THE ACTIVITY OF *COMMIPHORA MYRRH* AGAINST MICROORGANISMS RECOVERED FROM WOUNDS

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A Thesis Submitted For the Fulfillment of the Requirement for Master Degree in Microbiology

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June 2008

قال تعالي:

وَالْبَلَدُ الطَّيِّبُ يَخْرُجُ نَبَاتُهُ بِإِذْنِ رَبِّهِ وَالَّذِي خَبُثَ لاَ يَخْرُجُ
إِلاَّ نَكِداً كَذَلِكَ نُصَرِّفُ الآيَاتِ لِقَوْمٍ يَشْكُرُونَ)

صدق الله العظيم

(سورة الأعراف، الآية ٥٨)

DEDICATION

To my Mother and my Aunt

To my Husband

For their abundant support

To my lovely sons

Ahmed & Mohamed & Omar

Whom I had taken their time

Sarah

Acknowledgments

First of all unlimited thanks to ALLAH for giving me strength and power to complete this study.

Appreciable thanks to **Dr. EL Amin ELnima** (Department of Microbiology, Faculty of Pharmacy, University. of Khartoum), my major supervisor, for his genuine guidance, valuable advice and unfailing encouragement during the period of this work.

I would like to thank and express my gratitude to **Dr.Aisha Zoheir Almagboul**, Medicinal and Aromatic Plants Research Institute, National Center for Research (MAPRI) for giving me much of her time for suggestions of this work.

I would like to express my deep thanks to my colleagues especially Mr. **Mohamed Abdel Rahman 'Garja'** senior technologist in Epidemiology department and Miss. **Asma Hassan** & Miss **kawthar Abd Algalil** for her patience and tolerance in organizing this work & those in Microbiology Lab in National Health Laboratory for their financial support.

My gratitude to the staff of National Center for Research, from Departments of Microbiology and Phytochemistry for their kind help during the practical work.

Finally my deep thanks go to my current colleagues whom I didn't mention for their assistance during the period of this work. May Allah bless them all and I thank Allah for all my achievements.

ABSTRACT

This study done in Khartoum state, Clinical isolates were recovered from wounds of Sudanese patients visiting Khartoum Teaching Hospital and National Health Laboratory during January 2005 – September 2007. A total of 200 bacterial strains were isolated and identified.

The study showed that 62 of the isolates were *Staphylococcus aureus*, 44 *Escherichia coli*, 34 *Pseudomonas aeruginosa*, 30 *Klebsiella pneumoniae,* and 30 *Proteus vulgaris*. The study proved the volatile oil extract from *Commiphora myrrh* effective on clinical isolates especially against *Staphylococcus aureus* .

The broth dilution method was used for determing the activity of the extract against standard organisms and clinical isolates. Five concentrations (100, 50, 25, 12.5, 6.25mg/ml and control) of purified volatile oil of *Commiphora myrrh* were tested to determine their effects against standard organisms and clinical isolates that were recovered from patient wounds. The results revealed that the highest activity was shown against *Staphylococcus aureus*. Both 100 mg/ml and 50mg/ml (100%) inhibited the growth of all species. In contrast 6.25mg/ml showed no activity for all organisms. The effect of 25 mg/ml and 12.5mg/ml varied between different species.

The study proved that the antibacterial activity of the volatile oil extract from *Commiphora myrrh* against clinical isolates was high. These findings should stimulate the search for novel natural antibacterial agent.

المستخلص

هذه الدراسه اجريت في ولاية الخرطوم تم عزل ٢٠٠ عينة بكتيرية من مرضى سودانيين جمعت عشوائيا من المعمل القومي الصحي ومستشفى الخرطوم التعليمي. في الفترة من يناير ٢٠٠٥ إلى سبتمبر٢٠٠٧

تم عمل تنقية والتعرف على السلالات البكتيرية المعزولة . اثبتت الدراسة ان هناك : (٦٢ عينة من العنقودية الذهبية ,٤٤ عينة من الإشريكية القولونية ,٣٠ عينة من الكليبسيلا الرئوية .٣٤ عينة الزائفة الزنجارية ,٣٠ عينة من المتقلبة الرائعة).
لقد توصلت الدراسة الى ان للزيت الطيار المستخلص من المر الحجازي أثر فعال ضد الأنواع البكتيرية المذكورة وكان أكثر فعالية ضد العنقودية الذهبية.

كما تم أيضا دراسة تأثير مستخلص الكحول المائي والزيت الطيار تحت خمسة تراكيز مختلفة ١٠٠ , ٥٠ , ٢٥ , ١٢,٥ , ٦,٢٥ ملجم/مل + تحكم .

لقد عكست نتائج اختبار حساسية البكتيريا ان الزيت الطيار له تأثير ايجابي واضح ضد كل الأنواع البكتيرية المعزولة. أوضحت الدراسة أن اعلى نشاط كان في العنقودية الذهبية ,وجد ان التركيز١٠٠و ٥٠ ملجم/مل ثبط النمو البكتيري في جميع الأنواع البكتيرية المعزولة بنسبه ١٠٠%, وان التركيز ٦,٢٥ ملجم/مل لم يظهر اي نشاط واضح ضد اي من الأنواع البكتيرية المعزولة, والتركيز ١٢,٥ و٢٥ ملجم/مل كان لهما تأثير متغير على الأنواع البكتيرية المعزولة.
أثبتت الدراسة أن الذي للزيت الطيار المستخلص من المر الحجازي ذو أثر فعال و عا لى ضد كل النواع البكتيرية المذكورة.

LIST OF ABBREVIATIONS

ATCC	**American Type Culture Collection**
C.m	*Commiphora myrrh*
DNAse	DeoxyriboNucleicease
D.W	Distilled water
E.coli	*Escherichia coli*
K	*Klebsiella pneumoniae*
KIA	Kligler-Iron agar
MBC	Minimum *Bactericidal* Concentration
MIC	Minimum Inhibitory Concentration
n	Number
Ps	*Pseudomonas aeruginosa*
Pr	*Proteus vulgaris*
S	*Staphylococcus aureus*
V.P	Voges –Proskauer
UTI	Urinary Tract Infection
WHO	World Health Organization
Cfu	Colonies-forming units
CLED	Cystine-lactose-Electrolyte- Deficient
H_2S	Hydrogen sulfide

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CHAPTER ONE

1. Introduction

The interest in the study of medicinal plants as a source of pharmacologically active compounds has increased worldwide. It is recognized that in some African countries including Sudan, plants are the main medicinal source to treat infectious disease. Over the past few years, it has been increasingly evident that the production of medicinal plants is gaining economic importance particularly for some tropical African countries.

In Sudan a wide range of medicinal plants has been used in our Traditional Medicine for treatment of febrile patients. Large number of plants is used for the treatment of various ailments. The medicinal value of those plants was recognized since ancient times. These several natural products were obtained from medicinal plants. The natural products that can not be used as such, have offered a lead to the development of various pharmaceutical analogues or derivatives (Phillips, 1992).

Myrrh is an economically and culturally, valuable product obtained from *Commiphora myrrh* tree. It is an important natural plant product used in several industries that include pharmaceuticals, cosmetics, perfumery and others. It has several local applications in medicinal, hygienic, and insecticide areas that could be developed through research. It is widely used in traditional medicines of several countries for treatments of a wide variety of infectious diseases.

1.1 Justification:

In spite of the significant medicinal and anti bacterial behavior of *Commiphora* species and the attention gained worldwide, very little research was conducted in Sudan to understand the behavior of this species.

1.2 Objectives:

1. To investigate the antibacterial activity of *Commiphora myrrh.*
2. To determine the minimum inhibitory concentration (MIC) of *Commiphora myrrh*

against standard organisms and clinical isolates.

CHAPTER TWO

2. Literature Review

An organism living and multiplying inside the body is termed a parasite; the body in instance is referred to as a host. When harmless to the host the parasite is termed a commensal. When harmful, it is a pathogen; however, under certain conditions, commensals may become pathogens and rarely pathogens may assume a commensal role. Organisms living on dead matter are termed saprophytes. When both host and parasite mutually benefit; the association is often called symbiosis. This same term is used by some authorities irrespective of whether benefit occurs to either partner but satellitism is the more correct term in these instances.

If microbes penetrate the body surface, enter body tissue and multiply, infection is said to have occurred .An infection which causes noticeable impairment of the body function is called an infectious disease. A disease is a process resulting from the interaction between host and pathogen .The outcome of this relationship depends not only on the pathogenicity or virulence of the parasite but also on the resistance or susceptibility of the host .A pathogen must first gain access to the host tissues and multiply before producing disease. To do this, the organism must penetrate the surface, such as the skin which normally acts as a barrier. Many pathogens are helped at this early stage by attaching on to specific receptor on the cell surface and indeed many remain at the surface where they multiply, producing a spreading infection (Baker and silverton., 1980).

2.1 Classification of Hazardous Groups:

Organisms are classified into four hazardous groups

Group 1:

An organism that may cause human disease.

Group2:

An organism that may cause human disease and which might be hazardous to laboratory workers, but is unlikely to spread in the community. Laboratory exposure rarely produces infection and effective prophylaxis or effective treatments are usually available.

Group 3:

An organism that may cause human disease and that presents a serious hazard to laboratory workers. It may present a risk of spread in the community, but there is usually effective prophylaxis or treatment available.

Group 4:

An organism that causes severe human disease and is a serious hazard to laboratory workers. It may present a high risk of spread in the community and there is usually no effective prophylaxis or treatment (Baker and Silverton., 1980).

2.2 Infection:

A term considered by some to mean the entrance, growth, and multiplication of a microorganism (pathogen) in the body of a host, resulting

in the establishment of a disease process. Others define infection as the presence of a micro organism in host tissues whether or not it evolves into detectable pathologic effects. The host may be a bacterium, plant, animal, or human being, and the infecting agent may be viral, rickettsial, bacterial, fungal, or protozoan. (Encyclopedia of science and technology. 2005).

2.2.1 Pathogenesis of Infection:

The pathogenesis of bacterial infection includes initiation of the infectious process and the mechanisms that lead to the development of signs and symptoms of disease. Characteristics of bacteria that are pathogenic include transmissibility, adherence to host cells, invasion of host cells and tissues, toxigenicity, and ability to evade the hosts, immune system. Many infections caused by bacteria that are commonly considered to be pathogenic are in apparent or asymptomatic. Disease occurs if the bacteria or immunologic reactions to their presence cause sufficient harm to the person (Jawetz *et al*., 2004).

2.3 Skin:

The skin, which covers and protects the body, is the body first line of defense against pathogens. The skin is an inhospitable place for most microorganisms because the secretions of the skin are acidic, and most of the skin contains little moisture. Presently it was found that perspiration contains an antimicrobial peptide which the discoverers named dermicidin that is effective against a wide spectrum of bacteria more over much of the skin is exposed to radiation which discourages microbial life .Some parts of the body, however, such as the arm pit, have enough moisture to support relatively large bacterial populations and other regions, such as the scalp,

support rather small numbers of microorganisms. The skin is a physical as well as an ecological barrier and it is almost impossible for pathogens to penetrate it .However some can enter through skin breaks that are not readily apparent and the larval forms of a few parasite can penetrate intact skin (Tortora *et al*., 2004).

The skin serves as a barrier between the internal organs and external environment. Skin is not only subjected to frequent trauma and thereby is at frequent risk of the infection but also it can reflect internal disease (Bettya and Halic. ,2002).

2.3.1 Structure and Function of the Skin:

2.3.1.1 Anatomy of the Skin:

From inside out the skin is divided into three distinct layers: the subcutaneous tissue, the dermis and the epidermis .The subcutaneous tissue lies beneath the dermis and is rich in fat deeper hair follicles and sweat glands originate in thin fascial membranes that cover muscles, ligaments and other connective tissues. The fascia serves as a barrier to infection for the deeper tissues and organ of the body.

Above the subcutaneous tissue and fascial membranes lies the dermis which comprises dense connective tissue that is rich in blood and nerve supply. Shorter hair follicles and sebaceous (oil producing) glands originate in the dermis .Finally the epidermis which is the outer most layer of the skin is made of layered squamous epithelium. Hair follicles, sebaceous glands and sweat glands open to the skin surface through the epidermis (Bettya and Halic., 2002).

2.3.1.2 Function of the Skin:

The skin is the body's largest and thinnest organ. It forms a self-repairing and protective boundary between the body's internal environment and an often hostile external environment.

Skin plays a crucial role in the control of the body temperature, excretion of water and salts, synthesis of important chemicals and hormones, and as a sensory receptor. Of significance, the skin has an important protective function by virtue of the epidermis outer most epithelial layer, which comprises cell containing keratin a water-repellent protein .The skin normal microbial flora, pH, and chemical defenses also help to prevent colonization by many pathogens (Bettya and Halic., 2002).

2.3.1.3 Infections of the Skin:

Approximately 15% of all patients who seek medical attention have either some skin disease or skin lesion, many of which are infectious. Many different bacteria, fungi, and viruses may be involved. Also, these infections can result from one or several causative agents. Because of this great diversity of etiologic agents and the complexity of these infections, only the most common infections involving the skin and subcutaneous tissues are addressed.

Skin infections can arise from the invasion by certain organisms from the external environment through breaks in the skin or by organisms that reach the skin through the blood as part of a systemic disease. In some infections such as staphylococcal scalded-skin syndrome, toxins produced by the bacteria cause skin lesions. In others, lesions can also result from the host’s immune response to microbial antigens.

Because of the diversity of etiologic agents, clinicians will often describe the appearance of skin lesions to microbiologists for possible input as to appropriate culture techniques. The physical characteristics of the lesions can indicate the need for smear, culture, biopsy or surgery for instance (Bettya and Halic., 2002).

Skin protects the body from infection .Breaks in the skin can occur through punctures, abrasions or laceration. Healthy individuals can develop infections through wounds in the skin. However, it is more likely that persons with underling immune system problems will develop wound infections if a break in their skin occurs

(Torpy *et al*., 2005).

Although the skin is generally inhospitable to most microorganisms, it supports the growth of certain microbes which are established as part of the normal microbiota. On superficial skin surface, certain aerobic bacteria produce fatty acids from sebum.

These acids inhibit many microbes and allow better adapted bacteria to flourish. Microorganisms that find the skin a satisfactory environment are resistant to dry conditions and to relatively high salt concentrations. The skin normal micro biota contains relatively large numbers of gram +ve bacteria, such as staphylococci and micrococcus. Some of these are capable of growth at sodium chloride concentration of 7.5% or more .Scanning electron micrographs show that bacteria on the skin tend to be grouped into rashes &lesion on the skins which do not necessarily indicate an infection of the skin. However, many systemic disease affecting internal organs are manifested in skin lesions .Variations in these lesions are often useful in describing the symptoms of the disease (Tortora *et al*., 2004).

2.3.1.4 Microbial Disease of the skin:

Microbial Disease		
Bacterial disease	**Pathogen**	**Characteristics**
Impetigo	*Staphylococcus aureus, Streptococcus pyogenes*	Superficial skin infection , isolated pustules
Folliculitis	*Staphylococcus aureus*	Infection of hair follicle
Toxic shock syndrome	*Staphylococcus aureus*	Fever ,rash, shock
Necrotizing fascitis	*Streptococcus pyogenes*	Extensive tissue destruction
Erysipelas	*Streptococcus pyogenes*	Reddish patches on the skin ;often with high fever
Pseudomonas dermatitis	*Pseudomonas aeruginosa*	Superficial rash
Otitis externa	*Pseudomonas aeruginosa*	Superficial infection of external ear canal
Acne	*Propionibacterium acnes*	Inflammatory lesions originating with accumulations of sebum that rupture a hair follicle
Viral disease	**Pathogen**	**Characteristics**
warts	*Papillomavirus spp*	A horny projection of the skin formed by proliferation of cells
Small pox	*Small pox (variola)virus*	Pustules that may be nearly confluent on skin ;systemic viral infection affects many internal organs
Chickenpox	*Varicella-zoster virus*	Vesicles in most cases confined to face ,throat ,and lower back

Shingles	*Varicella-zoste rvirus*	Vesicles similar to chickenpox; typically on one side of waist ,face and scalp, or upper chest
Herpes simplex	*Herpes simplex virus type 1*	Most commonly as cold sores vesicle around mouth, can also affect other areas of skin and mucous membranes.
Measles	*Measles virus*	Skin rash of reddish macules first appearing on face and spreading to trunk and extremities.
Rubella (German measles)	*Rubella virus*	Mild disease with a rash resembling measles, but less extensive and disappears in 3 days or less
Fifth disease (erythema infectiosum)	*Human parvovirus B 19*	Mild disease with a facial rash
Roseola	*Human herpes virus*	Childhood disease; high fever followed by body rash
Fungal Diseases	**Pathogen**	**Characteristics**
Ringworm (tinea)	*Micros porum ,Trichophyton, Epidermophyton spp*	Skin lesion of highly varied appearance ,on scalp may cause local loss of hair
Sporotrichosis	*Sporothrix schenckii*	Ulcer at site of infection spreading into nearby lymphatic vessels

Candidiasis		Symptoms vary with infection site; usually affects mucous membranes or moist areas of skin.
Parasitic infestation	**Pathogen**	**Characteristics**
Scabies	*Sarcoptes scabiei(mite)*	Papules due to hypersensitivity reaction to mites
Pediculosis (lice)	*Pediculus humanus capitis*	Itching

(Tortora *et al.,* 2004).

2.4 Wounds:

2.4.1 Definition:

Wound is a break in the skin that can occur through punctures (like a nail or a thorn), abrasions (scrapes or scratches), or lacerations (rips in the skin tissue).Healthy individuals can develop infections through wounds in the skin. However, it is more likely that person with underlying immune system (the body's ability to fight infection) problems will develop wound infections if a break in their skin occurs (Torpy *et al*., 2005).

A wound is a breach in the normal tissue continuum, resulting in a variety of cellular molecular squeals. Wounds may be accidental, or as a result of planned surgical interventions of many different types in all the tissues of the body. The term" wound" is generally applied to more superficial forms of tissue damage whereas" injury "is used for damage to deeper structures (Charles *et al*., 1995).

Wounding has a variety of effects on the tissues:

1 Mechanical:

Separation of functional structures, such as blood vessels resulting in bleeding. Deformation occurs due to tissue tensions resulting in the gaping of skin wounds.

2 Biological:

Effects commence immediately, as in the resulting inflammatory response from the wounding process.

3 Secondary:

Effects occur at a later stage, such as infection (Charles *et al*., 1995).

Clinical features of wounds:

Signs of wound infections:

(Charles *et al*. 1995), listed the following wound infection signs

- Bleeding
- Pain
- Loss of function
- Swelling
- Inflammation

2.4.2 Risk Factors of wound infection:

(Torpy *et a*l., 2005) reported the following as risk factors.

- Old age
- Immune system disorders
- Cancer, human immunodeficiency

- Virus infection, and malnutrition,
- Diabetes mellitus
- Paralysis or other limited mobility
- Hospitalization, which increases risk for infection by
- Organisms that are resistant to antibiotics

2.4.3 Complications of wound infection:

- Death of surrounding tissue, including muscle, connective tissue, or bone, which may require surgical debridement.
- Spread of the infection to the bloodstream, involving other organs.
- Septic shock, a critical illness involving the whole body, which may require intensive care and life support and lead to multiple organ failure or death (Torpy *et al*., 2005).

2.4.4 Classification of wounds:

Acute

a) Closed wounds
   - bruise / contusion
   - haematoma

b) Open wounds
   - puncture wounds and bites
   - Abrasions and friction burns
   - Lacerations
   - Sharp
   - bursting type

c) Complex

- Crush / avulsion
- Internal organs
- War wounds and gunshot injuries
- Tissue loss

d) Injuries to special tissues

- Fat
- Muscle
- Bone
- Nerve
- Artery
- Vein.

Chronic wounds

- Ulcers
- Pressure sores (Charles *et al*., 1995).

2.4.6 Causative agent of wound infections:

Staphylococci are the most common causative agents of wound infection (30%-50%) other organisms involved in wound infections include, *klebsicalla spp, Pseudomans aeruginosa, E.coli , Bacteriods spp, streptococci anaerobic, Clostridium perfringens, and Entercocc*i .Wound infection can be caused by Vibrio vulnifcus which is a halophilic marine vibrio that produced infection (Elagal , 1994).

2.4.6.1 Organisms that cause wound Infection (Cheesbrough 2000)

Gram P+ve	**Gram N-ve**
Staphylococcus aureus.	*Klebsiella* spp
Streptococcus pyogenes	*E.coli*
Enterococci	*Pseudomonas* spp
Clostridium spp	*Proteus* spp
Actinomycetes	*Bacteroides fragilis* group

2.5 Laboratory Diagnosis:

A sample of material, such as pus or a portion of tissue, is taken from the wound, placed in a sterile container, and sent to the laboratory. In the laboratory, this material is spread over the surface of several different types of culture plates and placed in an incubator at 37ºc for one to two days (Pagana *et al*., 1998).

A skin or wound culture is a test to detect and identify organisms (bacteria or fungi) that may be infecting the skin or a wound. If no organisms grow, the culture is negative. If organisms that can cause infection (pathogenic organisms) grow, the culture is positive. The type of organism will be identified with a microscope, chemical tests, or both (Nissl and David., 2004).

The first step in identification of bacteria is a Gram stain which differentiates into G+ve and G-ve. If many white blood cells and bacteria are seen, it is an early confirmation of infection (Pagana *et al*., 1998).

A sensitivity test, also called an antibiotic susceptibility test, is also done. The susceptibility of bacteria to different antibiotics is carried to determine the best choice for treating the infection (Pagana *et al*., 1998).

2.6 Wound Treatment:

Current treatment using drugs may involve the use of irrigating solutions, compounded drug preparations and dressings. It is important to understand the role of the wound eschar and the factors impacting wound healing in therapeutic strategies to enhance wound healing. The wound eschar debries itself is a part of the wound healing
Process. The wound color will change from black to yellow to red to granular red during healing; generally a red/yellow/black color. Designation is used to describe the wound at each visit. Observations should be made daily for any change in the wound eschar. Complications and factors impacting wound healing include dryness, swelling, infection, incontinence, dead tissue, age, body type (i.e. obesity since fatty tissue has less blood supply), nutrition, vascular insufficiencies, suppressed immune system and chronic diseases. These factors will determine the therapeutic approach or changes during therapy (Isago *et al*., 2003).

2.6.1 Drugs Used to Treat Wounds:

Agents generally used to treat wounds include anti-infective (Hydrogen peroxide, sodium hypochlorite), antibiotics (aminoglycosides, Penicillins, cephalosporins, metronidazole), granulation stimulants (phonation), enzymes for debridement, anesthetics (lidocaine), and moisture

enhancers (dressings, humectants). In addition to preventing and treating infection, anti-infectives can, also be used to minimize odor emanating from some wounds. Many of these ingredients are illustrated in the formulations presented (Isago *et al*., 2003).

2.7 Medicinal Plants as source of antimicrobial agent:

Plants may represent a potential source of antimicrobial agents as evidenced by the huge number of studies dealing with the anti-microbial activities of medicinal plants (Al Magboul, 1998).

In all countries of the world there exists tradition as related to the health of humans and animals. According to the World Health Organization (WHO) knowledge and the definition of traditional medicine may be summarized as the sum total of all the knowledge and practices, whether explicable or not used in the diagnosis, prevention and elimination of physical, mental or social imbalance and relying exclusively on practical experience and observation handed down from generation to generation, whether verbally or in writing. Traditional medicine might also be considered as a solid amalgamation of dynamic medical know-how and ancestral experience (UNESCO, 1994).

Increased utilization of indigenous plant medicines in developing countries became a World Health Organization Policy in 1970s.The World Health Organization has estimated that 80%or more of the inhabitants of the world rely primarily on traditional medicine for primary health care needs. It can safely be presumed that a major part of traditional medicine involves the uses of plants and /or their derived active principles. Though their use is not always verified by the scientific means (Ibrahim, 1992); a large number of

plants are used for the treatment of various ailments. The medicinal value of those plants was recognized since ancient times. There are several natural products obtained from medicinal plants that can not be used as such, but have offered a lead to the development of various pharmaceuticals analogues or derivatives (Phillips, 1992).

2.7.1 Antimicrobial Activity of Medicinal Plants worldwide:

A large number of plants are used for the treatment of various ailments. The medicinal value of those plants was recognized since ancient times. There are several natural products obtained from medicinal plant which can not be used as such, have offered a lead to the development of various pharmaceuticals as analogues or derivatives. Antimicrobial agents may be defined as those chemical substances that interfere with the growth and activity of micro organisms. Generally the term denotes inhibition of microbial growth (biostatic) and /or microbial destruction (biocidal). Such terms as antibacterial or antifungal are frequently employed to refer to activities against specific groups of micro organisms (Mona, 2000).

One hundred and one crude extracts were obtained from various plant parts (leaves, roots, stems, flowers, seeds, or fruits) of 59 species representing mostly the plant families Scrophulariacaceae and Acanthaceae. These were investigated for their antimicrobial properties against *E.coli*, *Pseudomonas aeruginosa*, *Staph aureus* and *C.albicans* (using agar disk diffusion assays). Plants were selected using ethanol botanical and chemotaxonomic information .Growth inhibitor activity against one or more of the test species was detected in >40% of these sample (Meurer-Grimes *et al*., 1996).

Ali-Shtayeh *et al*., (1998) investigated ethanolic and aqueous extracts of 20 Palestinian plant species, used in folkloric medicine for treatment of dematomucosal infections, for their antimicrobial activities against 5 bacterial species *E.coli, S.aureus, Ps.aeruginosa, K. pneumoniae, P.vulgaris.*They found that 90% of the plants tested have anti-microbial activity.

Encarnacion-Dimayuga *et al*., (1998) screened ethanol extracts of 109 plants reported to be used in traditional medicine of Baja California Sur (Mexico) for anti-microbial activity against *S.aureus*, *B.subtilis*, *S.faecalis*, *E.coli* and *C.albicans*. Out of these, 64 were active against one or more test organisms.

Martinez *et al*., (1996) Screened 23 extracts of 12 Cuban medicinal plants species for anti-bacterial activity against. *S.aureus*, *B.subtilis*, *E.coli* and *C.albicans*, *Ps.aeruginosa*. The result showed that 9 extracts (from 7 species) were active against Gram+positive bacteria, but only two of these were also active against Gram-negative bacteria.

2.7.2 Medicinal plants in Africa:

In Africa, traditional healers and remedies made from plants play an important role in the health of millions of people. The relative ratios of traditional practitioners and university trained doctors in relation to the whole population in African countries are revealing.

It is important to note that even in contemporary urban Africa, there is no doubt about the efficacy of herbal medicine. Many Africans, especially

rural people and the urban poor, rely on the use of herbal medicine when they are ill. In fact, many rural communities in Africa still have area where traditional herbal medicine is the major and in some cases the only source of health care available. Thus there can be no doubt about the acceptability and efficacy of herbal remedy within African society (Sofowora, 1982).

In Africa and other developing countries, traditional medicines from plants continue to form the basis of rural medical care. This is due to the fact that these medicines are easily available and cheap.

There is no doubt that tropical flora are constantly exposed to attack by various parasites such as viruses ,bacteria, fungi ,protozoa and insects and confronted with much harsher conditions for survival than their temperate counterparts . This necessarily leads to efficient built- in defence mechanisms and it is presumable for this reason that African tropical flora offer a rich and intriguing source for isolating natural products possessing medicinal properties (Almagboul, 1992).

Desta (1993) assessed 63 plant species of Known therapeutic value in Ethiopian traditional medicine for their potential activity against *S.aureus, E.coli, P.vulgaris, P.aeruginosa, K.pneumoniae* and *C.albicans*. All species were active against at least one microorganism. The traditional therapeutic indications of some species were positively correlated with their anti-microbial activities.

Different extracts of 10 medicinal plants collected from Egypt screened by Ashour and Kheiralla (1995) for their anti-microbial activities against 7 bacteria and 6 fungi.Most extracts showed marked activity against most of the organisms tested.

2.7.3 Medicinal plants in Sudan:

In Sudan temperature as well as the water holding capacity of the soil varies from region to the other. This unique variable climate gives the opportunity to the growth of 3156 plant spp belonging to 1137 genera and 170 different families (Andrews, 1956). Plants used in Sudanese traditional medicine were investigated and out of 135 extracts of 31 plants belonging to 15 families were studied for their anti-microbial activity against (*B.subtilis, E coli, Ps.aeruginosa* and *S.aureus*). The result showed that 101 (75%) extracts exhibited anti-microbial activity against one or more microorgamisms (Almagboul *et al*., 1985).

More than five hundred extracts of 111 Sudanese medicinal plants were subjected to antibacterial activity against *S.aureus. E.coli. B.subtilis and Ps.aeruginosa*. Out of 573 extracts screened 433 extracts exhibited inhibitory activity against one or more of the four tested bacteria. These investigations lead to the isolation of two sesquiterpene lactones, venodalin and vernolepin from Vernonia amygdaline (Asteraceae). Both sesquiterpene and lactones have significant activity against the four bacterial strains tested (Almagboul, 1992).

Farouk *et al*., (1983) investigated 76 extracts of 31 plants belonging to 21 families for their anti-bacterial activity against four organisms (*E.coli, S.aureus, B.subtilis, and Ps.aeruginosa).* The result was encouraging as about 64(84%) extracts exhibited inhibitory effects against at least one organism.

El Egami *et al*. (1998) screened 114 extracts of Sudanese plant used in folkloric medicine for their anti-bacterial activity against *(E.coli, S.aureus, B.subtilis, and Ps.aeruginosa)*. Of these 83(73%) extracts showed significant anti-bacterial activity.

Sixty-six extracts from 15 Sudanese plants belonging to 12 families were screened against 4 standard organisms *(E.coli, S.aureus, B.subtilis, and P.aeruginosa*) by Omer *et al*. (1997). The result indicated that 53 (80%) extracts showed significant anti-bacterial activity.

2.8 Herb information

Commiphora mol mol (Engl.) Ageless herbal.

http://en.wikipedia.org/wiki/Talk:Myrrh

Scientific classification	
Kingdom:	Plantae
Division:	Magnoliophyta
Class:	Magnoliopsida
Order:	Sapindales
Family:	Burseraceae
Genus:	*Commiphora*
Species:	***C. myrrha***

Description:

Species are small trees or shrubs with short, thorny branches. True *myrrh* is produced by *C. myrrha*, a variable species found in southern Arabia and

northeast Africa (chiefly Somalia) as far south as northeast Kenya. Other resin-producing *Commiphora* occur
in southern Arabia, Sudan, Ethiopia, Eritrea, Somalia and Kenya. *C. erythraea* and *C. kataf*, the main sources of opopanax, are abundant in many parts of southern
Arabia, Somalia, eastern Ethiopia and Kenya.(Hanus *et al*., 2005)

Myrrh is a reddish-brown resinous material, the dried sap of the tree Commiphora myrrha, native to Yemen, Somalia and the eastern parts of Ethiopia. The sap of a number of other Commiphora and Balsamodendron species are also known as *Myrrh*, including that from C. erythraea (sometimes called East Indian myrrh), C. opobalsamum and Balsamodendron kua. Its name entered English via the Ancient Greek, μύρρα, which is probably of Semitic origin. *Myrrh* is also applied to the potherb *Myrrh is odorata* otherwise known as "Cicely" or "Sweet Cicely".

High quality *myrrh* can be identified through the darkness and clarity of the resin. However, the best method of judging the resin's quality is by feeling the stickiness of freshly broken fragments directly to determine the fragrant-oil content of the *myrrh* resin. The scent of raw *myrrh* resin and its essential oil is sharp, pleasant, and somewhat bitter and can be roughly described as being "stereotypically resinous". When burned, it produces a smoke that is heavy, bitter and somewhat phenolic in scent, which may be tinged with a slight vanillic sweetness. Unlike most other resins, *myrrh* expands and "blooms" when burned instead of melting or liquefying.

The scent can also be used in mixtures of incense, to provide an earthy element to the overall smell, and as an additive to wine, a practice alluded to

by ancient authorities such as Fabius Dorsennus. It is also used in various perfumes, toothpastes, lotions, and other modern toiletries.

Myrrh was used as an embalming ointment and was used, up until about the 15th century, as penitential incense in funerals and cremations. The "holy oil" traditionally used by the Eastern Orthodox Church for performing the sacraments of chrismation and unction is traditionally *scented with myrrh*, and receiving either of these sacraments is commonly referred to as "receiving the *Myrrh*".

Actions: antimicrobial, antifungal, astringent and healing, tonic and stimulant, carminative, stomachic, anticatarrhal, expectorant, diaphoretic, vulnerary, local antiseptic, immune stimulant, bitter, circulatory stimulant, anti-inflammatory, antispasmodic.

Indications: aphthous ulcers, pharyngitis, respiratory catarrh, furunculous; topically for wounds and abrasions .

Therapeutics and Pharmacology: *Commiphora myrrh* achieves its antimicrobial action by stimulating the production of white blood corpuscles and also by its direct antimicrobial effect. It is of great value in the treatment of mouth infections such as ulcers, gingivitis and pyorrhea, as well as the catarrhal problems such as pharyngitis and sinusitis. Systemically, it is used in the treatment of boils and similar conditions as well as glandular fever and brucellosis. Externally, it is healing and antiseptic for wounds and abrasions and is suitable as a douche. It is a useful tonic in dyspepsia. The oil is used in chest rubs for bronchitis and catarrhal colds.

Combinations: *Commiphora myrrh* may be combined with Echinacea for infections and as a mouthwash for ulcers, or with Echinacea and Baptisia in tonsillitis and pharyngitis. It can be used with Achillea, Sambucus, Hyssopus and Capsicum in the common cold. Tincture of *myrrh* with Achillea can be painted onto infected gums.

Caution: avoid in pregnancy as it is a uterine stimulant.

2.8.1 History:

Since Bible times it has been used as a medicine and wound dressing and has been closely associated with the health and purification rituals of women and for embalming and fumigations in Ancient Egypt. In folk tradition it was used for muscular pains and in rheumatic plasters.. It was first described in the Chinese medical literature. The use of *myrrh* medicinally was recorded in China in A.D. 600 during the Tang Dynasty. *Myrrh* is used today in Chinese medicine to treat wounds, relieve painful swelling, and to treat menstrual pain due to blood stagnation. *Myrrh* is called *mo yao* in China. It has long been used in the Ayurvedic system of medicine. *Myrrh* oil, distilled

from the resin, has been used since ancient Greek times to heal wounds it's said that the Greek soldiers would not go into battle without a poultice of *myrrh* to put on their wounds. (Mills, 1993; Ody, 1993).

Myrrh is a constituent of perfumes and incense, was highly valued in ancient times, and was often worth more than its weight in gold. The Greek word for *myrrh, μύρον*, came to be synonymous with the word for "perfume". In Ancient Rome *myrrh* was priced at five times as much as frankincense, though the latter was far more popular. *Myrrh* was burned in ancient Roman funerals to mask the smell emanating from charring corpses. It was said that the Roman Emperor Nero burned a year's worth of *myrrh* at the funeral of his wife, Poppaea. Pliny the Elder refers to *myrrh* as being one of the ingredients of perfumes, and specifically the "Royal Perfume" of the Parthians. He also says *myrrh* was used to fumigate wine jars before bottling .In Arabic the word *murr* means "bitter", it has been used throughout history in incense and as a perfume.

***Myrrh* Oil:**

In order to collect gum, the natives make incisions into the bark, causing the exudation of a yellowish oleores in.Exposed to the air, this dries, hardens and turns reddish-bron.*Myrrh* consists of water-soluble gum, alcohol-soluble resins and volatile oil. The gum contains polysaccharides and proteins, while the volatile oil is composed of steroids, sterols and terpenes. *Myrrh's* characteristic odor is derived from furanoses quiter penes. (Hanus *et al*., 2005).

2.8.2 Chemistry:

Myrrh chemical composition can be divided into three active compounds groups:

1. **Essential oil (2-10%):** The main components in this group are furano-sesquiterpenes, derivatives of germacrane, eudesmane, elemane and guaiane (furanoeudesma-1,3- diene the main one, lindestrene, furanodiene). Sesquiterpenes such as α-copaene, δ-elemene, β-bourbonene and humulene can also be found. This fraction is soluble in alcohol.
2. **Resin (25-40%):** The characteristic components of resin are α-, β- and γ-commiphoric acids, α- and β-heerabomyrrhol, heeraboresene and burseracin. Terpenes and the sesquiterpene lactone commiferin are also present. This fraction is soluble in alcohol too.

3. **Gum (30-60%):** Mainly composed of a proteoglycan with arabinose or galactose side chains alternate with 4-O-methylglucuronic acid. The side chains bind hydroxyproline residues on proteins. This fraction is water soluble (Koelblin-Fortuna-Druck, 2001).

2.8.2 Traditional Uses:

Myrrh has been used since the ancient times. It is very well known from references in the Bible. Egyptians used it in embalming mixtures, Persians in wound healing treatments and Romans in medicines for the eyes due to its antiseptic and anaesthetic properties. Contemporarily, *myrrh* tincture is used in mouthwash preparations aimed at treating mild oral and pharyngeal inflammation. It is also used to treat skin abrasion, wounds and inflammation because of its anti-inflammatory properties. Anthelmintic properties had been recently described (Sheir 2001).

In Chinese medicine, *myrrh* is classified as bitter, spicy, neutral in temperature and affecting the heart, liver, and spleen meridians. Its uses are similar to those of frankincense, with which it is often combined in decoctions, liniments and incense. *Myrrh* is said to be blood-moving, while frankincense is said to move the *Qi* more, and is better for arthritic conditions. It is said to be useful for amenorrhea, dysmenorrhea, menopause and uterine tumors, as it is said to purge stagnant blood out of the uterus *Myrrh* is said [to help toothache pain, and can be used in liniment for bruises, aches and sprains. *Myrrh* is most commonly used in Chinese medicine for rheumatic, arthritic and circulatory problems. It is combined with such herbs as notoginseng, safflower stamens, Angelica sinensis, cinnamon and Salvia miltiorrhiza, usually in alcohol, and used both internally and externally.

Myrrh is used more frequently in Ayurveda, Unani medicine and Western herbalism, which ascribe to it tonic and rejuvenative properties. A related species, known as guggul in Ayurvedic medicine is considered one of the best substances for the treatment of circulatory problems, nervous system disorders and rheumatic complaints, *Myrrh* (Daindhava) is used in many rasayana formulas in Ayurveda

However rasayana herbs have special processing. Outside of this form *myrrh* is said to be contraindicated for pregnant women or women with excessive uterine bleeding, and not be used with evidence of kidney dysfunction or stomach pain.

2.8.3 Cosmetic Properties:

Myrrh astringent properties, attributed to the resin components (Council of Europe, 2001) are useful in cosmetics. These properties, together with the antibacterial action of its sesquiterpenes (Dolara 1999), make *myrrh* a good active ingredient in formulations aimed at greasy skin and hair. Therefore, it is suitable for anti-acne treatments or for hair treatments intended to palliate sebum excessive production and restore it to normal levels.

Anti-inflammatory action on acute and subacute inflammation phases has also been demonstrated using mice as experimental models. The immuno-stimulant effects of some *myrrh* extracts and the analgesic activity of furanoeudesma-1,3-diene have been demonstrated with the same experimental model. Some other studies provide data about *myrrh* cytoprotective effects on gastric mucosa (ESCOP, 2003). Furanoeudesma -1, 3-diene Lindestrene.

2.8.4.1 Skins:

On the skin, it is wonderful to treat wounds and sores, especially weeping eczemas, skin ulcers and bed sores; it is used effectively on mature skin, as well as sorting out deep cracks on the feet and hands. In Western countries, *myrrh* probably is used most often as a soothing agent for mouth and skin tissues. In mouthwashes, it can relieve mouth and throat irritations. *Myrrh* is an astringent that shrinks and tightens the top layers of skin or mucous membranes, thereby reducing secretions, relieving irritation, and improving tissue firmness. It may have slight antibacterial effects, which could help to prevent infections on the skin or in irritated mouth tissue, as well. As a mouth rinse, *myrrh* is approved for treating mouth inflammation by the German Commission E, the German governmental agency that

evaluates the safety and effectiveness of herbal products used in Europe. In addition to relieving inflammation, using myrrh as a mouthwash also it is thought to improve bad breath. Undiluted *myrrh* tincture can be applied directly to sores inside the mouth.

2.8.4.2 Skin cosmetics:

In Wet wounds or eczema *Myrrh* is one of the oils some people think of when faced with weepy wet wounds or eczema. It dries, clears toxins, helps resist putrefaction and infection, and promotes tissue repair, among other things. Steam distilled oil from gum oleoresin in used normally in cosmetics (Baser *et a l*., 2002).

2.8.5 Toxicity:

Limited scientific studies suggest that *myrrh* (*Commiphora molmol*) has antibacterial and anti-inflammatory activities. Based on the MTT assay, 24- and 48-h exposures to 0.001% MO had little effect on fibroblast and epithelial cell (24-h only) viability. At 48 h, 0.0005–0.001% MO decreased epithelial cell viability by 30–50%. After 24 and 48 h, MO, at 0.005%, maximally decreased viability of all cell lines. In the LDH release assay, exposure to 0.0001% MO caused <10% cytotoxicity to all cells. At 24 h, 0.0025% MO caused maximal cytotoxicity; 0.001% MO caused 10–70% cytotoxicity. At longer exposure times, epithelial cells were more susceptible to cytotoxic effects of MO. There was little or no detectable IL-1β-stimulated production of IL-6 or IL-8 by cells exposed to 0.0025% MO, probably reflective of loss of viability. At subtoxic MO levels (0.00001–0.001%), there was a significant reduction of IL-1β-stimulated IL-6 and IL-8 production by fibroblasts, but not by epithelial cells (Tipton *et al*., 2003).

2.9 Preparation and Dosage:

Infusion: Dissolves in water with difficulty; should be powdered well. Pour cup of boiling water onto 1-2 teaspoons and infuse for 10-15 minutes. Drink three times a day.

Gargle: Steep 1 teaspoon myrrh and 1 teaspoon boric acid in half a liter of boiling water. Strain after 30 minutes. Alternatively use 5ml tincture in a tumbler of water.

Tincture: 1-2ml three times a day. Used for infectious, feverish conditions, from head colds to glandular fever. It is ideal for upper respiratory catarrhal and can be added to expectorant mixtures.

Capsules: more palatable than the tincture; take one 200mg capsule up to five times a day.

Douche: use the diluted tincture for thrush.

Oil: dilute 10 drops in 25ml water and apply to wounds and chronic ulcers or in lotions to hemorrhoids.

Chest rub: 1ml oil in 15ml almond or sunflower oil for bronchitis and colds with thick phlegm.

Pessaries: 10 drops of oil to 30g cocoa butter in a 24-pessary mould. Use for thrush.

CHAPTER THREE

3. Materials and Methods

In the present study, 200 clinical isolates were randomly obtained from the National Health Laboratory, Khartoum Teaching Hospital during the period January 2005 – September 2007. The isolates were purified through proper streaking on appropriate selective and differential culture media and selection of typical colonies of each of purified organisms. The purified cultures of the different isolates were then subjected to identification procedures based on the cultural characteristics, the microscopic examination and the biochemical characteristics.

Two hundred samples were randomly collected from males and females wound swabs. Collection of organisms was carefully done by taking samples from wound discharge on a sterile cotton wool swab. The specimens were immediately taken for culture to ensure no contamination of samples. Work was done in a controlled environment to minimize variation.

Fresh plant materials were carefully collected from one site near Galabat city (Gadarif State, Eastern Sudan). To ensure homogeneity of samples, identification and classification were done by experts from the herbarium, medicinal and Aromatic Plants Research Institute, National Research Center, Khartoum, Sudan.

Volatile oil of *commiphpra myrrh* (resin) used was obtained by water distillation technique using Clevenger s apparatus .The plant parts were cut

into small pieces, an accurate weight 250gms was taken from the cut material and was introduced in 2 liters round bottomed flask, and distilled water was added and mixed thoroughly.

The contents of the flask were boiled gently for four hours until the volatile oil has been distilled. The crude volatile oil obtained was transferred by means of a pipette in a dark bottle. Anhydrous sodium sulphate was added and agitated gently to absorb the water and the clear oil was decanted into dark bottle and kept in the refrigerator till used. Yield was calculated as v/w %.

3.1 Biological Materials:

The following biological materials were used:

Biological Material	Source
Blood	National Health Laboratory, Khartoum
Plasma	National Health Laboratory, Khartoum

3.2 Chemicals and Reagents:

The following chemical reagents were used:

Chemicals and Reagents	Source
Methanol	The British Drug House Ltd, United Kingdom
Crystal Violet	The British Drug House Ltd, United Kingdom
Hydrochloric Acid	The British Drug House Ltd, United Kingdom
Hydrogen Peroxide	The British Drug House Ltd, United Kingdom

Immersion Oil	The British Drug House Ltd, United Kingdom
Lugol's Iodine	The British Drug House Ltd, United Kingdom
Methyl Red Indicator	The British Drug House Ltd, United Kingdom
Safranin Red	The British Drug House Ltd, United Kingdom
Sodium Chloride	Oxoid Ltd, United Kingdom
Tetra methyl –p-phenylene- diamine Dihydrochloride (oxidase reagent)	The British Drug House Ltd, United Kingdom
Urea	Abbott Ltd .U.K

3.3 Culture Media:

Culture Media	Source
Bacteriological Peptone	Oxoid Ltd, , United Kingdom
Blood Agar base	Oxoid Ltd, United Kingdom
Eosin Methylene blue agar	Oxoid Ltd, United Kingdom
Kligler Iron Agar	Oxoid Ltd, United Kingdom
MacConkey Agar	Oxoid Ltd, United Kingdom
Mannitol salt Agar	Oxoid Ltd, United Kingdom
Muller & Hinton Medium	Oxoid Ltd, United Kingdom
Nutrient Agar	Oxoid Ltd, United Kingdom
Nutrient Broth	Oxoid Ltd, United Kingdom

Peptone water	Oxoid Ltd, United Kingdom
Simmons's Citrate Medium	Plasmatic Labs, , United Kingdom
Urea Agar Base	Plasmatic Labs

3.4 Equipment and Instruments:

Autoclave	Baird &Tatlock, England
Balance	Adam Equipment Co .England
Colony counters	Gallenkam P. England
Glassware etc……	Griffin &George Ltd England and Kim ax Ltd USA
Hot Air Oven	Mummers, Germany.
Incubator	Baird & Tatlock, England
Microscope and Microscope Slides	Olympus, type CH20, Japan
1mm thick	Horwell Limited, London, U.K
Swabs	Medical Disposable Industrial Complex (MDIC), Saudi Arabia
Water Bath	Grant Ltd. England.
Wires Loop Holder	Baird &Tat lock, England

3.5 Test Control Organisms:

Standard strains of the test organisms were obtained from the American Type Culture Collection (ATCC). The following organisms were used.

Escherichia coli	ATCC 25922
Staphylococcus aureus	ATCC 25923
Proteus vulgaris	ATCC 6380
Pseudomonas aeruginosa	ATCC 27853
Klebsiella pneumoniae	ATCC 35657

3.6 Isolation of Clinical isolates:

The wound swab was inoculated on sterile blood agar plate and MacConkey agar plate and then streaked by means of a wire loop. The plates were incubated aerobically at 37°C overnight. On MacConkey agar plate, the lactose and non-lactose fermenting colonies were isolated. Subculture was made on MacConkey agar and nutrient agar. On blood agar plate the B-hemolytic and alpha-hemolytic and non-hemolytic colonies were isolated. Subculture was made on blood agar, mannitol salt agar and nutrient agar.

3.6.1 Types of Culture Media:

In this study, the following different types of culture media were used to differentiate many types of bacteria.

1. **MacConkey Agar:**

It is a differential and selective medium used for cultivation of *Enterobacteria* and for differentiating lactose fermenting from non-lactose fermenting *Enterobacteria.*

2. **Nutrient Agar:**

It is a basal ordinary culture medium used for culturing bacteria that have no special nutritional requirements .The plates were incubated at 37° C for 24 hours. All samples were cultured and incubated under aerobic conditions.

3. **Blood Agar:**

It is used to differentiate organisms that may be Beta heamolytic or Alpha heamolytic or non –heamolytic.

4. **Mannitol Salt Agar:**

It is a selective medium used for isolation of *staphylococci* which are salt tolerant and some are mannitol fermenters.

5. **Eosin Methylene Blue Agar:**

Eosin methylene blue agar is the selective media used to identify *E.coli*

3.6.2 Purification of Isolated Colonies:

Isolated colonies were purified by streaking the organisms on nutrient agar. Single colonies of purified cultures were further sub cultured on sloping agar incubated aerobically at 37 ^{0}C for 24 hours and then preserved in refrigerator at 4^{0}Cuntil they were used.

3.6.3 Microscopical examination of the Clinical Isolates:

All these isolates were subjected to microscopical examination to study their morphology and staining properties using the Grams staining technique.

3.6.4 Cultural characteristics of the Clinical Isolates:

In this study many types of bacteria were isolated:

3.6.4.1 Staphylococcus *aureus* Isolates:

It is a Gram positive coccus that grows in irregular, grape like clusters, facultatively anaerobic, form acid from glucose under either aerobic or anaerobic conditions. Staphylococci are salt tolerant and grow on mannitol salt agar very well, in which they produce 1-2mm in diameter yellow to cream or occasionally white colonies by fermenting of mannitol. Some strains are heamolytic and non –lactose fermenters. They are non – capsulated, coagulase positive. It occurs as a parasite on the skin in man and other vertebrate animals. (Greenwood.,*et al*., 2002).

3.6.4.2 *Escherichia coli* Isolates:

E.coli is a Gram negative rod, aerobic and facultative anaerobe. Most strains are motile, some strains are capsulate. Usually ferments lactose,and produce Indole (Sleigh, 1994). It causes UTI, wound infection, peritonitis, sepsis, meningitis, septicemia, and diarrhoeal diseases. On MacConkey agar they were lactose fermenting, producing smooth pink colonies, on KIA most produce acid deep and acid slope with gas production and no blackening and hence H_2S negative.

3.6.4.3 *Klebsiella pneumoniae* Isolates:

Klebsiella pneumoniae are Gram Negative non-motile, capsulated rods. Normal habitat is intestinal tract of human and water .It causes urinary tract infection (UTI) especially in hospital, chest infections, pneumonia and wound infections and cause rinoscleromatis of the nose and pharynx which leads to chronic inflammatory growths by *Klebsiella pneumoniae* rinoscleromatis. On MacConkey *Klebsiella pneumoniae* produce mucoid pink colonies (lactose fermenters), always large and mucoid (due to possession of a prominent capsule) on blood agar, CLED (Sleigh, 1994).

3.6.4.4 *Proteus vulgaris* Isolates:

It is gram negative rod, aerobic and facultative anaerobe, non capsulated, motility is not easily observed at 37°C. It causes UTI abdominal and wound infections, chest infections, septicaemia and meningitis. Natural habitats include; Intestinal tract, soil, water and vegetables. It produces highly motile organisms that swarm on nutrient and blood agar; however, swarming is not observed on MacConkey agar. Low convex and small circular non lactose fermenting colonies are shown on MacConkey medium. *Proteus vulgaris* cultures have a characteristics fishy odour, colourless colonies on MacConkey agar and blue green colonies on CLED agar (Sleigh, 1994).

3.6.4.5 *Pseudomonas aeruginosa* Isolates:

It is gram negative rod, motile, non sporing, non capsulated, attacks sugars oxidatively and grows aerobically in wide variety of laboratory media. On blood agar large flat heamolytic colonies are produced. On

MacConkey agar, they are non-lactose fermenting colonies. *Ps-aeruginosa* is an opportunistic pathogen in urinary tract and, wounds. Most strains produced pyocyanin Pigment, typically the colony and surrounding is greenish-blue. The most common colonial form is relatively large, low convex with an irregular surface, energy from carbohydrate by an oxidative rather than a fermentative metabolism. All strain give a rapid positive oxidase reaction (Greenwood.,*et al*., 2003).

3.7 Biochemical Test Adopted for Identification of Bacterial Isolates:

3.7.1 Catalase Test:

This test acts as a catalyst in breakdown of hydrogen peroxide to oxygen and water is carried out by pouring 2-3 ml hydrogen peroxide in test tube and using sterile wooden stick or glass rod to remove several colonies which were grown on blood –free media then immersed in hydrogen peroxide solution. Bubbles of oxygen are released if the organism is a catalase producer. It is used to differentiate bacteria that produce a catalase enzyme such as *staphylococci* from non – Catalase producer e.g. *streptococci* (Cheesbrough, 2000).

3.7.2 Coagulase Test:

Coagulase causes clotting of plasma by converting fibrinogen to fibrin .It is carried out by pouring 0.2 ml of plasma in test tube then adding 0.8 ml of test broth culture and incubating 37°C then examining for clot after 1hour, 3 hours and over night. It is used to identify *Staph. aureus*, which is coagulase enzyme producer (Cheesbrough, 2000).

3.7.3 Mannitol fermentation

Mannitol salt agar (Annex No. 6) was a useful differential and selective medium for differentiation *Staphylococcus aureus* from mixed isolates with other gram positive cocci. *S.aureus* ferment mannitol sugar producing an acid medium, which was recognized by the change of colour in the medium from pink to yellow (Collee *et al*., 1996).

3.7.4 Citrate Utilization Test:

This test is based on the ability of an organism to use citrate as the only source of carbon and ammonia as the only source of nitrogen. The test organism was inoculated in a medium, which contains sodium citrate, ammonium salt and the Bromothymol blue as indicator (Simmons citrate medium which is a modification of koser's medium with agar and an indicator) then incubated at 37°C for up to four days. Citrate utilization appeared by a change in color of indicator from light green to blue due to alkaline reaction (Cheesbrough, 2000).

3.7.5 DeoxyriboNuclease (DNAse) Test:

The test is used to demonstrate the DNAse producing organisms and is carried out by culturing an organism in a medium containing DNA and incubating at 37º C over night. The colonies are tested for DNAse production, by flooding the plate with a weak hydrochloric acid solution, the acid precipitates unhydrolized DNA therefore clear zones surround DNAse producing colonies. It is used to differentiate *Staph. aureus* from other *Staphylococci* (Cheesbrough, 2000).

3.7.6 Indole Test:

This test demonstrates the ability of certain bacteria such as *E.coli* to break down the amino acid tryptophane with the release of indole, which accumulates in the medium. The test organism was cultured on a suitable tryptophane rich medium, mainly peptone water for 48 hours, then, 0.5ml of Kovac's reagent is added, a ring of red color in the alcohol layer indicates a positive reaction (Cheesbroug, 2000).

3.7.7 Kligler-Iron agar:

It is a differential slope medium for fermentation of lactose and glucose, production of hydrogen sulphide and production of gas; the reactions are used in identification *Enterobacteriaceae*. A yellow butt and red slope indicate the fermentation of glucose only .A yellow slope and a yellow butt indicate the fermentation of glucose and lactose. A red slope and a red butt indicate fermentation of neither glucose nor lactose. Cracks and bubbles indicate gas production where blackening indicates hydrogen sulphide (H_2S) production (Cheesbrough, 2000).

3.7.8 Oxidase Test:

This test depends on the presence of Oxidase enzyme, in certain bacteria that will catalyze the transport of electrons between electron donors in the bacteria and a redox -dye (tetra methyl –P-phenylene-diamine Dihydrochloride) (Cruickshank *et al*., 1975). A piece of filter paper is soaked with few drops of Oxidase reagent (tetra methyl-P-phenylene-diamine Dihydrochloride). A colony of the test organism is then smeared on the filter paper. If the organism is oxidase – producer, the phenylene diamine in the reagent will be oxidized to a deep purple colour (Cheesbrough, 2000).

3.7.9 Urease Test:

This test was carried to asses urease enzyme activity, and it is important in recognizing Enterobacteria .The test is carried out by inoculating slopes of medium, which contain urea, with test organism and incubating at 37c for up to five days and examining daily. If the strain is urease producer, the enzyme will breakdown the urea to give ammonia and carbon dioxide .The releasing of ammonia makes the medium alkaline so the colour of the indicator (phenol Red) changes to pink-red (Cheesbroug,1996).

3.7.10 Voges –Proskauer (VP):

Many bacteria ferment carbohydrates with the production of acetyl methyl carbinol (acetoin). The test is carried out by culturing test organism in glucose phosphate peptone water for 48 hours .Sodium hydroxide and small amounts of creatinine were added. Under alkaline conditions and exposure to the air, a pink color develops within 2-5 minutes which becomes crimson in 30 minute indicating a positive reaction for acetoin production (Cheesbrough, 2000).

3.8 Preparation of the test organisms:

3.8.1 Preparation of standard bacterial suspension:

One ml aliquots of 24 hours broth cultures of the test organisms were aseptically distributed onto nutrient agar slopes and incubated at 37 ^{o}C for 24 hours. The bacterial growth was harvested and washed off with sterile normal saline; to produce a suspension containing about 10^{10} colony forming units per ml .The suspension was stored in the refrigerator at 4^{o}C till used.

The average number of viable organisms per ml .of the stock suspension was determined by means of surface viable counting technique (Miles and Misra, 1938). Serial dilutions of the stock suspensions were made in sterile normal saline, and 0.02 ml (one drop) volumes of the appropriate dilution were transferred on to the surface of dried nutrient agar plates. The plates were allowed to stand for two hours at room temperature for the drop to dry, and then incubated at 37 ^{o}C for 24 hours. After incubation, the number of developed colonies in each drop was counted.

The average number of colonies per drop (0.02ml) was multiplied by 50 and by the dilution factor to give the viable count of the stock suspensions, expressed as the number of colony forming units (cfu) per ml of suspensions.

Each time a fresh stock suspension was prepared. All the above experimental conditions were maintained constant so that suspensions with very close viable counts would be obtained (Raja, 2000).

3.9 Measurement of antimicrobial activity:

3.9.1 Incorporation of drugs in media:

The tube dilution test is the standard method for determining levels of resistance to an antibiotic. Serial dilutions of the antibiotic are made in a liquid medium which is inoculated with a standardized number of organisms and incubated for a prescribed time. The lowest concentration (highest dilution) of antibiotic preventing appearance of turbidity is considered to be the minimum inhibitory concentration (MIC). At this dilution the antibiotic is bacteriostatic. Additionally, the minimum bactericidal concentration (MBC) can be determined by subculturing the contents of the negative tubes onto antibiotic-free solid medium and examining for bacterial growth.

3.9.2 Determination of minimum inhibitory concentration (MIC) by agar plate dilution method:

The principle of the agar plate dilution method is the inhibition of growth of the seeded bacteria on the surface of the agar by the plant extract incorporated into the medium. The organisms tested were grown in broth overnight and diluted in normal saline to contain 10^8 cfu. /ml organisms per ml. Plant extract were prepared in the following concentrations; 6.25, 12.5, 25, 50, and 100 mg/ml. Each concentration was marked off the bottom of each plate.

A test solution of volatile oil for antimicrobial activity was prepared in Methanol as dilution medium, since it was devoid of any antimicrobial activity. Dilution tests on media involve addition of varying concentrations of drug to measure volume of agar medium which was melted and cooled to 45-50ºC, the resultant mixtures were then poured as plates into Petri dishes. Standardized inoculates were seeded onto the surface of the medium and MIC read after an appropriate incubation period (Garrod *et al*., 1981). In this method, it is essential to test strains of known susceptibility with each series of unknowns in order to minimize error due to drug deterioration, inaccuracies in dilution or variation in the medium, (Cruickshank *et al*., 1975).

A loop-full of the diluted culture is spotted with standard loop that delivers 0.01 ml onto the surface of each segment and then incubated at 37°C overnight. Break point concentration of antibiotics is used to characterize antibiotic activity, the highest dilution showing no growth is the minimum inhibitory concentration.

3.9.3 Agar dilution Method to the plant:

The principle of the agar plate dilution method is the inhibition of growth on the surface of the agar by the antimicrobial agent (plant extract) incorporated into the medium.

The organisms tested were prepared in sterile normal saline to an opacity of McFarland standard (ca10^9cFU/ml).(158,161).

A loop full of the diluted culture is spotted with a standard loop that delivers 0.001ml on the surface of the media .

Sterile screw cap tubes were numbered .All the following steps were cared out using aseptic technique.

* 1ml of *Commiphora myrrh* extract was added to 10 ml of methanol as the first tube and 10ml of sterile distilled water was added to all other tube.
* 10 ml transferred from the first tube to the second tube and mixed well with separate pipette and transferred 10 ml to the third tube.
* The dilution was continued in this manner to tube number 5 and the pipettes changed between tubes to tube to prevent carry-over of extract on the external surface of the pipette.
* 10 ml removed from tube 6 and discarded.
* Tube 6 which served as a control, no *Commiphora* added to it.

Muller-Hinton media prepared in dabble concentration for amount of the media to 20 ml of D.W only 10 ml were used and autoclaved and cooled and the diluted extract added to it , mixed and transferred to the plate.

After one drop of the suspension transferred to the plate .Incubated at 37^0C for 18-24 hrs the result observed by growth or no growth (NCCLS ,2003), Chaves,*et al*.,(1999)

Data management

Statistical Analysis: Statistical Analysis System (SAS) software was used for analysis, and using statistical package for Social Science for Personal Computer (SPSS/PC).

CHAPTER FOUR

RESULTS AND DISCUSSION

4. Result and discussion

4.1. Identification of Clinical isolates:

Identification of the clinical isolates was always started with microscope examination of properly prepared films which had been stained by Grams staining technique (Raja, 2000). The isolates were purified by proper streaking on appropriate selective and differential culture media and selection of typical colonies of each of purified organisms. The purified cultures of the different isolates were then subjected to identification procedures based on the cultural characteristics, the microscopic examination and the biochemical characteristics.

On the basis of the result of these identification tests, it was found that of the 200 clinical isolates, 62 were *staph.aureus*, 44 *Escherichi coli, 34 Pseudomonas aeruginosa*, 30 *Klebsiella pneumoniae*, and 30 *Proteus vulgaris*.

Table (1): Descriptive statistics of 200 isolated bacterial organisms used:

Species	**No of isolates**	**Percent**
Escherichia coli	44	22%
Klebsiella pneumonia	30	15%
Pseudomonas aeruginosa	34	17%
Proteus vulgaris	30	15%
Staphylococcus aureus	62	31%

4.1.1 Identification of *Staphylococcus aureus:*

Cultural Characteristics:

On nutrient agar, golden yellow colonies were observed. On mannitol salt agar, it changed the colour of medium from red to yellow.

Microscopically Examination:

With Gram's staining technique, Gram-positive cocci arranged in grape – like clusters.

Biochemical Reactions:

The isolates fermented lactose with production of acid and didn't form gas. All of them fermented sucrose and mannitol with acid production .Most of them fermented glucose with acid production.

Almost all of isolates were Catalase-positive, Coagulase-positive, and DNAse-positive, Table (2).

The production of coagulase identifies *staphylococcus aureus*, and also DNAse test is a confirmatory test.

4.1.2 Identification of *Klebsiella pneumoniae*:

Cultural characteristics:

On MacConkey's agar medium, large pink and mucoid colonies were seen.

Microscopical examination:

With Gram's staining technique, Gram-negative capsulated rods were seen

Biochemical Reaction:

All isolates were lactose fermenting with acid and gas production. Most of them fermented glucose and sucrose with acid or gas production.

All isolates were Indole-negative, Methyl red-positive, and Voges-Proskauer negative. Almost all of them were Citrate-positive, Table (6)

Klebsiella pneumoniae gives a positive urease test after 18-24 hours and positive citrate.

4.1.3 Identification of *Escherichia. coli*:

Culture characteristics:

On MacConkey agar medium, large red colonies were observed as a result of lactose fermentation.

Microscopical examination:

With Gram's staining technique Gram-negative rods were seen.

Biochemical Reactions:

All the isolates fermented lactose with production of acid and gas. Most of them fermented glucose and sucrose with acid production and gas formation.

All the isolates give indole –positive result .All of them were Methyl red-positive and Voges Proskauer-negative. None of the isolates produced urease, utilized citrate or give positive Oxidase.

All isolates did not change the yellow colour of K.I.A both in slope and butt, with the absence of H_2S production, but all isolates produced gas.

Escherichia coli are a Gram-negative, unicellular, rod –shaped organism. It is an aerobe and facultative anaerobe.

An *Escherichia coli* strain is the production of indole from peptone water containing trytophane.

4.1.4 Identification of *Pseudomonas aeruginosa*:

Cultural Characteristics:

On MacConkey agar .discrete pale colonies were observed. On nutrient agar most of the isolates produced blue –green pigment which diffused in surrounding medium.

Microscopical Examination:

With Gram's staining technique, Gram-negative rods were seen.

Biochemical Reactions:

Regarding K.I.A, all of them changed the slope colour to red, and most change the butt to red without H_2S or gas production.

Pseudomonas aeruginosa is non-lactose fermenting .All isolates fermented glucose and none of them fermented sucrose. All isolates were Oxidase-positive, indole-negative, Methyl red and Voges Proskauer negative.

4.1.5 Identification of *Proteus vulgaris*

Cultural Characteristics:

On nutrient agar, fishy smell and swarming appearance was seen. On MacConkey agar and deoxycholate citrate agar, pale coloured colonies were observed as a sign of non –lactose fermenting and with no swarming.

Microscopical Examination:

With Grams staining technique, Gram-negative rods were seen.

Biochemical Reactions:

All isolates were non-lactose fermenters .Most of the isolates fermented sucrose with acid production and some of them formed gas .All isolates fermented glucose with acid production.

Most of isolates were Methyl red positive, and all of them were Voges-Proskauer negative. Almost all of them were urease positive.

All isolates changed the colour of slope of K.I.A from yellow to red and maintained the yellow colour of butt. Most were H_2S producers and non gas –producers.

4.2 Inhibitory effectiveness of volatile oil of *Commiphora myrrh* against standard organisms was determined by using the tube dilution method:

Using the tube dilution method the inhibitory effectiveness of volatile oil of *Commiphora myrrh* against standard organism's *pseudomonas aeruginosa, Staph. aureus, Escherichia coli, and Proteus vulgaris* was determined and the results were shown in table(2).

The volatile oil had inhibitory effect on the standard organisms at concentration of 100mg/ml (100%), 50mg/ml (100%) and 25mg/ml showed week growth. At

Table (2) Inhibitory effectiveness of the volatile oil of *Commiphora myrrh* against Standard organisms

Standard organisms	**Serial dilutions**					
	100mg/ml	**50mg/ml**	**25mg/ml**	**12.5mg/ml**	**6.25mg/ml**	
Staph. aureus	N.G	N.G	N.G	W.G	G	G
Escherichia coli	N.G	N.G	W.G	W.G	G	G
Pseudomonas aeruginosa	N.G	N.G	W.G	W.G	G	G
Proteus vulgaris	N.G	N.G	W.G	W.G	G	G

Key:

N.G = No growth

W.G = Weak growth

G = growth

4.3. Inhibitory effectiveness of volatile oil of *Commiphora myrrh* against bacterial isolates:

4.3.1. The Inhibitory effectiveness of the volatile oil of *Commiphora myrrh* against bacterial isolates was determined using the tube dilution method.

4.3.1.1. Inhibitory effectiveness of the volatile oil of *Commiphora myrrh* against *Staphylococcus aureus* isolates:

Using the tube dilution method the inhibitory effectiveness of volatile oil of *Commiphora myrrh* against 62 bacterial isolates of *Staphylococcus aureus* was determined and the results were shown in Table (3).

The volatile oil had inhibitory effect on the isolates of *Staphylococcus aureus*

at concentrations of 100 mg/ml (100%), 50mg/ml (100%), 25mg/ml (46.7%), 12.5mg/ml (33.8%). Table (4)

The volatile oil showed no activity at concentration of 6.25mg/ml, and some of 25 mg/ml, 12.5 mg/ml

Table (3): Inhibitory effectiveness of the volatile oil of *Commiphora myrrh* against *Staphylococcus aureus isolates*.

Isolates	Serial dilutions					Control
	100mg/ml	50mg/ml	25mg/ml	12.5mg/ml	6.25mg/ml	
S1	-	-	-	+	+	+
S2	-	-	-	+	+	+
S3	-	-	-	+	+	+
S4	-	-	-	+	+	+
S5	-	-	-	+	+	+
S6	-	-	-	+	+	+
S7	-	-	-	+	+	+
S8	-	-	-	+	+	+
S9	-	-	-	+	+	+
S10	-	-	-	+	+	+
S11	-	-	-	+	+	+
S12	-	-	-	+	+	+
S13	-	-	-	+	+	+
S14	-	-	-	+	+	+
S15	-	-	-	+	+	+
S16	-	-	-	+	+	+
S17	-	-	-	+	+	+
S18	-	-	-	+	+	+
S19	-	-	+	+	+	+
S20	-	-	+	+	+	+
S21	-	-	+	+	+	+
S22	-	-	+	+	+	+

Table (3): continue						
Isolates	Serial dilutions					Control
	100mg/ml	50mg/ml	25mg/ml	12.5mg/ml	6.25mg/ml	
S23	-	-	+	+	+	+
S24	-	-	+	+	+	+
S25	-	-	+	+	+	+
S26	-	-	+	+	+	+
S27	-	-	+	+	+	+
S28	-	-	+	+	+	+
S29	-	-	-	+	+	+
S30	-	-	-	+	+	+
S31	-	-	-	+	+	+
S32	-	-	-	+	+	+
S33	-	-	-	+	+	+
S34	-	-	-	+	+	+
S35	-	-	-	+	+	+
S36	-	-	-	+	+	+
S37	-	-	-	+	+	+
S38	-	-	-	+	+	+
S40	-	-	-	+	+	+
S41	-	-	+	+	+	+
S42	-	-	+	+	+	+
S43	-	-	+	+	+	+
S44	-	-	+	+	+	+
S45	-	-	+	+	+	+
S46	-	-	+	+	+	+
S47	-	-	+	+	+	+
S48	-	-	+	+	+	+

Table(3): continue						
Isolates	Serial dilutions					Control
	100mg/ml	50mg/ml	25mg/ml	12.5mg/ml	6.25mg/ml	
S50	-	-	+	+	+	+
S51	-	-	+	+	+	+
S52	-	-	+	+	+	+
S53	-	-	+	+	+	+
S54	-	-	+	+	+	+
S55	-	-	+	+	+	+
S56	-	-	+	+	+	+
S57	-	-	+	+	+	+
S58	-	-	+	+	+	+
S59	-	-	+	+	+	+
S60	-	-	+	+	+	+
S61	-	-	+	+	+	+
S62	-	-	+	+	+	+

(-): No growth **(+): Growth**

S: *Staphylococcus aureus*

Table (4): Effect of different *Commiphora myrrh* concentrations on *Staph. aureus* growth:

Concentration (mg/ml)	**Sample Size**	**%Inhibited**
100	62	100
50	62	100
25	62	66
12.5	62	53
6.25	62	0
Control	**62**	**0**

This result is in alignment with most clinical studies from wound swab in Sudan that showed predominant presence of Staphylococcus aureus. Elegail, 1994 found that in wound infection, Staph .aureus contributes to 52% of the total samples collected.

The inhibitory effectiveness of volatile oil of Commiphora myrrh against 62 bacterial isolates of Staphylococcus aureus showed that the volatile oil had inhibitory effect on the isolates at concentrations of 100 mg/ml (100%) and 50mg/ml (100%), 25mg/ml (46.7%) and 12.5mg/ml (33.8%). The volatile oil showed no activity at concentration of 6.25mg/ml (Table 4).

Similar results were reported by Atta and Alkofahi. (1998) who studied the anti-inflammatory and analgesic effects of *Commiphora myrrh*. They found that *Commiphora myrrh* has significant effect when used against induced, localized acute and chronic inflammation in rodents. The anti-

nociceptive and anti-inflammatory effects were dose dependent. These data affirm the traditional use of some of these plants for painful and inflammatory conditions.

4.3.1.2 Inhibitory effectiveness of the volatile oil of *Commiphora myrrh* against *Klebsiella pneumoniae* isolates:

Using the tube dilution method the inhibitory effectiveness of the volatile oil of *Commiphora myrrh* against 30 bacterial isolates of *Klebsiella pneumoniae* was determined and the results were shown in Table (5).

The volatile oil had inhibitory effect on the isolates of *Klebsiella pneumoniae* at concentrations of 100 mg/ml (100%), 50mg/ml (100%), and 25mg/ml (23.3%).

Table (6)

The volatile oil had no activity at concentration of 12.5mg/ml, 6.25mg/ml and some of 25 mg/ml.

Table (5): Inhibitory effectiveness of the volatile oil of *Commiphora myrrh* against *Klebsiella pneumoniae* isolates:

Isolates	Serial dilution					Control
	100mg/ml	50mg/ml	25mg/ml	12.5mg/ml	6.25mg/ml	
K1	-	-	+	+	+	+
K2	-	-	+	+	+	+
K3	-	-	+	+	+	+
K4	-	-	+	+	+	+
K5	-	-	+	+	+	+
K6	-	-	+	+	+	+
K7	-	-	+	+	+	+
K8	-	-	+	+	+	+
K9	-	-	+	+	+	+
K10	-	-	+	+	+	+
K11	-	-	+	+	+	+
K12	-	-	-	+	+	+
K13	-	-	+	+	+	+
K14	-	-	+	+	+	+
K15	-	-	+	+	+	+
K16	-	-	+	+	+	+
K17	-	-	-	+	+	+
K18	-	-	+	+	+	+
K19	-	-	+	+	+	+
K20	-	-	+	+	+	+
K21	-	-	+	+	+	+

Table(5) continue:						
Isolates	Serial dilution					Control
	100mg/ml	50mg/ml	25mg/ml	12.5mg/ml	6.25mg/ml	
K23	-	-	+	+	+	+
K24	-	-	+	+	+	+
K25	-	-	+	+	+	+
K26	-	-	+	+	+	+
K27	-	-	-	+	+	+
K28	-	-	-	+	+	+
K29	-	-	-	+	+	+
K30	-	-	-	+	+	+

(-): No growth

(+): Growth

K: *Klebsiella pneumoniae*

Table (6): Effect of different *Commiphora myrrh* concentrations on *Klebsiella pneumoniae* growth*:*

Concentration (mg/ml)	Sample Size	%Inhibited
100	30	100
50	30	100
25	30	76
12.5	30	0
6.25	30	0
Control	**30**	**0**

4.3.1.3 Inhibitory effectiveness of the volatile oil of *Commiphora myrrh* against *Escherichia coli* isolates:

Using the tube dilution method the inhibitory effectiveness of volatile oil of *Commiphora myrrh* against 44 bacterial isolates of *Escherichia coli* was determined and the results were shown in Table (7).

The volatile oil had inhibitory effect on the isolates of *Escherichia coli* at concentration of 100 mg/ml (100%), 50mg/ml (100%), 25mg/ml (22.7%).

Table (8)

The volatile oil showed no activity at concentration of 12.5mg/ml, 6.25mg/ml and some of 25 mg/ml

Table (7): Inhibitory effectiveness of the volatile oil of *Commiphora myrrh* against *Escherichia coli* isolates.

Isolates	Serial dilutions					control
	100mglml	50mg/ml	25mg/ml	12mg/ml	6.25mg/ml	
E1	-	-	+	+	+	+
E2	-	-	+	+	+	+
E3	-	-	+	+	+	+
E4	-	-	+	+	+	+
E5	-	-	+	+	+	+
E6	-	-	+	+	+	+
E7	-	-	-	+	+	+
E8	-	-	+	+	+	+
E9	-	-	+	+	+	+
E10	-	-	+	+	+	+
E11	-	-	+	+	+	+
E12	-	-	+	+	+	+
E13	-	-	+	+	+	+
E14	-	-	+	+	+	+
E15	-	-	+	+	+	+
E16	-	-	+	+	+	+
E17	-	-	+	+	+	+
E18	-	-	+	+	+	+
E19	-	-	-	+	+	+
E20	-	-	-	+	+	+

Table(7) continue:						
Isolates	Serial dilutions					Control
	100mglml	50mg/ml	25mg/ml	12mg/ml	6.25mg/ml	
E22	-	-	-	+	+	+
E23	-	-	+	+	+	+
E24	-	-	+	+	+	+
E25	-	-	+	+	+	+
E26	-	-	+	+	+	+
E27	-	-	+	+	+	+
E28	-	-	+	+	+	+
E29	-	-	-	+	+	+
E30	-	-	+	+	+	+
E31	-	-	+	+	+	+
E32	-	-	-	+	+	+
E33	-	-	+	+	+	+
E34	-	-	-	+	+	+
E35	-	-	+	+	+	+
E36	-	-	+	+	+	+
E37	-	-	+	+	+	+
E38	-	-	+	+	+	+
E39	-	-	-	+	+	+
E40	-	-	-	+	+	+
E41	-	-	+	+	+	+
E42	-	-	+	+	+	+
E43	-	-	+	+	+	+

E44	-	-	+	+	+	+

(-): No growth **(+): Growth**

E: *Escherichia coli*

Table (8): Effect of different *Commiphora myrrh* concentrations on *Escherichia coli* growth:

Concentration (mg/ml)	**Sample Size**	**%Inhibited**
100	44	100
50	44	100
25	44	77
12.5	44	0
6.25	44	0
Control	**44**	**0**

The effect of *Commiphora myrrh* concentrations on *Escherichia coli* showed similar results to *Staph.aureus* at 100 and 50 mg/ml concentrations where growth of *E. coli* was inhibited. At 25 mg/ml concentration, partial growth was observed. Normal growth of *E. coli* was found at lower concentrations of 12.5 mg/ml, 6.25 mg/ml and the control (Table 8). Similarly, Obame, *et al*. (2007) found that Dacryodes buettneri (Burseraceae) essential oils tested against twelve strains of bacteria exhibited antibacterial activity against almost all microorganisms tested.

4.3.1.4 Inhibitory effectiveness of the volatile oil of *Commiphora myrrh* against *Pseudomonas aeruginosa isolates*:

Using the tube dilution method the inhibitory effectiveness of the volatile oil of *Commiphora myrrh* against 34 bacterial isolates of *Pseudomonas aeruginosa* was determined and the results were show in Table (9).

The volatile oil had inhibitory effect on the isolates of *Pseudomonas aeruginosa*
at concentrations of 100 mg/ml (100%), 50mg/ml (100%), 25mg/ml (17.6%).
Table (10)

The volatile oil showed no activity at concentration of 12.5mg/ml, 6.25mg/ml and some of 25 mg/ml.

Table (9): Inhibitory effectiveness of the volatile oil of *Commiphora myrrh* against *Pseudomonas aeruginosa* isolates.

Isolates	Serial dilution					Control
	100mg/ml	50mg/ml	25mg/ml	12.5mg/ml	6.25mg/ml	
Ps1	-	-	+	+	+	+
Ps2	-	-	+	+	+	+
Ps3	-	-	+	+	+	+
Ps4	-	-	+	+	+	+
Ps5	-	-	+	+	+	+
Ps6	-	-	+	+	+	+
Ps7	-	-	+	+	+	+
Ps8	-	-	+	+	+	+
Ps9	-	-	+	+	+	+
Ps10	-	-	+	+	+	+

Ps11	-	-	+	+	+	+
Ps12	-	-	+	+	+	+
Ps13	-	-	-	+	+	+
Ps14	-	-	+	+	+	+
Ps15	-	-	+	+	+	+
Ps16	-	-	+	+	+	+
Ps17	-	-	+	+	+	+
Ps18	-	-	+	+	+	+
Ps19	-	-	-	+	+	+
Ps20	-	-	+	+	+	+
Ps21	-	-	+	+	+	+
Ps22	-	-	-	+	+	+
Ps23	-	-	+	+	+	+
Ps24	-	-	+	+	+	+
Ps25	-	-	-	+	+	+
Ps26	-	-	+	+	+	+
Ps27	-	-	+	+	+	+
Ps28	-	-	-	+	+	+
Ps29	-	-	-	+	+	+
Ps30	-	-	+	+	+	+
Ps31	-	-	+	+	+	+
Ps32	-	-	+	+	+	+
Ps33	-	-	+	+	+	+
Ps34	-	-	+	+	+	+

(-): No growth **(+): Growth**

Ps: *Pseudomonas aeruginosa*

Table (10): Effect of different *Commiphora myrrh* concentrations on *Pseudomonas aeruginosa* growth:

Concentration (mg/ml)	Sample Size	%Inhibited
100	34	100
50	34	100
25	34	82
12.5	34	0
6.25	34	0
Control	**34**	**0**

4.3.1.5 Inhibitory effectiveness of the volatile oil of *Commiphora myrrh* against *Proteus vulgaris isolates*:

Using the tube dilution method the inhibitory effectiveness of the volatile oil of *Commiphora myrrh* against 30 bacterial isolates of *Proteus vulgaris* was determined and the results were presented in Table (11).

The volatile oil had inhibitory effect on the isolates of *Proteus vulgaris* at concentrations of 100 mg/ml (100%), 50mg/ml (100%), and 25mg/ml (10%).

Table (12)

The volatile oil had no activity at concentration of 12.5mg/ml, 6.25mg/ml, and some of 25 mg/ml.

Table (11): Inhibitory effectiveness of the volatile oil of *Commiphora myrrh* against *Proteus vulgari isolates*:

Isolates	Serial dilution					Control
	100mg/ml	50mg/ml	25mg/ml	12.5mg/ml	6.25mg/ml	
Pr1	-	-	+	+	+	+
Pr2	-	-	+	+	+	+
Pr3	-	-	+	+	+	+
Pr4	-	-	+	+	+	+
Pr5	-	-	+	+	+	+
Pr6	-	-	+	+	+	+
Pr7	-	-	+	+	+	+
Pr8	-	-	+	+	+	+
Pr9	-	-	+	+	+	+
Pr10	-	-	+	+	+	+
Pr11	-	-	+	+	+	+
Pr12	-	-	-	+	+	+
Pr13	-	-	+	+	+	+
Pr14	-	-	+	+	+	+
Pr15	-	-	+	+	+	+
Pr16	-	-	+	+	+	+
Pr17	-	-	-	+	+	+
Pr18	-	-	+	+	+	+
Pr19	-	-	+	+	+	+
Pr20	-	-	+	+	+	+
Pr21	-	-	+	+	+	+

Table (11)continue:						
Isolates	Serial dilution					Control
	100mg/ml	50mg/ml	25mg/ml	12.5mg/ml	6.25mg/ml	
Pr23	-	-	+	+	+	+
Pr24	-	-	+	+	+	+
Pr25	-	-	+	+	+	+
Pr26	-	-	+	+	+	+
Pr27	-	-	+	+	+	+
Pr28	-	-	+	+	+	+
Pr29	-	-	+	+	+	+
Pr30	-	-	+	+	+	+

(-) No growth

(+): Growth

Pr: *Proteus vulgaris*

Table (12): Effect of different *Commiphora myrrh* concentrations on *Proteus vulgaris* growth:

Concentration (mg/ml)	Sample Size	%Inhibited
100	30	100
50	30	100
25	30	86
12.5	30	0
6.25	30	0
Control	**30**	**0**

Both *Pseudomonas aeruginosa* and *Proteus vulgaris* showed similar trends for all concentrations of *Commiphora myrrh* used (Tables 10 and 12). At 25 mg/ml concentration, growth of 82 and 87 for *Ps. aeruginosa* and *P. vulgaris* respectively were observed.

Banso and Mann 2006, working with isolated fraction of *Commiphora africana* (*Myrrh*) and assayed against *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa Bacillus subtilis, and Streptococcus pyogenes* found antimicrobial activities against all the test microorganisms. They observed that *Bacillus subtilis* was the most susceptible to the alkaloids followed by *Staphylococcus aureus, Escherichia coli, Streptococcus pyogenes and Pseudomonas aeruginosa* respectively. The minimum inhibitory concentration ranged between 3.5mg/ml and 4.5mg/ml while the minimum bactericidal concentration ranged between 4.0mg/ml and 5.0mg/ml. They concluded that alkaloids fraction from *Commiphora africana* could be a potential source for chemotherapeutic agents.

In a study by Tariq.,*et al*, (1986) *Commiphora myrrh* was found to significantly inhibit localy induced inflammation in mice. It also exhibited significant anti-pyretic (fever-reducing) activity in mice.

Nomicos, *et al.,* (2007). Showed that in the study the genus *Commiphora myrrh* is composed of more than 200 species, and has been exploited as a natural drug to treat pain, skin infections, inflammatory conditions, diarrhea, and periodontal diseases. In more recent history, products derived from *Commiphora myrrh* and various other species of *Commiphora* are becoming recognized to possess significant antiseptic,

anesthetic, and antitumor properties. Traditional practice and evidence-based research have supported that these properties are directly attributable to terpenoids (especially furanosesquiterpenes), the active compounds present in *myrrh* essential oil. More recently, current studies have focused on applying clinical trial methodologies to validate its use as an antineoplastic, an antiparasitic agent, and as an adjunct in healing wounds.

Figure 1: Number of *bacterial Isolates*

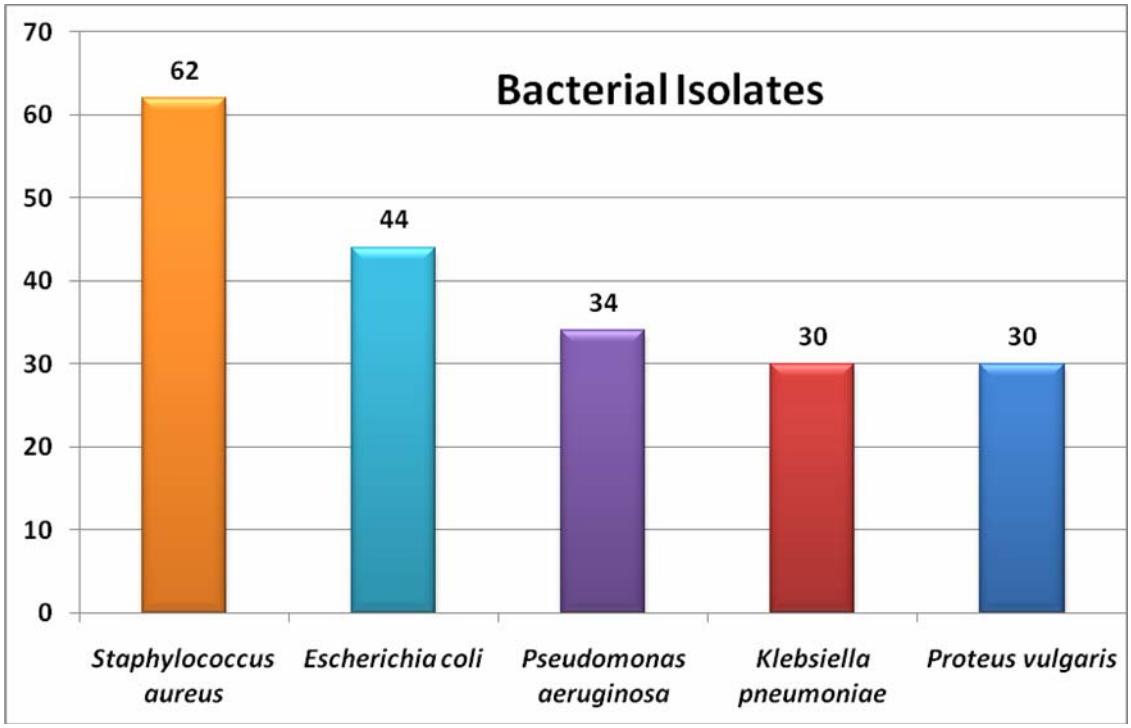


Figure 2: Inhibition of *Staphylococcus aureus* by different concentrations of *Commiphora myrrh*. n=62.

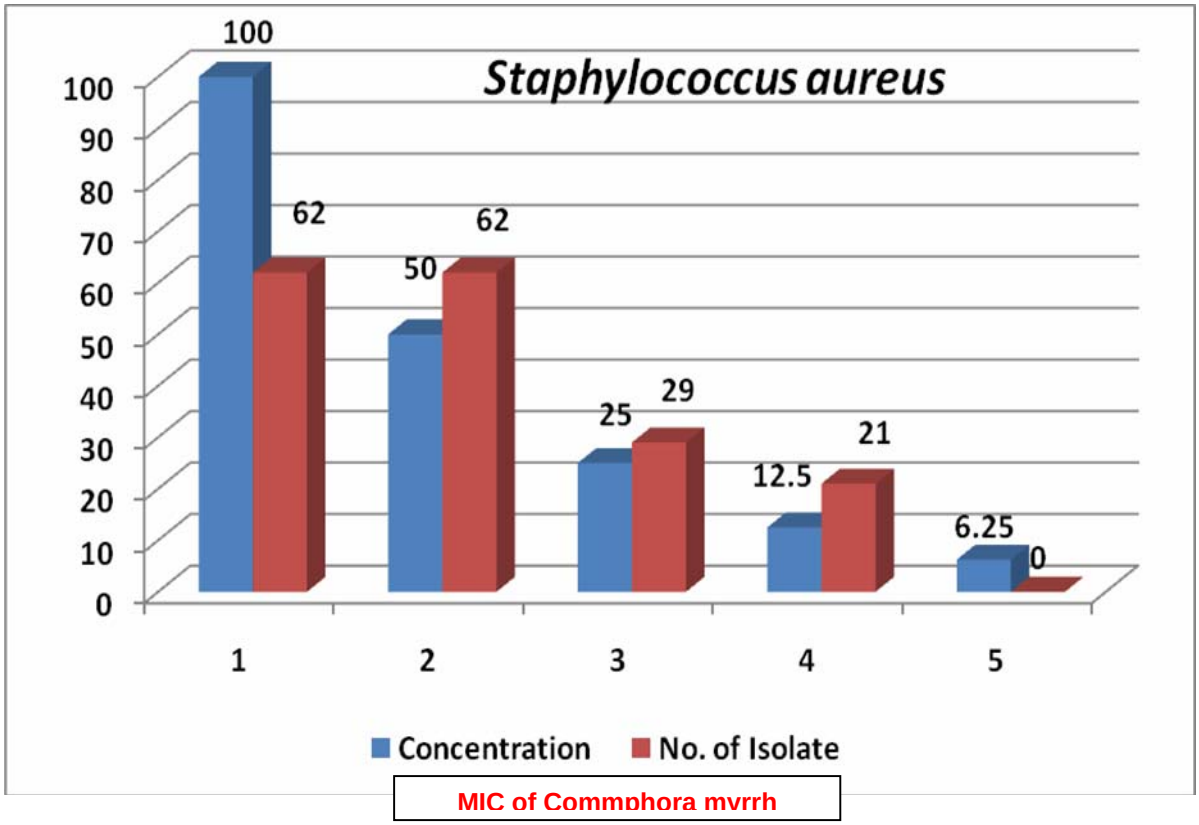


Figure 3: Inhibition of *Escherichia.coli* by different concentrations of *Commiphora myrrh*. n = 44

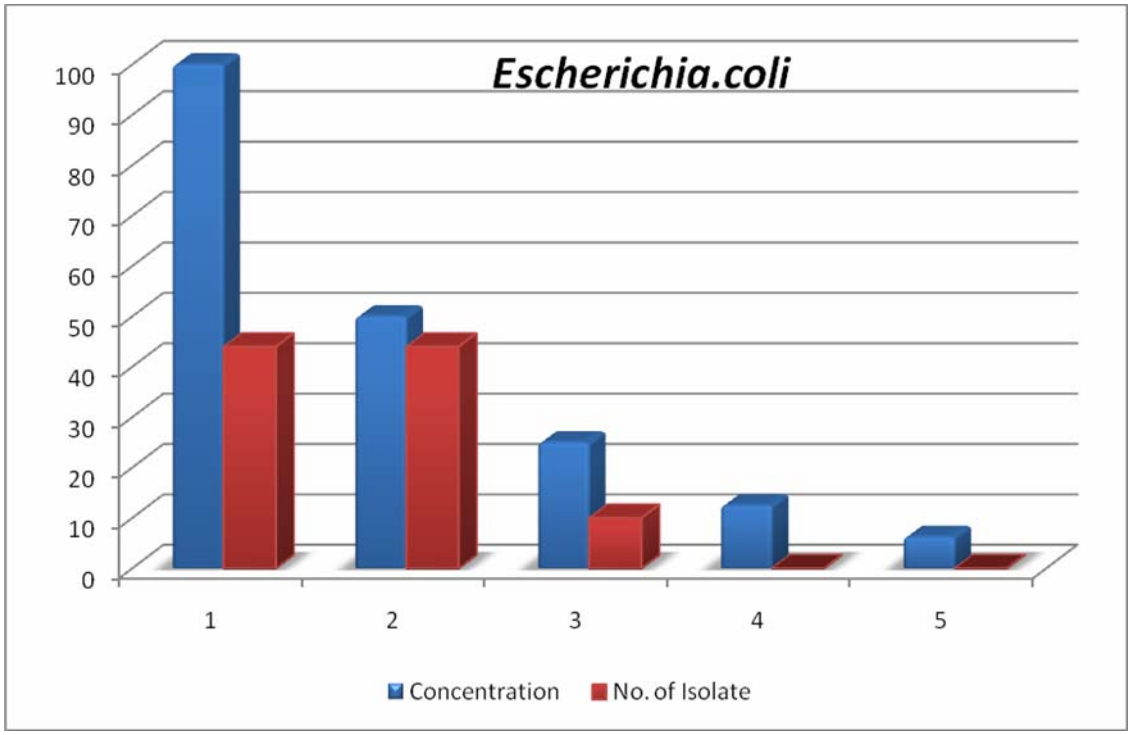
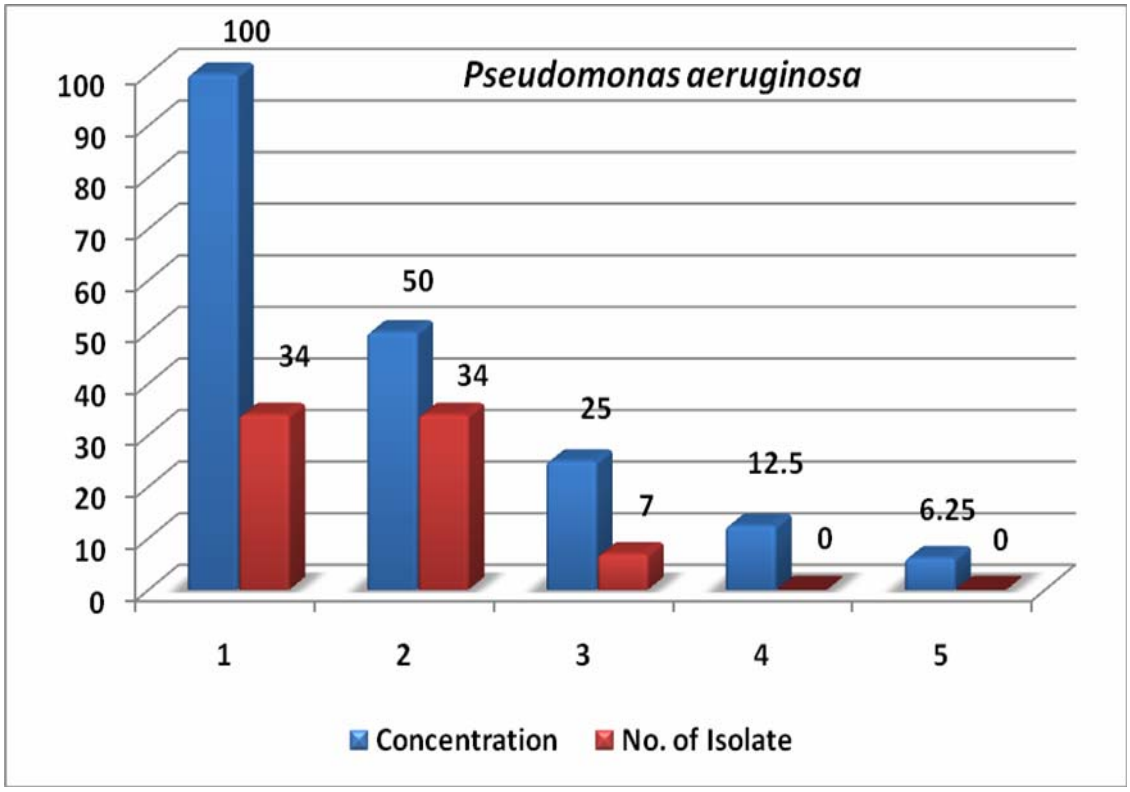


Figure 4: Inhibition of *Pseudomonas aeruginosa* by different concentrations of *Commiphora myrrh*. n = 34



MIC of Commphora myrrh

Figure 5: Inhibition of *Klebsiella pneumoniae* by different concentrations of *Commiphora myrrh*. n =30.

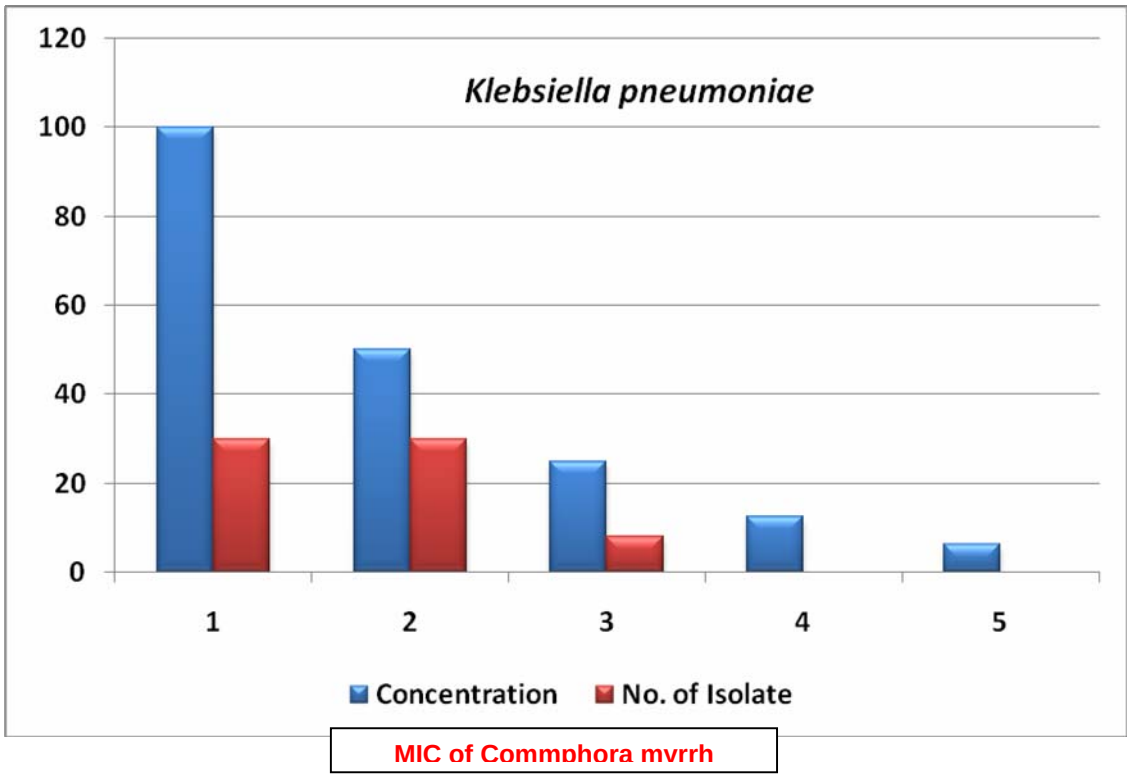
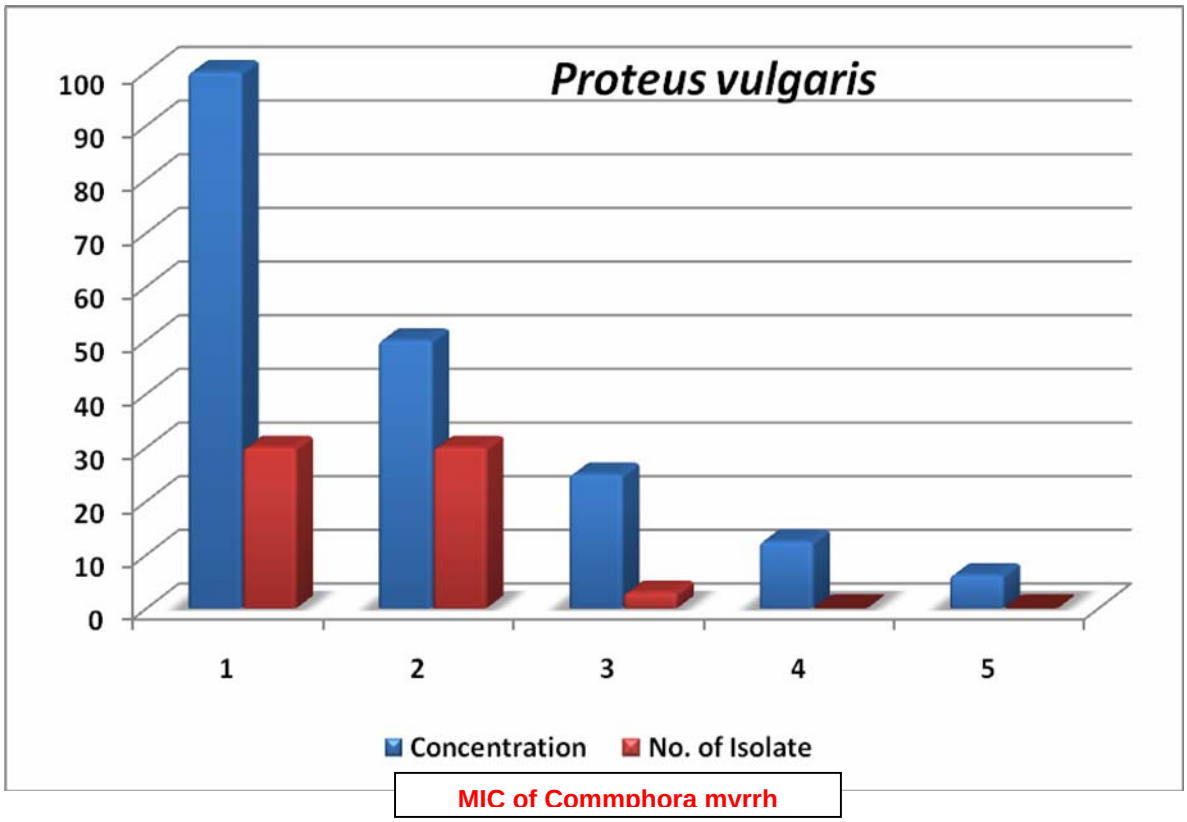


Figure 6: **Inhibition of *Proteus vulgaris* by different concentrations of *Commiphora myrrh*. n =30.**



CONCLUSIONS & RECOMMENDATIONS

Conclusions

Two hundred clinical isolates were obtained from wounds swab after bacteriological procedure, antimicrobial test of *Commiphora myrrh* were subjected by tube dilution method technique against Gram Negative and Gram Positive organisms, this study concluded that:

A, The most common pathogen isolated positive cocci from wounds was S*taphylococcus aureus* .

B, *Commiphora myrrh* is suitable antimicrobial agent against *Staphylococcus aureus* .

C. Medicinal plants remain as a potential source of antimicrobial agents.

Recommendations

According to this study the following recommendations should be considered:

1. Availability and cost of antimicrobial drugs are a real problem for many people in Sudan .Development of resistance to many drugs in current use is another problem that emphasized the need for urgent search for a new and cheap antimicrobial drug.

2. Sudan has huge resources of medicinal plants. Exploitation of these medicinal plants represents an important means of obtaining cheap and effective drugs necessary to resolve the health problems in the Sudan.

3. The results of the present work indicates that there is a promising plant with high antimicrobial activity.

SUGGESTION FOR FUTURE WORK

Pharmacological, toxicological and clinical studies should be carried out on the selected medicinal plants (*Commiphora Myrrh*) to assess their safety, therapeutic efficacy and potential for commercial utilization, as it proved to be a potent wound healing agent.

CHAPTER FIVE

REFERENCES

Ageless. A. (2006). Herbal productsWeb site 04.

Ali-shtayeh,M.S.; Yaghmoar,R.M.R.;Salem,K.and AI Nuri,M.A.(1998) Antimicrobial activity of 20 plants used in folkoric medicine in the Palestinian area.Journal of Ethanopharmacology 60:(3)265-271

Almagboul, A., Z., Farouk, A., & Salih, A., M. (1985). Antimicrobial activity of certain Sudanese plants used in folkloric medicine (1111). *Fitoterapia,* **4**: Pages 195-200.

AL Magboul, A.Z. (1992).Antimicrobial and phytochemical investigation .of Vernonia amygdaline and other Sudanese medicinal plants. PhD, Faculty of pharmacy, university of Khartoum.

Amina, A. (2006). In-Vitro Activity of the third Generation Cephalosporin against Pseudomonas. Msc thesis. Sudan University of science and Technology.

Andrews, **(**1956) .The flowering plants of Anglo-Egyptian, Sudan. Vol I.T. Buncle and co. I TD. Scotland, 237, Pages 485-579.

Ashour, M.S.and Kheiralla, Z.H. (1995) Study of antimicrobial activity of some medicinal plants .Egyptian Journal of Microbiology 30: (3) 455-469.

Atta, A.H., Alkofahi, A. (1998).Anti-nociceptive and anti-inflammatory effects of some Jordanian medicinal plant extracts. Ethnopharmacol, 60(2):117-24.

Baker; F.J. & Silverton, R. E. (1980). Introduction to microbiology In: Introduction to Medical Laboratory Technology 16th (ed). Page 247-286.

Banso & Mann. (2006). *Commiphora Africana*; Alkaloids; Antimicrobial activity Journal of Pharmacy and Bioresources Vol. 3 (2) ; p. 98-102

Baser, K.H.C., Demirci, B., Dekebo, A. & Dagne, E. (2002). Essential Oils of Frankincense, Myrrh and Opopanax Perfu. Flav.

Bettya, F., Daniel, F., Sahm, & Alice, S. W. (2002). Skin, Soft Tissue, and wound infection. In:Diagnostic Microbiology 11th (ed). Page 975.

Charles, V.M., Russell, R.C.G. & Williams, N.S. (1995).Wounds, tissue repair and scars. In:Bailey &love's Chapter 2, 22nd (ed),Page 8 -11. Published in 1995 by Chapman& Hall.London.

Chaves, M. S., Gadanho, R. T. and Jose, C. (1999). Assessment of Metronidazole Susceptiblity in H. pylori :Statistical Validation and Error Rate Analysis of Breakpoints Determied by the Disk Diffusion test American Society for Microbiology.Jan.

Cheesbrough, M. (2003). Biochemical tests to identify bacterial isolates. In: District Laboratory Practice in Tropical Countries part II, Cambridge University press, UK ,page 80.

Cheesbrough, M. (1996).Examination of pus, ulcer material and skin specimens In: District laboratory Practice in Tropical countries part 2.

Cheesbrough,M.(1984) Medical Labrotary Manual for Tropical Countries.

Collee, J. G., Fraser, A. G. Marmion, B. P. & Simmons, A. Practical medical microbiology. Churchil Livingstone. (1996).

Council of Europe. Commiphora myrrha. In: Plants in Cosmetics -Volume II. Germany:

Cruickshank,R.;Duguid,JP.;Marmion,BP.;Swain,RH(1975)Medical Microbiology.12th Edn.
Churchill Livingstone (Pub) Edinburg,London andNewyork.Vol,II

David, I. E. (1980).Assessment of antimicrobial drug action In: Antimicrobial Drug action .D.I. Edwards (ed).The MacMillan Press LTD, London and Basingstoke. Pages 16-18.

David, I E. (1980). Mechanisms of drug resistance. In: Antimicrobial Drug action.
D.I. Edwards (ed).The MacMillan Press LTD, London and Basingstoke. Pages 55-56.

David, I E. (1980).Mechanisms of action .In: Antimicrobial Drug Action .D.I. Edwards (ed).The MacMillan Press LTD, London and Basingstoke. Pages 107-135.

Designed & Maintained by David M. Rollins Copyright © 2000, D.M. Rollins and S.W. Joseph Revised: (2000UR*)*. *http://life.umd.edu/classroom/bsci424*.

Desta,B.(1993)Ethiobia Traditional Herbal Drugs,part II:Antimicrobial activity of 63 medicinal plants .Journal of Ethanopharmacology 39: (2) 129-139.

Dolara, P. (2000).Local anaesthetic, antibacterial and antifungal properties of Sesquiterpenes from *myrrh*. *Plant Media*, 66: 356 – 358 (ref.4120).

Elegail, M.Asrar (1994). Bacteriological analysis and antibiotics sensitivity isolate from wound and ear infection .M.Sc thesis. Khartoum University. Page: 15

El Egami,A.A:Almagboul,A.Z:Omer,M.E.A and El Tohami,M.S. (1998)Sudanase plants used in folkoric medicine:Screening for antimicrobial activity part VIII.Fitoterapia (4) 369-373.

Encarnacion,Dimayuga,R.;Virgen,M.andOchoa,N.(1998)Antimicrobial activity of medicinal plants from Baja California Sur (Mexico).Pharmaceutical Biology.36:(1)33-34.

ESCOP. (2003). Myrrha. In: Monographs. 2nd ed. Exeter: ESCOP, p. 340 - 344 (ref.633.8 (031) ENC).

Farouk,A.;Bashir,A.K.and Salih,A.M.(1983)Antimicrobial activity of certain Sudanase plants used in folkloric medicine,Screening for antibacterial activity part I.Fitoterapia(1) 3-7

Fransworth, N. (1984). Howcan the well be dry when it is full of water? Econ.Botany, 38: pages 4-13

Gad, A. M., El-Dakhakhny, M. & Hassan, M. M.(1963).
Studies of chemical constituents of Egyptian Nigella sativa oil. Plant Med., 11(2): pages 134-136.

Garrod,LP.;Lambert,HP.;O Grady,F.(1981)Antibiotic and chemotherapy 5th Edn Churchill Livingstone (Pub).Edinburg.

Gilman, A.G., Goodman, L.S. & Gilman, A. (1980).Chemotherapy of microbial disease. In: The Pharmacological Basis is of Therapeutic .A.G. Gilman, L.S. Good man &A. Gilman (ed), MacMillan Pub. Co., London, 6 th(ed).1080.

Greenwood, D., Richard. S .C.B. & Peutherer, J .F. (2002).Bacterial genetics .In: Medical Microbiology 16th(ed)
David,Greenwood,Richard.C.B.Slack&John.F.peutherer(eds).Churchill Livingstone. Pages 61-72.

Greenwood, D. Richard, C., Slack, B., John, F. & Peuthere (2003). Medical Microbiology. Churchill Livingstone.

Greenwood, D.;Slack, R.and Pentherer, J.F.(1997)Medica l Microbiology.Churchill,. Livingstone,NewYork,Edinburgh,London,Madridand Tokyo. Fifteen Editions

Hanus, L.O., Rezanka, T., Dembitsky, V. M. & Moussaieff, A. (2005*).Myrrh – Commiphora* Chemistry. In: Biomed. Papers 149(1), 3-2

Isago, T., Nozaki, M., Kikuchi, Y., Honda, T. & Nakazawa, H. (2003).Negative-pressure dressings in the treatment of pressure ulcers. *J Dermatology*, 30(4): pages 299-305.

Jawetz, M. N. & Adel, B. (2004).Pathogenesis of Bacterial Infection. In: Medical Microbiology 23th (Ed) Pages 147.

Jawetz,E.;Melnick,J.L.;Adelberg,E.A.;Brooks,G.F.;Butel,J.S.and Ornston,L.N.(1995) Medical Microbiology. 20th.Ed.Brooks,GF;Butel,Js; Omston,LN.Appleton and Lange,Norwalk,California;Librarie du Liban,Beirut,Lebanon.

Kavanagh, E. (1972) Analytical microbiology.Vol.11, Academic Press, New York and London.

Koelblin-Fortuna-Druck, 2001; Council of Europe. *Commiphora myrrha*. In: Plants in Cosmetics - Volume II. Germany: p.57 - 60 (ref.68*1ANT).

Martinez,M.J.:Betancourt,J.:Alonso-Gonzalez,N.and Jauregai,A.(1996) Screening of some Cuban medicinal plants for antimicrobial activity. Journal of Ethanopharmacology 52: (3) 171-174.

Meurur-Grimes,B;McBeth,D.L.;Hallihan,B.and Delph,S.(1996) .Antimicrobial plants of the Scorphulariaceae and Acanthaceae International Journal of Pharmacognosy 34:(4)243-248

Mile,A.A.;Misra,S.S (1938) .The estimation of the bactericidal power of the blood.Journal of Hygein 38:732.

Mitscher, L.A., Park, Y.H., Clark, D.,Clark, G.W., Hammersfaler, W.U.W.N.& Beal, J.L.(1978). Antimicrobial agents from higher plants. An investigation of *Hunnemannia fumerariaefolia.* In: Lioydia. 41: pages 145-148.

Mills, S.Y. (1993). *The A-Z of Modern Herbalism*, Diamond Books, London .

Mona, A. Mohamed. (2000). Anti microbial agents, preparation of extract. In: Biological Activity of Nigella Sativa. Page 20. PhD, Faculty of pharmacy, university of Khartoum.

NCCLS MIC Testing Supplemental Tables M 100-S13 (M7) January 2003

Nissl, J. R.N. & David, B.S.W. C. (2004) .Infectious Disease. MSc, MD, FRCPC

Nomicos, E. Y (2007). Myrrh: medical marvel or myth of the Magi?. Holist Nurs Pract . (21). 308-23.

Obame, L. C., Koudou, J., Chalchat, J. C., Bassolé, I., Edou, P., Ouattara, A., S., & Traore, A. S. (2007) Volatile components, antioxidant and antibacterial activities of *Dacryodes buettneri* H. J. Lam. essential oil from Gabon. *Scientific Research and Essay*; 2 (11): 491-495.

Ody, P. (1993). *The Herb Society's Complete Medicinal Herbal*, Dorling Kindersley, London.

Omer,M.E.A;Al Magboub,A.Z.and El Egami,A.A.(1997)Sudanese plants used in folkloric medicine:Screening for antibacterial activity part (VII).Fitoterapia(6) 549-554.

Pagana, K. D. (1998).Wound culture. In: Mosby's Manual of Diagnostic and laboratory tests.

Pagana, K. D. Mosby's(1998).Manual of Diagnostic and Laboratory Tests. St. Louis: Mosby, Inc.

Pelczar, M.J. Reid, R. D. & Chan,E.S.I.(1977). Microbiology.M.J.Pelczar;R.D.Reeir and E.S.I.chan (Eds)Mc.Graw-Hill Book Company.New York.4th edition. Page Page.414

Phillips, J.D. (1992).Medicinal plants. Biologist.39:187-191.

Raja, E. O. (2000) Preparation of standard bacterial suspensions in: Antimicrobial Activity of Sudance Medicinal Plant .Page 25

Science and Technology Encyclopedia information about infection McGraw-Hill Encyclopedia of Science and Technology. Copyright © 2005 by The McGraw-Hill Companies, Inc

Sheir, Z. A. (2001). Safe, effective, herbal antischistosomal therapy derived from *myrrh* Amer. *J. Trop. Med. Hyg*, 65(6): 700 – 704 (ref. 6753).

Sleigh,J.D; and Timbury , M (1994).Notes on medical microbiology.4th edition.Churchill Livingstone,UK.

Sofowora, A.,(1982).Medicinal plants and traditional medicinal in Africa John Wiles and Sons Limited, chichester.

Tariq,M.,Ageel,A.M.,Al-Yahya,M.A.,Mossa,J.S.,Al-Said,M.S.,Parmar,N.S.(1986).Anti-inflammatory activity of *Commiphora molmol.*Agents&Actions,17(3-4):381-2.

Torpy, J.M., Alison Burke, B. M.A. & Glass, R.M. (2005).Wound infections. *JAMA*, 294 (16): page2122.

Tortora, J. & Funke, C. (2004). Microbial disease of the skin. In: Microbiology an Introduction 8th (ed). Page 590-594.

Tipton, D.A., Lyle, B., Babich, H. & Dabbous, Kh.M. (2003). In vitro cytotoxic and anti-inflammatory effects of *Myrrh oil* on human gingival fibroblasts and epithelial cells, Toxicology, 17 :(3), pages 301- 310.

UNESCO. (1994).Traditional Knowledge in tropical Environment, Natural &Resource, volume 30, No1, UNESCO, Paris.

Yang Yfang (2000) Chinese Herbal Medicine Comparisons and Characteristics ,Churchill Livingstone , London.
http://en.wikipedia.org/wiki/Talk:Myrrh