isenberg (1992) and Noor (2016), $\sum FIC \leq 0.5$ describes Synergism, $0.5 < \sum FIC \leq 1$ describes Additive, Indifference if $1 < \sum FIC \leq 4$ and Antagonism as $\sum FIC > 4$.

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FIC indices of aqueous *Senna alata* (aqSA) and aqueous *Lannea barteri* (aqLB) ranged from 0.4992 to 0.5616 (Table 4.20a). It was observed that, two interactive mechanisms between the bioactive constituents of the extracts and the microorganisms had occurred with the prominent being synergistic interactions against *S. aureus*, *P. aeruginosa, K. pneumoniae and C. albicans* resulting in the $\sum FIC \leq 0.5$, whilst additive interaction was observed against *E. coli*. The observed results showed by the combined plant extracts could be that, crude extracts of different phytochemicals inhibited the microorganisms by different mechanisms such as protein synthesis, folic acid synthesis, cell membrane

disruptions, complexing with the cell wall and improvement of efficacy through such interactions (Aiyegoro and Okoh, 2009). Research reports on combination studies have revealed that, drug-drug combinations improve the efficacy of antimicrobials on pathogenic microbes (Stermitz *et al.,* 2000; Aliyegoro and Okoh 2009). It is also possible that less efficacious drugs (antimicrobials) could have been enhanced through combinations (Mabrouk, 2012). The findings in (Tables 4.18a to 4.19b) revealed that there was a significant reduction in the minimum inhibitory concentration (MIC) of the combined extracts compared with the individual extracts or when used alone.

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The combined extracts of ELB and aqSA against the selected microorganisms revealed that the FIC index ranged from 0.4992 to 0.5610. The observed interactions that was more prominent was synergistic effect and was observed against *S. aureus, P. aeruginosa*, *K. pneumonas* and *C. albicans* with ∑FIC ≤ 0.5. Also, the combined interactions between the plant extracts and the microorganisms exhibited additive effect against *E. coli* with ∑FIC between 0.5 and 1 (Table 4.20b). The results demonstrated that the extracts used in the current research could be combined to treat multiple infections because they may have different mode of mechanisms against the selected microorganisms based on the observed extract combination results. In a similar research earlier, Ali and Dixit (2012) observed that, combination of two flavonoids: *Orientin* and *Viceinin* from *Ocimum sanctum* leaves resulted in positive interaction against pathogenic microorganisms.

The combined extract of aqLB and aqRC studied against the selected microorganisms revealed that the combination had lower MIC values than the individual extracts (Table 4.18b). The combined interaction of the plant extract against the selected microorganisms resulted in ∑FIC index ranged from 0.4992 to 0.7790. Synergistic interaction was

observed against *E. coli, K. pneumoniae and C. albicans* with ∑FIC index ≤ 0.5. Also additive interaction was recorded against *S. aureus, P. aeruginosa* with ∑FIC index > 0.5 and ≤ 1 (Table 4.20c). The double attack by the respective plant extract in combination on the microorganisms could have different target sites thereby reducing their mechanisms of resistance.

Synergism resulting from the combination of ethanol *Lannea barteri (ELB)* and aqueous *Ricinus communis (aqRC)* extracts was also studied. The results showed lower MIC values as a result of the effect of the combination compared with the individual extract activity (Table 4.18c). The results further revealed that, ∑FIC index of the combined effect ranged from 0.239744 to 0.779003. The MIC of the combination gave ∑FIC index values ranging between 0.239744 and 0.4992 resulting in synergistic effect against *E. coli, P. aeruginosa, K. pneumoniae and C. albicans* whereas, additive effect was determined against *S. aureus* with ∑FIC > 0.5 and ≤ 1 (Table 4.20d). The combined extract influence on the microorganisms could be due to different phytochemicals present in the crude extracts which might have inhibited the microorganisms by different mechanisms (Duke *et al.,* 2003). There are several reports that indicated that combination of known antibiotics with plant extracts against pathogenic microorganisms has led to a reduction in MIC values (Betoni *et al.,* 2006).

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When the combined effect of aqLB and ESA was investigated against the selected microorganisms, the results revealed that, MIC values were lower than the individual extract activity and thus a positive interaction between the two plant extracts resulted. The results further indicated that the ∑FIC index for each of the test microorganism was 0.4992. There was synergistic effect against *E. coli, S. aureus, P. aeruginosa, K.*

pneumoniae and C. albicans with ∑FIC index being ≤ 0.5 (Table 4.20e). In addition, the results showed that synergistic effect had occurred against the Gram positive and Gram-negative bacteria as well as the fungus. The MIC values were reduced, comparing the combined extracts and that of the individuals, by a number of folds indicating that the combined extracts were more sensitive to the microorganisms than the individual plant extracts.

The combination effect of ELB and ESA against the selected microorganisms resulted in a significant reduction in MIC values as compare when used alone (Table 4.20f). The result indicates that, there was positive interaction of the combined extracts against each of the microorganism and thus resulted in ∑FIC index ranged from 0.4992 to 0.5616. Synergistic interaction was observed against *E. coli, P. aeruginosa C. albicans* with ∑FIC index ≤ 0.5, whilst interaction was observed against *S. aureus*, *K. pneumonia with* ∑FIC> 0.5 and ≤ 1 (Table 4.20f). The use of multiple drugs with different mechanisms or modes of action may directly have effect against single target or a disease and thus treat it more effectively (Chou, 2006).

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The effect of combined extract of aqRC and aqSA was investigated against the test microorganisms. The results showed that, the extracts significantly reduced the microbial activity resulting in lower MIC values when compared to the individual effects. The results further showed ∑FIC index ranged from 0.2808 to 0.5610 with the better interactive effect being synergistic (∑FIC index ≤ 0.5) which was expressed against *E. coli, P. aeruginosa and C. albicans* whilst additive effect against *S. aureus with* ∑FIC index > 0.5 and ≤ 1 (Table 4.20g).

The combined extracts of ERC and aqSA effectively inhibited the growth of the respective microorganisms with a varying reduction of MIC values demonstrating high potency of combined extracts against the test microorganisms. The combinations of the plant extracts (ERC + aqSA) exhibited synergistic (∑FIC index ≤ 0.5) effect against all the test microorganisms: *E. coli, P. aeruginosa S. aureus and P. aeruginosa* and *C. albicans* (∑ FIC index ≤ 0.5) (Table 4.20h). The ∑ FIC index of the combined extract ranged from 0.281 to 0.468 which indicates the best combination of the crude extracts. The activity of the combined extract could be due to multiple compounds found in them and multiple antimicrobial actions ensued. Also, the combined had a broad-spectrum activity as it influenced Gram positive and Gram-negative bacteria as well as the fungus (Table 4.20h).

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The combination of aqLB and ERC demonstrated significant activity against the test microorganisms with a reduction of the MIC values in about 4 folds and thus showed a positive interaction. The observed activity could be attributed to various phytochemicals in the respective plant extracts. The results further revealed that the ∑FIC index for each of the test microorganism was ranged from 0.2928 to 0.5628. The observed interactions showed synergistic effect against *E. coli, P. aeruginosa, K. pneumoniae and C. albicans* with ∑FIC index being ≤ 0.5 (Table4.20i), whilst Additive interaction occurred against *S. aureus* with ∑FIC index > 0.5 and ≤ 1. In addition, the results showed that synergism and additive effects have occurred in Gram positive and Gram-negative bacteria as well as the fungus. Double attack by both plant extracts on different target sites on the test microorganisms could either lead to an additive or a synergistic effect (Esimone *et al.,* 2006).

The results of antimicrobial activity of the combination of ELB and ERC against the test microorganisms showed a decrease in MIC values which is indicative of high potency of the combined extract. The combined plant extract interaction exhibited synergistic effect against *E. coli, S. aureus, K. pneumoniae* and *P. aeruginosa* with ∑FIC index ≤ 0.5, whilst additive effect was recorded against *C. albicans* with ∑FIC index > 0.5 and ≤ 1 (Table 4.20j). Synergistic effect may be attributed to blocking of metabolic pathway (Bassole and Jullian, 2012). On the other hand, additive effect occurs when the activity of the combined action is equivalent to the sum of the activity of each drug when used alone (Bassole and Jullian, 2012)

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Combining plant extracts of aqRC and ESA against the studied microorganisms significantly reduced the respective MIC values and is indicative of positive interactions. The results further showed that, the ∑FIC index ranged from 0.4992 to 0.7790 with synergistic interaction observed against *E. coli, K. pneumoniae and C. albicans* with ∑FIC index ≤ 0.5, whiles additive interaction was observed against *S. aureus and P. aeruginosa* with ∑FIC index > 0.5 and ≤ 1 (Table 4.20k). The use of multiple drugs is capable of increasing the efficacy of therapeutic effect, decreasing the dosage but increasing or maintaining the same efficacy to avoid toxicity, minimizing or slowing down the development of drug resistance and providing selective synergism against target host (Chou, 2006). The results confirm previous research which indicates that synergistic effect resulting from combination of plant extracts and known antimicrobial led to significant reduction in MICs values (Bentoni *et al*., 2006).

The combined extract interaction between ERC and ESA against the selected microorganisms revealed about 4 folds reduction of MIC values when compared to the

individual effect. The reduction in the MIC values was significant as it gave ∑FIC index ranges from 0.2808 to 0.5616. The results of the ∑FIC index showed that the combined extract exhibited synergistic interaction against *E. coli*, *S. aureus, P. aeruginosa and C. albicans* (∑FIC range ≤ 0.5). Meanwhile, additive effect was recorded against *K. pneumoniae* ∑FIC range 0.5 and ≤ 1 (Table 4.20l). This could be as a result of multiple compounds present in the respective extract having different mechanism of action against the selected microbes. Numerous compounds present in the respective plant extracts when used in combination may interfere with the activity of the microorganisms thereby inhibiting the growth. The benefits derived from combinations of drugs which may include interactions of antibiotics or plant extracts or plant extracts and antibiotics have advantages such as increase in efficiency, reduction of undesirable effects, increase in stability or bioavailability of free agent and obtaining an adequate therapeutic effect with relatively small doses when compared with a synthetic medication (Inui *et al.,* 2007; Chanda and Rakholiya, 2011).

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4.2.9.1 Bactericidal effect of combined extracts

The antimicrobial studies conducted on the respective crude extracts on the test microorganisms indicated that, the crude extracts were effective and thus inhibited growth of the microbes. The MBC/MFC of the combined extracts was determined against the test microbes and the results revealed that, the combined extracts effectively suppressed the growth and killing of the microorganisms resulting in bactericidal effects. Bactericidal activity for all the test microorganisms was recorded at 150 mg/ml (Table4.21a to 4.21b).

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Combinations of drugs is said to prevent resistance of emerging treatment and also increase efficacy against disease causing microorganisms (Chanda and Rakholiya, 2011).

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CHAPTER FIVE

5.0 CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

The phytochemicals screened from the plant extracts showed the presence of tannins, saponins, reducing sugars, polyuronoids, terpenoids, flavonoids, alkaloids and anthraquinones. From the current research, the antimicrobial assay indicated that, all the microorganisms were susceptible to the plant extracts used at the various concentrations considered. The present research also indicates that, *Senna alata, Lannea barteri* and *Ricimus communis* extracts inhibited the test microorganisms in vitro at different MIC values. The low MIC values recorded against the test microorganisms indicated high potency of the extracts and thus required small amount of extract to inhibit the microbes.

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The MBC and MFC evaluated showed a range between 200–400 mg/ml of the respective plant crude extracts against the test microbes. The formulated ointments (PEG, Shea butter based) showed antimicrobial activity against the test microbes with PEG ointment indicating higher activity than the Shea butter ointment at the various concentrations studied. The MIC values of PEG ointment were lower values than the Shea butter ointment exhibited. The MBC/MFC of the formulated ointment, up to a concentration of 400 mg/g revealed that PEG ointment showed both bactericidal and fungicidal activity whilst Shea butter ointment was only bacteriostatic. The combination studies revealed synergistic and additive effects with no antagonistic effect and also both bactericidal and

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fungicidal activity against the test microbes. The MBC/MFC of combined extracts for the test microorganisms was recorded at 150 mg/ml.

The activities manifested by the plant extracts against the test microorganisms testify to the fact that, these plants could be used as antimicrobial agents.

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5.2 Recommendations

From the study conducted, the following recommendations are suggested:

1. Other parts of the respective plants should be investigated to determine their effectiveness.
2. Toxicity of the extracts plants should be investigated to determine safety indices of the extracts.
3. The plant extract- PEG ointment should be explored in vivo to determine the effectiveness of the ointments for possible use in treating infectious diseases.
4. Combination studies of the plant extracts should be carried out to determine their potency against other types of multi-cellular fungi and Gram positive bacteria.
5. Isolation, characterization and identification of bioactive compounds found in the respective extracts should be investigated.

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APPENDICES

APPENDIX

Table 1a: Antimicrobial susceptibility test (mm) of methanol *Ricinus communis* on microorganisms

	Methanol extract of *Ricinus communis* inhibition zone in mm		
Test organism	50 mg/	100 mg/ml	200 mg/ml
E. coli	16.0±2.83	17.5±2.12	24.0±1.41
S. aureus	17.5±0.71	18.5±2.12	21.5± 2.12
P. aeruginosa	19.5±2.12	30.5±0.71	31.0 ±1.41
K. Pneumoniae	24.0±1.41	26.0±1.41	29.0 ±1.41
Fungus			
C. albicans	26.0 ±1.41	26.0±2.83	28 ± 2.83

The antimicrobial test in table 1a revealed the plant extract inhibited the test organisms

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Table 1b: Antimicrobial susceptibility test (mm) of petroleum ether *Ricinus communis* Organisms

	Petroleum ether extract of *Ricinus communis* inhibition zone in mm		
Test organism	50 mg/ml	100 mg/ml	200 mg/ml
E. coli	16.0 ± 0.00	18.5 ± 0.71	21 ± 2.83
S. aureus	15.5 ± 0.71	18.5 ± 0.71	20 ± 1.41
P. aeruginosa	14.5 ± 0.71	17.0 ± 2.83	21 ± 1.41
K. Pneumoniae	14.5 ± 0.71	24.0 ± 1.41	29 ± 1.41
Fungus			
C. albicans	13.5 ± 0.71	14.5 ± 0.71	17 ± 1.41

The experimental works showed that extract had effect as it inhibited the respective microorganisms as shown in table 1b.

Table 1c: Antimicrobial susceptibility test (mm) of ethyl acetate *Ricinus communis* on microorganisms

	Ethyl acetate extract of *Ricinus communis* inhibition zone in mm		
Test organism	50 mg/ml	100 mg/ml	200 mg/ml
E. coli	11.5 ± 0.71	15.0 ± 1.41	24.5 ± 0.71
S. aureus	12.0 ± 1.41	16.0 ± 1.41	25.0 ± 1.41
P. aeruginosa	14.5 ± 2.12	16.0 ± 1.41	17.0 ± 1.41
K. Pneumoniae	14.5 ± 0.71	22.0 ± 0.71	25.0 ± 1.41
Fungus			
C. albicans	11.0 ± 1.41	14.0 ± 1.41	22.0 ± 1.41

The ethyl acetate extract of *Ricinus communis* equally suppressed the growth of organisms as observed in table 1c.

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Table 2a: MIC and MBC of the plant extract *Ricinus communis* on microorganisms (mg/ml)

	Aqueous *Ricinus Communis*		Methanol *Ricinus Communis*	
Test Microorganism	MIC	MBC	MIC	MBC
E. coli	6.25	UD	6.25	400
S aureus	3.13	400	3.13	UD
P. aeruginosa	12.5	200	12.5	200
K pneumoniae	12.5	400	12.5	UD
Fungus		MFC		MFC
C albicans	12.5	300	12.5	200

MIC = Minimum inhibitory concentration, MBC=Minimum Bactericidal Concentration, MFC=Minimum Fungicidal Concentration. UD= Undetected

Both extracts expressed activity against the test microorganisms with low MIC values and bactericidal and bacteriostatic properties as shown in table 2a.

Table 2b: MIC and MBC of the plant extract *Ricinus communis* on microorganisms (mg/ml)

	Ethanol *Ricinus Communis*		Petroleum Ether *Ricinus Communis*	
Test Microorganism	MIC	MBC	MIC	MBC
E. coli	6.25	UD	6.25	UD
S aureus	3.13	400	3.13	200
P aeruginosa	12.5	400	12.5	400
K pneumoniae	12.5	UD	12.5	300
Fungus		MFC		MFC
C albicans	12.5	300	12.5	300

MIC = Minimum Inhibitory Concentration, MBC=Minimum Bactericidal Concentration, MFC=Minimum Fungicidal Concentration. UD= Undetected at 400 mg/ml

The respective extracts of *Ricinus communis* in table 2b showed that both extracts are sensitive to the test microorganisms with low MIC values and its bactericidal properties.

The ethyl acetate extract showed activity against the entire selected microorganisms with low MIC values and bactericidal properties as well.

Table 2c: MIC and MBC/MFC of the plant extract *Ricinus communis* on microorganisms (mg/ml)

	Ethyl acetate *Ricinus Communis*	
Test Microorganism	MIC	MBC
E.c.oli	6.25	300
S. aureus	3.13	400
P. aeruginosa	12.5	400
K. pneumoniae	12.5	UD
Fungus		MFC
C. albicans	12.5	300

MIC = Minimum Inhibitory Concentration, MBC= Minimum Bactericidal Concentration, MFC=Minimum Fungicidal Concentration. UD= Undetected at 400 mg/ml

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