

# 1. INTRODUCTION

## 1.1. Introduction

Wound healing can no longer be thought of as a generic term. Non healing wound found elevated levels of inflammatory cytokines, proteinases and low level of growth factor activity compared with fluids collect from acute. The wounds healing society has stated that when wounds proceed through these processes in an orderly and timely manner and achieve sustained anatomic and functional integrity, they are considered acute infection. When they either do not proceed in an orderly and timely fashion or do so without achieving sustained anatomic and functional integrity, they are considered chronic (Martain and Shaw, 2009).

Nosocomial infection represents an important public health problem in developing countries. It is one of the key reasons why wound healing may still leading to increased risk of patient morbidity and mortality (Yalcin , 2003).

The impact of microorganism on chronic wound has been extensively studied and reviewed using different approaches to elicit their possible role in non-healing, these have a range from highlight the occurrence of particular species (Falange, 2004).

For a long period of time, a plant has been a valuable source of natural products for maintaining human health, especially in the last decade, with more intensive studies for natural therapies. The use of plant compound for the pharmaceutical purpose has gradually increased in the world according to the World Health Organization (WHO, 2005).

The use of plant extract and phytochemicals, both with known antimicrobial properties can be of great significance in therapeutic of the traditional medicinal plant in Sudan (Elhassan *et al.*, 2016).

## **1.2. Rationale:**

The problem of microbial resistance is growing and the outlook for use of antimicrobial drugs in the future is still uncertain. Therefore, action must be taken to reduce this problem, for example, to control the use of antibiotic, develop research to better understand the genetic mechanism of resistance, and to continue studies to develop new drugs, either synthetic or natural (Spellberg *et al.* , 2008).

Furthermore, Antibiotic resistance is a global challenge that impacts all pharmaceutically used antibiotic. In recent years' pharmaceutical companies have almost stopped producing new antibiotics which have led researchers to look for alternative antimicrobial. Herbs were used for the treatment of infectious diseases in many developing countries ( Khetan and Collins., 2007).

Therefore, in Sudan, with high percentage of multidrug resistant bacteria, we in urgent need to develop new drug from our traditional medicine.

Three Sudanese medicinal plants namely *Azadirachta indica*, *Cymbopogon proximus* and *Guiera senegalensis* may possess antibacterial activity because it used in rural medical care for treatment of many infectious and chronic diseases. Thus, to verify the antibacterial activity of those plant against resistant bacteria isolated from wound infection.

### **1.3. Objectives:**

#### **1.3.1. General objective:**

To study the antimicrobial activity of *Guiera Senegalensis*, *Azadirachta indica* and *Cymbopogon proximus* methanolic extracts on selected multidrug resistant pathogenic bacteria from wound infection in Khartoum State, Sudan using agar diffusion method during the period from February to May 2018.

#### **1.3.2. Specific objectives:**

- 1- To isolate and identify the pathogenic bacteria from wound infection.
- 2- To evaluate *in vitro* susceptibility pattern of bacterial isolated from wound specimen against multidrug.
- 3- To determine the prevalence of resistant bacteria in wound infection.
- 4- To compare between the antibacterial activity of *Guiera senegalensis*, *Azadirachta indica* and *Cymbopogon proximus* extracts on selected pathogenic bacteria.
- 5- To determine the minimum inhibitory concentration (MIC) of *Guiera senegalensis*, *Azadirachta indica*, and *Cymbopogon proximus* extracts and compare their activity with the common used antibacterial agent in Sudan.

## **2. LITERATURE REVIEW**

### **2.1. Herbal Medicines:**

#### **2.1.1. Definition:**

Herbal Medicine, the global popularity of herbal supplements and the promise they hold in treating various disease states has caused an unprecedented interest in understanding the molecular basis of the biological activity of traditional remedies. Herb plants produce and contain a variety of chemical substance that acts upon the body. The substance derived from the plants remain the basis for a large proportion of the commercial medications used today for the treatment of heart disease, high blood pressure, pain, asthma and other problem. For example, ephedra is herb used in traditional Chinese Medicine for more than two thousand years to treat asthma and other respiratory problem (Benzie and Galor. 2011).

#### **2.1.2. Antimicrobial Properties of Medicinal Plants:**

Medical plants represent a rich source of antimicrobial agent. Plants are used medicinally in different countries and are a source of many potent and powerful drugs.

A wide range of medicinal plant parts is used for extract as raw drugs and they possess varied medicinal properties. The secondary metabolism of the plant was found to be a source of various phytochemical that could be directly used as intermediates for the production of new drugs (Chandra, 2013).

For a long time, a plant has been a valuable source of natural products for maintaining human health, especially in the last decade, with more intensive studies for natural therapies. The plant compounds for

pharmaceutic purposes has gradually increased in the world according to the World Health Organization (Gislene *et al.*, 2000).

### **2.1.3. *Cymbopogon proximus***

*Cymbopogon proximus*, Family Poaceae, is a perennial aromatic plant, mostly native to the Old World Tropics, and constitutes an important proportion of Savannah grass. In Sudanese traditional medicine, *Cymbopogon proximus* is commonly known as Maharieb which is used in same part of Sudan to treat intestinal spasm and kidney stone, the plant inflorescence decoction is used to treat kidney pains and urethritis. Also, this plant is intensively used in the folk medicine in Sudan for gout, renal colic, helminthiasis, diuresis, inflammation of the prostate and as antipyretic. (Elhassan *et al.*, 2016).

Proximadiol is a C<sub>15</sub> H<sub>20</sub>O<sub>2</sub> dicyclic sesquiterpenediol separated from the unsaponifiable matter fraction of the petroleum ether extract of *Cymbopogon proximus*, this compound proved to have unique antispasmodic characteristics and could be used for the propulsion of renal and ureteric calculi (Maghrabi *et al.*, 2014).

*Cymbopogon proximus* (Gramineae) is a weed known as Halfabar that grows widely in Sudan. Bioactivity- assisted fractionation of the hexane extract leads to isolation of an active principle, proximal (0.02% yield) and bicyclic sesquiterpene diol chemical structure was confirmed by the spectral data. In addition, two sesquiterpenes, elemol and bata-eudesmol, were also isolated from the unsaponifiable fraction of the fatty matter of the plant (Hesham *al et.*, 2003).

There were previous studies shown of the *Cymbopogon proximus* capable of induced hypoglycemia on the activity enzymes in the liver of diabetic rats and treatment increased the hepatic activity ( Hamdy *et al.*, 2002).

#### **2.1.4. *Azadirachta indica*:**

Neem (*Azadirachta indica*) is perhaps the most useful traditional medicinal plant in India. Each part of the neem tree has some medicinal property and is thus commercially exploitable. During the last five decades, apart from the chemistry of the neem compound, considerable progress has been achieved regarding the biological activity and medicinal applications of neem. It's now considered as a valuable source of unique natural products for the development of medicines against various disease and also for the development of the industrial product (Kauslk *et al.*, 2002).

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The various chemical agent has been evaluated over the years with respect to their antimicrobial effects in the oral cavity; however, all are associated with side effects that prohibit regular long-term use. Therefore, the effectiveness of neem (*Azadirachta A. Juss*) leaf extract against plaque formation was assessed in males between the age group of 20-30-years over a period of 6 week. The present study includes the formulation of mucoadhesive dental gel containing *Azadirachta indica* leaf extract to evaluate the efficacy o f neem and treat the dental bacteria carrier like *Streptococcus mutans* and *Lactobacilli* (Raveendra , 2004 and Joshi *et al.*, 2011).

It provides many useful compounds that are used pesticides and could be applied to protect stored seed against the insect. However, in addition to

the possible beneficial health effect, such as blood sugar lowering, antiparasitic, anti-inflammatory, antiulcer and hepatoprotective (Daniel *et al.*, 2001).

*Azadirachta indica* were screened and evaluated for their antimicrobial activity against pathogenic fungi and bacteria. and shown the most activity against all gens of *Streptomyces* and *E. coli* and *Pseudomonas aeruginosa* (Vijary *et at.*, 2009).

#### **2.1.5. *Guiera Senigalensis*:**

It plays an essential and multiple roles in the balance of the Sahelian and Sudanese ecosystems exploited by man and his animal, of the behavior of cattle, sheep and goats on natural pasture ( Sanon *et al .*, 2007).

Effective treatment of diabetes is increasingly dependent on active constituents of *Guiera senigalensis* capable of controlling hyperglycemia as well as is secondary complications (Arulrayan *et al .*, 2007).

## **2.2. Wound infection**

### **2.2.1 Skin and Soft Tissue Infection**

Skin and soft tissue infection are some of the most common infectious diagnoses and result in hundreds of thousands of medical office and emergency room visit each year. These infections often occur following a break in normal skin integrity from either trauma or skin disease. The vast majority of these infections are caused by *Staphylococcus aureus* and *Streptococcus pyogenes*. Hematogenous seeding of organism into the skin can occur but is uncommon (Brain and Schwart, 2016).

Infections among surgical patients remain a serious problem today. Urinary tract infection accounts for approximately 40 percent of nosocomial infection among surgical patients. Surgical wound and skin infections

account for one-third of the nosocomial infections among surgical patients. Rates for wound infection rise with age, with increased length of hospitalization before surgery, and with increased duration of surgery. They are higher for the patient who has an infection at a distant site and for those who have the more hazardous surgical procedures as determined by risk categories. Gram-negative are organism more prevalent than gram-positive organism (Philip , 2002).

### **2.2.2. Clinical signs and symptoms of wound infection**

It is uncertain how accurately classic signs of acute infection identify infection in the chronic wound, or if the signs of infection specific to secondary wounds are the better indicator of infection in these wounds. The examine the validity of the classic signs (i.e., pain, erythema, edema, heat and purulence) and the signs specific to secondary wound (i.e., serous exudate, delayed healing, discoloration of granulation tissue, friable granulation tissue, foul odor and wound breakdown (Sue *et al.*, 2001).

## **2.3. Types and epidemiology wound infection:**

### **2.3.1. Surgical wound infection:**

Surgical site infections (SSIs) are defined as infections occurring up to 30 days after surgery (or up to one year after surgery in patients receiving implants) and affecting either the incision or deep tissue at the operation site. Despite the improvement in prevention, SSIs remain a significant clinical problem as they are associated with substantial mortality and morbidity and impose severe demands on healthcare resources (Owens and Stoessel, 2008).

From the previous study included 676 surgery patients with signs and symptoms indicative of wound infection, who presented over the course of 6 years. They reported that, the bacterial pathogen was isolated from 614



individuals. A single etiologic agent was identified in 271 patients, multiple agents were found in 343, and no agent was identified in 62. A high preponderance of aerobic bacteria was observed. Among the common pathogen were *Staphylococcus aureus* (191 patients, 28.2%), *Pseudomonas aeruginosa* (170 patients, 25.2%), *Escherichia coli* (53 patients, 7.8%) *Staphylococcus epidermidis* (48 patients, 7.1%) and *Enterococcus faecalis* (38 patients, 5.6%) (Giacometti *et al.*, 2000).

An estimated 2 million patients develop nosocomial infections in the United States annually. The increasing number of the antimicrobial agent-resistant pathogen and high-risk patients in the hospital are challenging to progress in preventing and controlling these infections. While *Escherichia coli* and *Staphylococcus aureus* remain the most common pathogen isolated overall from nosocomial infection, coagulase-negative *Staphylococci* (CoNS), organisms previously considered contaminant in most cultures, are now the predominant pathogens in bloodstream infection. The growing number of antimicrobial agent-resistant organisms is troublesome, particularly vancomycin-resistant, CoNS and *Enterococcus spp* and *Pseudomonas aeruginosa* resistant to imipenem. The active involvement and cooperation of the microbiology laboratory are important to the infection control program, particularly in surveillance and the use of laboratory service for epidemiologic purposes (Huang and platt, 2003 ).

### **2.3.2. Burn wound infection:**

The burn wound infection represents a susceptible site for opportunistic colonization by organisms of endogenous and exogenous origin. Burn wound infections are serious complication of thermal injury, although pneumonia is now most important infection in patient with burns (Weinstein and Mayhall 2003).

Burn infections an important cause of mortality in burns emerging nosocomial pathogens and problem of multi drug resistance. Diagnostic procedure and therapy must be based on an understanding of the pathophysiology of burn wound and the pathogenesis of various forms of burn wound infection. The time-related changes in the predominant flora of burn wound from gram-positive to gram-negative recapitulate the history of burn wound infection. Proper clinical and culture surveillance of the burn wound permits early diagnosis of gram-positive cellulitis and the stable susceptibility of Beta hemolytic *Streptococci* to penicillin has eliminated the threat of this once common burn wound pathogen. Selection and dissemination of intrinsic and acquired resistance mechanisms increase the probability of burn wound colonization by resistant species such as *Pseudomonas aeruginosa* (Mehta *et al.*, 2007).

*Pseudomonas aeruginosa* plays a prominent role as an etiological agent involved in serious infection in burned patients. In previous studied, *P. aeruginosa* infection was analyzed at the Motahari Burn Center in Tehran (from 22 December 1998 to April 1999) to estimate their frequency, antibiotic susceptibility, and serotypes. One hundred and eighty-four positive culture and 205 bacterial strains were isolated among swabs or biopsy specimens during the study period. *Pseudomonas* was found to be the most common (57%) followed to be the *Acinetobacter* (17%), *Escherichia coli* (12%), *Staphylococcus aureus* (8%) and other organisms (6%) (Hamid *et al.*, 2002).

A retrospective study of major aerobic bacterial isolated from pus/wound swabs taken from patients admitted to burn unit at Govt. Medical College Hospital, Chandigarh, India, over the period of 5 years (June 1997-May 2002) was undertaken. The study was carried out to determine the bacterial profile and antimicrobial susceptibility of the isolates and to describe the

change in trends over the study period. The pus/wound swabs yielded very high culture positivity (96%) for 665 total isolates. *Pseudomonas aeruginosa* was found to be the most common isolate (59%) followed by *Staphylococcus aureus* (17.9%), *Acinetobacter spp.* (7.2%), *Klebsiella spp.* (3.9%), *Enterobacter spp.* (3.9%), *Proteus spp.* (3.3%) and other (4.8%). Although *P. aeruginosa* continued to remain the predominant isolate over the five years, constant and significant increase in the incidence of *Acinetobacter spp* (Agnihotri *et al.*, 2004).

### **2.3.3. Diabetics wound infection:**

One of the leading causes of impaired wound healing is diabetes mellitus, unlike other cause of altered tissue repair and multiple factors that contribute to the impairment. However, only bacteriuria can be documented to occur with increases in frequency in diabetic compared with non-diabetic (Greenhalgh, 2003).

Infection foot ulcer is a common cause of morbidity in diabetic patients, ultimately leading to the dreaded complication like gangrene and amputation. Lifetime risk to a person with diabetes for developing a foot ulcer could be as high as 25%. Infection is most often is that a consequence of foot ulceration, which typically follows trauma to a neuropathic foot (Ekta *et al.*, 2008).

The polymicrobial nature of diabetic foot infection has been a good document in the literature. Patients with diabetic foot infection do not expose to antibiotics are not well studied before. The relative frequency of bacterial isolates cultured from the community-acquired foot infection that are not exposed to antimicrobial; agent for 30 days is studied. In addition, the bacterial comparative *in vitro* susceptibility to the commonly used (Abdulrazak *et at.*, 2005).

One of the previous study was a prospective study in which the infected wound of 86 consecutive diabetic patient seen in the diabetic foot clinic in Adan Teaching Hospital was cultured when visiting the clinic. The patients did not receive antimicrobial therapy 30 days prior taken cultured using aerobic and anaerobic microbiological techniques. Isolates were tested for susceptibility therapy. *Staphylococcus aureus* was the most common isolate, being recovered from 38% of cases. Other organisms were *Pseudomonas aeruginosa* (17.5%) and *Proteus mirabilis* (18%), anaerobic gram-negative organisms (10.5%), mainly *Bacteroides fragilis* (Abdulrazak *et al.*, 2005).

As part a United States-based multicenter clinical trial, conducted from 2001 to 2004, they obtained 454 pretreatment specimens from 433 patients. After were collected from wound specimens, isolation and identification Among the 427 positive culture, 83.8% were polymicrobial, 48% grew only aerobes, 43.7% had both aerobes and anaerobes, and 1.3% had only anaerobes. The predominant aerobic organisms were oxacillin-susceptible *Staphylococcus aureus* (14.4%) oxacillin-resistant *Staphylococcus aureus* (4.4%) coagulase-negative *Staphylococcus* species (15.3%), Streptococcus species (15.5%), Enterococcus species (13.5%), *Corynebacterium* species (10.1%), member of the family Enterobacteriaceae (12.8%), and *Pseudomonas aeruginosa* (3.5%). The predominant anaerobic were gram-positive cocci (45.2%), *Provetella* species (13.6%), *Porphyromonas* species (11.3%), and the *Bacteroides fragile* group (10.2%) (Citron *et al.*, 2007).

#### **2.4. Complication of bacterial in wound infection:**

The incidence of wound infection consider as one of the most serious common complication, the frequency and spectrum of antimicrobial-

resistant infection have increased in both the hospital and community (Yadla *et al.*, 2011).

Burn is one of the most common and devastating forms of trauma. Patients with serious thermal injury require immediate specialized care in order to minimize morbidity and mortality. Significant thermal injuries induce a state of immunosuppression that predisposes burn patients to infectious complication. A current summary of classification of burn wound infection, including their diagnosis, treatment, and prevention is given. Early excision of the Escher has substantially decreased the incidence of invasive burn wound infection and secondary sepsis, but most deaths in severity burn-injured patients are still due to burn wound sepsis or complication due to inhalation injury. Burn patients are also at risk for developing sepsis secondary to pneumonia, catheter-related infection and suppurative thrombophlebitis (Deirdre *et al.*, 2006).

Chronic ulceration of the lower leg is a frequent condition, with a prevalence of 3-5% in the population over 65 years of age. The incidence of ulceration is rising as a result of the aging population and increased risk factors for atherosclerotic occlusion such as smoking, obesity, and diabetes. Ulcers can be defined as wound with a full thickness depth and a slow healing tendency ( Gohel and Poskitt, 2010).

In general, the slow healing tendency is not simply explained by depth and size, but caused by an underlying pathogenic factor that needs to be removed to induce healing. The main causes are venous valve insufficiency, lower extremity arterial disease, and diabetes. Less frequent conditions are an infection, vasculitis, skin malignancies and ulcerating skin diseases such as pyoderma gangrenous (Mekkes *et al.*, 2003).

Necrotizing fasciitis is an uncommon soft tissue infection, usually caused by toxin-producing, virulent bacteria, which is characterized by widespread fascial necrosis with relative sparing of skin and underlying muscle. It accompanied by local pain, fever, and systemic toxicity and is often fatal unless promptly recognized and aggressively treated (Wong *et al.*, 2003).

Foot infection in patients with diabetes cause substantial morbidity and frequent visit to health care professionals and may lead to amputation of a lower extremity. The major predisposing factor in these infections is foot ulceration, which usually related to peripheral neuropathy. Peripheral vascular disease and various immunological disturbance play a secondary role ( Boulton *et al.*, 2005)

Aerobic gram-positive cocci especially *Staphylococcus aureus* are predominant pathogens in diabetic foot infection. Patients who have the chronic wound or have recently received antibiotic therapy may also be infected with gram-negative rods, and those with foot ischemia or gangrene may have obligate anaerobic pathogen (Benjamin *et al.*, 2004).

## **2.5. Resistant bacterial cause wound infection:**

### **2.5.1. *Staphylococcus aureus*:**

It is Gram-positive, aerobic and also grow anaerobically but less well. Temperature range for growth (10-42°C), with optimum of (35-37°C). It grows on blood agar producing creamy to yellow colonies, occasionally white with diameter 1-2 mm. In MacConky's agar, it produces smaller colonies (0.1-0.5mm). It ferments mannitol to give yellow color, it is coagulase, catalase and DNase positive. It causes boils, pustules, impetigo, infection of wound, ulcer and burns, osteomyelitis, mastitis, septicemia, meningitis and pneumonia. It is carried in the nose of 40% or more of healthy people. (Cheesbrough, 2005).

More than 90% of *S. aureus* strain contain plasmids that encode beta-lactamase, the enzyme that degrades many, but not all, penicillin. Some strain of *S. aureus* are resistant to beta-lactamase-resistant penicillins, such as methicillin and nafcillin, by virtue of the change in the penicillin-binding protein (PBP) in their cell membrane. Gene on the bacterial chromosome called *mecA* genes encode these altered PBPs (Levinson, 2016).

*S. aureus* has several important wall components and antigens:

(1) Protein A is the major protein in the cell wall. It is an important virulence factor because it binds to the Fc portion of IgG at the complement-binding site, thereby preventing the activation of complement.

(2) Teichoic acids are polymers of ribitol phosphate. They mediate adherence of the *Staphylococci* to mucosal cells. Lipoteichoic acids play a role in the induction of septic shock by inducing cytokines such as IL-1 and TNF from macrophage.

(3) Polysaccharide capsule is also an important virulence factor.

(4) Surface receptors for specific *Staphylococcal* bacteriophage permit the phage typing of strain for epidemiologic.

(5) The peptidoglycan of *S. aureus* has endotoxin-like properties and stimulate macrophage to produce cytokines, activate the complement and the coagulation cascade (Levinson, 2016).

### **2.5.2. *Pseudomonas aeruginosa*:**

*P. aeruginosa* is a versatile pathogen associated with a broad spectrum of infection in humans. It is gram-negative, non-spore forming rod; usually a single polar flagellum. Fimbriae may be present and usually polar and non-haemagglutination (Kerr and Snelling, 2009).

*P. aeruginosa* are resembled the members of Enterobacteriaceae by differing in that they are strict aerobes (i.e. they derive their energy only by oxidation of sugars rather than fermentation). Because they do not ferment glucose, they are called nonfermenters, in contrast to members of the Enterobacteriaceae, which do ferment glucose. Oxidase-positive (Levinson, 2016).

*P. aeruginosa* is obligatory aerobe. It usually recognized by the pigment it produces including pyocyanin a blue-green pigment, and pyoverdine (fluorescein) a yellow-green fluorescent pigment. A minority of strain is pigment. Culture has a distinctive smell due to the production of 2- amino acetophenone. *P. aeruginosa* is growing over a wide range 6-42°C with an optimum of 35-37°C. It is grown in blood ager produce large, flat, spreading colonies which are often hemolytic and usually (90% of strain) pigment producing. Some strain produces small colonies or mucoid colonies when the culture is left at room temperature. And in MacConkey agar produce pale color colonies. In CLED agar produce a metallic appearance), and pink-red butt is produced. No gas is formed and no H<sub>2</sub>S is produced. (Cheesbrough, 2000).

*P. aeruginosa* causes infection sepsis, pneumonia, and urinary tract infection. Primary in patients with lowered host defenses. It also chronic lower respiratory tract infection in patients with cystic fibrosis, wound infection (cellulitis) in burn patients and malignant otitis externa in diabetic patients (Levienson, 2016).

### **2.5.3. *Proteus spp*:**

It's gram-negative, actively motile, non-capsulate. In blood agar when cultured aerobically, most *proteus* culture have a characteristic fishy odour and swarming on blood agar. Its MacConkey produce individual non-lactose fermenting colonies after overnight incubation at 35-37°C.



Swarming is prevented on MacConkey agar because these media contain bile salt (Cheesbrough, 2000).

*Proteus spp* is distinguished from other members of the Enterobacteriaceae by their ability to produce the enzyme phenylalanine deaminase. In addition, they produce the enzyme urease, which cleaves urea to form  $\text{N H}_3$  and  $\text{CO}_2$  (Levienson, 2016).

#### **2.5.4. *Escherichia coli*:**

The primary habitat of *E. coli* is the vertebrate gut, where it is the predominant aerobic organism, living in symbiosis with its host, non-spore-forming bacilli that grow both aerobically and anaerobically on ordinary laboratory media, including MacConkey's lactose bile –salt agar; they are oxidase negative and catalase positive; they ferment glucose and other carbohydrates in peptone water with production of acid or acid and gas, they reduce nitrates to nitrites and either motile with peritrichous flagella or non- motile ( Denamur, 2010).

#### **2.5.5. *Klebsiella pneumonia*:**

*K. pneumonia* is non-motile, it's facultative anaerobe, but growth under aerobic conditions is rare. It ferments glucose and produce urease enzyme, but negative for methyl red, and now recognized as an urgent threat to human health because of the emergence of multidrug- resistant strains associated with hospital outbreaks and hypervirulent strains associated with sever community-acquired infection (Holt *et al.*, 2015).

### **3. Material and Method**

#### **3.1. Study type and design**

The study was a descriptive and cross-sectional study.

#### **3.2. Study area and duration:**

This study was carried out in Omdurman Military Hospital and Jabir Abulaiz in Khartoum State, Sudan, during the period from February to May 2018.

#### **3.3. Study population:**

Patients admitted with symptom of chronic diseases of wound infections.

#### **3.4. Inclusion criteria:**

Patients admitted with wound infection.

#### **3.5. Exclusion criteria:**

Patient under antimicrobial treatment were excluded.

#### **3.6. Sampling:**

Simple random sample.

#### **3.7. Sample size:**

A total of one hundred sample (n=100) were collected.

#### **3.8. Data collection:**

Data were collected from the patients using data collection form containing all study Variables (Appendix 2)

#### **3.9. Ethical considerations:**

Permission was issued by the Medical Laboratory Science Collage's Ethical Committee, Sudan University of Science and Technology.

### **3.10. Specimen Processing:**

#### **3.10.1. Collection of specimens:**

Under the aseptic condition, wound swabs was collected using sterile cotton swabs moistened with sterile normal saline.

#### **3.10.2. Cultivation of specimens:**

Wound specimens were cultured on Blood ager and MacCnoky's ager, inoculated plates were incubated aerobically and facultative an aerobically at 37°C for 18-24 hours (Angel *et al.*, 2011).

#### **3.10.3. Identification of the isolates:**

##### **3.10.3.1. Culture characteristics:**

After the incubation period, the plates were examined for the size, color, edges, side views, odor and surface of the colonies.

##### **3.10.3.2. Gram stain:**

Smears from the growth were prepared and stains by Gram's stain as follow: fixed by heat, after cooling covered by crystal violets stain for 60 second, washed off stain by cleaned water, covered with Iogl's iodine for 60 second, washed with clean water, covered with safranine stain for 2 minutes, then washed and left to air dry and microscopically examined using oil immersion objective (100X) to observed morphological appearance, Gram-positive reaction and Gram-negative. The result of Gram's stain was reported (Carter and Cole, 2012).

##### **3.10.3.3. Biochemical tests for Gram's positive cocci:**

###### **3.10.3.3.1. Catalase test:**

Apure of 2-3 ml of hydrogen peroxide solution was added in a test tube, by sterile wooden stick several colonies of test organism was immersed in

hydrogen peroxide solution. The positive result indicated by immediate bubbling (Cheesbrough, 2000).

#### **3.10.3.3.2. Coagulase test:**

A drop of physiological saline was placed on each end of the slide, a colony of the of the test organism was emulsified in each of drops to make two thick suspensions and a drop of plasma was added to one of the suspensions and mixed gently by rotating. The positive result indicated by producing clump within 10 seconds (Cheesbrough, 2000).

#### **3.10.3.3.3. Deoxyribonuclease (DNAase) test:**

The test organism was cultured on a medium which contains DNA. After overnight incubation, the colonies were tested for DNAase production by flooding the plate with a weak hydrochloric acid solution. The acid precipitated unhydrolyzed DNA. DNAse producing colonies were, therefore, surrounded by clear areas due to DNA hydrolysis (Cheesbrough, 2000).

#### **3.10.3.3.4. Mannitol Salt Ager:**

This medium was used to differentiate *S.aureus* from other *Staphylococci* species. A portion of the colony was incubated aerobically at 37°C for 18-24 hours. *S.aureus* ferment mannitol producing yellow colonies (Cheesbrough, 2000).

#### **3.10.3.4. Biochemical test for Gram's negative rods**

##### **3.10.3.4.1. Indole test:**

The test colony was inoculated in sterile peptone water using a sterile wire loop and then incubated at 37°C aerobically overnight. Few drops of Kovac's reagent were added to medium and shaken gently to test for indole. A positive result was indicated by the production of red color in the surface layer within 10 minutes (Cheesbrough, 2000).

#### **3.10.3.4.2. Citrate utilization test:**

Slopes of Simmon's citrate agar medium were prepped, by using sterile straight wire loop the slope was streaked the butt was stabbed with a small part of the test colony. Then the slops of medium were incubated overnight at 35°C. A positive reaction was indicated by the change in the color of the medium into blue color while the negative reaction was indicated by no change in the color (Cheesbrough, 2000).

#### **3.10.3.4.3. Urease test:**

The test colony was inoculated on the surface of the slope of Christensen's urea agar medium by a sterile straight loop in zigzagging manner and then incubated over night at 37°C aerobically. The positive reaction was indicated by the color change in the indicator (phenol red) to pink color and negative reaction as indicated by no change in the color (Cheesbrough, 2000).

#### **3.10.3.4.4. Motility test:**

The tested colony was taken by a sterile straight loop and inoculated by stabbing a semi-solid media, then incubated aerobically at 37°C overnight. The motility was shown by spreading turbidity from the stab- line or turbidity throughout the medium (Cheesbrough, 2000).

#### **3.10.3.4.5. Kligler Iron Ager:**

A small part of the tested colony was picked off using a straight loop and inoculated in KIA medium. First stabbing the butt, then streaking the slope in the zigzag pattern, and then incubated at 37°C aerobically overnight. Then the results were interpreted as following:

A yellow butt red –pink slope indicated the fermentation of glucose only.

A yellow slope and butt indicated the fermentation of lactose and glucose.

A red-pink slope and butt indicated no fermentation of glucose and lactose.

Blackening along the stab line or throughout the medium indicated H<sub>2</sub> S production. Cracks and bubbles in the medium indicated gas production from glucose fermentation (Cheesbrough, 2000).

#### **3.10.3.4.6. Oxidase test:**

A piece of filter paper was placed on a clean glass slide and three to four drops of freshly prepared oxidase reagent (tetra methyl para phenylene diamine dihydrochloride) were added using sterile Pasteur pipette, a wooden stick was used to pick a colony of the test organism and placed on filter paper. The positive reaction was indicated by the production of blue-purple color within 10 seconds (Cheesbrough, 2000).

#### **3.10.4. Antimicrobial Sensitivity:**

Modified Kirby Bauer Disc Diffusion Method, by using sterile wire loop 3-5 colonies of similar appearance were selected and emulsified in 2-4 ml of sterile physiological saline and compared with McFarland's turbidity standard, by sterile swab, the suspension was inoculated in Mueller Hinton agar plate and by sterile forceps, an antibiotic disc was placed in the inoculated plate, incubated aerobically at 37°C for overnight. Then the inhibition zone measured (Reller *et al.*, 2009).

Interpretation of the result was done with the aid of an interpretative chart. By using the chart, the organism was characterized as being resistant, intermediate, or susceptible to the specific antibiotic. The following antimicrobial agents were tested: Cotrimoxazole(25mcg), Ciprofloxacin(5mcg), Gentamicin(10mcg), Imipenem(10mcg), Erythromycin(15mcg), Tetracycline(30mcg), Methacillin(5mcg), Ceftazidime(30mcg).

### **3.10.5. Preservation of Organism:**

After the identification of microorganisms (wound isolates), glycerol peptone broth was prepared 80ml from peptone and 20ml from glycerol, inoculated with the organism, then preserved in a refrigerator at 4°C

### **3.11. Extraction of *Guiera senegalensis*, *Azadirachta indica* and *Cymbopogon proximus*:**

Ethanollic extracts of *Guiera senegalensis*, *Azadirachta indica* and *Cymbopogon proximus* was obtained from Medicinal and Aromatic Plant and Traditional Medicine Research Institute, National Center for Research, Khartoum, Sudan.

#### **3.11.1. Preparation of *Guiera sanegalansis*, *Azadirachta indica* and *Cymbopogon proximus* extracts for testing the Antibacterial Activity:**

The crude extracts were diluted into different concentration as follows: 100%, 50%, 25% and 12.5% to be used against the selected organisms.

#### **3.11.2. Preparation of isolation bacterial suspension:**

The new subculture of the selected resistant bacterial strains (*Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumonia*, *Proteus mirabilis*, *Proteus vulgaris*, *Pseudomonas aeruginosa* and *Citrobacter spp*) including standard bacteria which brought from Microbiology Department of National Institute for Research and Bacteria Isolated from the clinical sample were inoculated into 3.0 ml of sterile normal saline. Inoculum density compared with McFarland stander solution.

#### **3.11.3. Agar disc diffusion assay:**

The ager diffusion technique has been widely used to assay plant extract for antimicrobial activity. In this method, 6mm dimeter sterilized filter papers disc was saturated with filter sterilized plant extract of the desired concentration. The impregnated disc is then placed onto the surface of

suitable solid agar medium like Muller Hinton. The media has been pre-inoculated with test organisms. The standard inoculum size is  $1 \times 10^8$  CFU/ml of bacteria for inoculating diffusion plates which is equal to McFarland 0.5 turbidity standard. Some researchers impregnate the paper disk with plant extract before putting on the inoculated plates (Lourens *et al.*; 2004 and Salie *et al.*, 2007) while others prefer after. The drying time of impregnated paper disk varies among researchers from 2h to overnight under a laminar flow cabinet. Plates are then inoculated for 24h at 37°C. The numbers of bacterial isolates inhibited by extracts of *Cymbopogon Proximus* inactive 11, moderate 18 and active 21, *Azadirachta indica* inactive 14, moderate 28 and active 6 and *Guiera senegalensis* inactive 20, moderate 22 and active 5 at concentration 100 mg/ml. The inhibition zone with the diameter less than 14 mm were considered as inactive, 14-18mm considered moderate antibacterial, more than 18 were considered as having antibacterial activity. (Das *et al.*, 2009).

#### **3.11.4. Determination of Minimum Inhibition Concentration:**

Determination of Minimum Inhibition Concentration (MICs) by broth media used tube method incubated overnight at 37°C were gave different results of inhibition bacteria by extracts at concentration 25%, 12.5%, and 6.25%.

#### **3.12. Data analysis**

Data were entered, checked and analyzed using Microsoft Excel 2016 and SPSS (Statistical Package of Social Science) software program version 16.



## 4. RESULTS

### 4.1 Frequency of sampling according to age group:

Out of 100 patients 61(61%) were males whereas 39 (39%) females. 61age were belong to 20-40 age group, 29 from 41- 60 age group, 10 from > 61 age group as shown in table 1. The age grouped into three group majority samples taken from age group 20-40 years.

**Table 1: Frequency and percentage of patients with wound infection according to age group:**

Age group	Frequency	Percentage
20- 40	61	61%
41-60	29	29%
>61	10	10%
Total	100	100%

### 4.2. Bacteriological Result:

#### 4.2.1. Frequency and percentage of bacterial growth:

Regard to bacterial growth, which showed 94/100 (94%) growth compared with 6/100 (6%) with no bacterial growth.

#### 4.2.2. Gram's stain for isolated bacteria species:

Out of the 94 positive culture for bacterial growth 40 were Gram-positive (42.6%) and 54 were Gram-negative (57.4%) Table 2.

**Table 2: Gram stain isolated bacteria:**

<b>Gram reaction</b>	<b>Frequency</b>	<b>Percentage</b>
Gram positive cocci	40	42.6%
Gram negative bacilli	54	57.4%
<b>Total</b>	<b>94</b>	<b>100%</b>

**4.2.3. Frequency and percentage of isolated bacteria species:**

Identify and isolated bacteria showed: *Staphylococcus aureus* was most abundant 40(42.6%), 22 of *S. aureus* was found resistant to Methicillin and consider as *MRSA*, while 54 Gram-negative follow by *P. aeruginosa* 15(27.7%), *Proteus mirabilis* 8(14.8%), *Proteus vulgaris* 5 (9.2%), *E. coli* 12 (22.2%), *K.pneumonia* 10 (18.5%), and *Citrobacter freundii* 4 (7.4%) Table 3.

**Table 3: Frequency and percentage of isolated bacteria:**

<b>Isolate</b>	<b>Frequency</b>	<b>Percentage</b>
<i>S. aureus</i>	40	42.6%
<i>P. aeruginosa</i>	15	27.7%
<i>Proteus mirabilis</i>	5	14.8%
<i>Proteus vulgaris</i>	8	9.2%
<i>E. coli</i>	12	22.2%
<i>K. pneumonia</i>	10	18.5%
<i>Citrobacter freundii</i>	4	7.4%
<b>Total</b>	<b>94</b>	<b>100%</b>

#### 4.2.4. Frequency and percentage of Isolated bacteria species according gender

Out of 94 isolated bacteria species 59/94 isolated from males, and 35/ 94 isolated from females, as shown in table 4. There was insignificant association between the isolated bacteria and the gender in the studies group (*p. value* 0.370)

**Table 4. Frequency and percentage of isolated bacteria species according gender**

Gender	Isolate bacteria						Total
	<i>S. aureus</i>	<i>Ps. aeruginosa</i>	<i>Proteus spp</i>	<i>E.coli</i>	<i>K. pneumoniae</i>	<i>Citrobacter freundii</i>	
Male	28	9	10	6	4	2	59
	47.5%	15.2%	16.9%	10.1%	6.7%	3.4%	62.8%
Female	12	6	3	6	6	2	35
	34.2%	17.1%	8.6%	17.1%	17.1%	5.7%	37.2%

#### 4.2.5. Frequency and percentage of isolated bacteria species according to ages group:

Out 94 isolated bacteria 60 (63.8%) were the majority age grouped 20-40 age, 27 (28.7%) from 41-60 age grouped and 7 (7.4%) from more than 61 age group, as shown in table 5. There was in significant association between isolated bacteria and age grouped (*p.value* .898)

**Table 5: Frequency and percentage of isolated bacteria species according to ages group**

Age	Isolate bacteria						Total
	<i>S. aureus</i>	<i>Ps. aeruginosa</i>	<i>Proteus spp</i>	<i>E.coli</i>	<i>K. pneumoniae</i>	<i>Citrobacter freundii</i>	
20-40	23 38.3%	11 18.3%	9 15%	7 11.7%	6 10%	4 6.7%	60 63.8%
41-60	13 48.1%	4 14.8%	3 8.6%	4 14.8%	3 11.1%	0 0%	27 28.7%
>61	4 57.1%	0 0%	1 14.3%	1 14.3%	1 14.3%	0 0%	7 7.4%

#### **4.3. Susceptibility test of bacterial isolated from wound isolate:**

***Staphylococcus aureus*:** Forty strains were tested, they all showed variable resistance pattern to tested antibiotics. Seven were resistant to 3 antibiotics and 16 were resistant to 4 antibiotics. Resistance was highest to Ceftazidime and Erythromycin followed by Penicillin and Cotrimoxazole. 22 of these were Methicillin resistance (Table 6).

***Pseudomonas aeruginosa*:** Out of 15 strain tested 3 were resistant to 3 antibiotics, 2 were resistant to 4 antibiotics. Resistance was highest for Cotrimoxazole and Tetracycline followed by Gentamycin and Ciprofloxacin (Table 6).

***Proteus mirabilis*:** Out of 5 strains tested, 5 were resistant to 3 antibiotics 1 was resistant to 4 antibiotics. Resistance was highest for Tetracyclin and Cotrimoxazole followed ciprofloxacin (Table 6).

***Escherichia coli*:** Out 12 strains tested 5 were resistant to 3 antibiotics, 1 was resistant to 4 antibiotics. Resistance was highest for Cotrimoxazole and Tetracyclin followed Ciprofloxacin (Table 6).

***Klebsiella pneumonia*:** Out 10 strains tested only 1 was resistant to 4 antibiotics. Resistance was highest for Tetracycline and Cotrimoxazole followed Gentamycin (Table 6).

***Citrobacter freundii*:** Out 4 strains tested only one were resistant to 3 antibiotics. Resistance was highest for Tetracycline. (Table 6).

**Table 6: Sensitivity test of Known antibiotics and the number of bacterial strains which were resistant to them:**

No. of isolates showing resistance to an antibiotic										
Code of Name of bacteria	Total	Ciprofloxacin	Cotimoxazole	Gentamycin	Tetracycline	Erythromycin	Imipenem	Ceftazidime	Penicillin	Methicillin
<i>Staphylococcus aureus</i>	40	1	18	-	-	24	-	25	24	22
<i>Pseudomonas aeruginosa</i>	15	5	8	6	8	-	0	-	-	-
<i>Proteus mirabilis</i>	5	2	5	1	5	-	0	-	-	-
<i>Proteus vulgaris</i>	8	3	4	0	7	-	0	8	-	-
<i>Escherichia coli</i>	12	7	9	0	10	12	0	12	-	-
<i>Klebsiella pneumoniae</i>	10	3	5	1	7	9	0	-	-	-
<i>Citrobacter freundii</i>	4	2	2	0	4	-	0	-	-	-

#### **4.4. Antimicrobial activities of *Cymbopogon proximus*, *Azadirachta indica* and *Guiera senegalensis*:**

Only the isolated bacteria which showed resistant to three antibiotic or more were tested for their sensitivity to *Cymbopogon proximus*, *Azadirachta indica* and *Guiera senegalensis* extracts, these include 22 *Staphylococcus aureus*, 11 *Pseudomonas aeruginosa*, 5 *Proteus mirabilis*, 5 *Escherichia coli* and 5 *Klebsiella pneumonia*

Ethanollic extracts of *Cymbopogon proximus*, *Azadirachta indica* and *Guiera senegalensis* showed a manifest effect on bacterial growth of wound infection isolates (Table 7).

#### **4.5. The activity ethanolic extracts and minimum inhibitory concentration of *Cymbopogon proximus*, *Azadirachta indica* and *Guiera senegalensis* on wound isolates:**

*Cymbopogon proximus*: The antimicrobial test showed the strong inhibited against that growth of multidrug resistant bacteria studies, which showed activity in all different concentration 100%, 50%, 25%, 12.5% and 6.25%. The *Cymbopogon proximus* was effective against 17 *Staphylococcus aureus*, 9 *Pseudomonas aeruginosa*, 3 *Proteus mirabilis*, 3 *Escherichia coli* and 5 *Klebsiella pneumonia*, at the highest concentration applied of 100mg/ml were exhibited strong inhibition zone against *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Klebsiella pneumonia* 22 mm in diameter at 100mg/ml, followed by *Staphylococcus aureus*, *Escherichia coli* 21mm in diameter at 100mg/ml. The MIC values were as follows: 12.5mg/ml for *Staphylococcus aureus* and *Proteus mirabilis*, 6.25mg/ml for *Pseudomonas aeruginosa*, 25mg/ml for *Escherichia coli* and *Klebsiella pneumonia* ethanol extract of *Cymbopogon proximus* (Table 9).

***Azadirachta indica***: The results showed that extract of neem possesses antimicrobial activity against multidrug resistant bacteria and less effected against same multidrug resistant bacterial studies. which showed activity in all concentration 100%, 50%, 25%, 12.5% and 6.25%. The *Azadirachta indica* was effected against 16 *Staphylococcus aureus*, 9 *Pseudomonas aeruginosa*, 3 *Escherichia coli*, 2 *Proteus mirabilis* and 3 *Klebsiella pneumonia*. The higher concentration gave the high effect on the growth bacteria and the largest inhibition zone obtained by ethanolic extract *Azadirachta indica* against *Staphylococcus aureus* 22mm in diameter at 100 mg/ml followed by *Escherichia coli* 21mm in diameter at 100 mg/ml then *Pseudomonas aeruginosa* 20mm in diameter at 100 mg/ml and showed was moderate activity against *Proteus mirabilis* and *Klebsiella pneumonia* 15mm in diameter 100mg/ml. The MIC value were as follows: 12.5mg/ml for *Staphylococcus aureus*, *Proteus mirabilis* and *Klebsiella pneumonia*, 25 mg/ml for *Pseudomonas aeruginosa* and *Escherichia coli* (Table 9).

***Guiera senegalensis***: The antimicrobial effected of ethanolic extract of *Guiera senegalensis* were showed weak activity inhibition against multidrug resistant bacteria studies. Which showed activity only in 100% concentration. The *Guiera senegalensis* was effected against 11 *Staphylococcus aureus*, 9 *Pseudomonas aeruginosa*, 1 *Proteus mirabilis*, 2 *Escherichia coli* and 4 *Klebsiella pneumonia*. The higher concentration gave the high effect on the growth bacteria and the largest inhibition zone obtained by ethanolic extract *Guiera senegalensis* against *Staphylococcus aureus* 21mm in diameter 100mg/ml, *Pseudomonas aeruginosa*, *Proteus mirabilis* 20mm in diameter 100mg/ml followed by *Klebsiella pneumonia* 18mm in diameter 100mg/ml and *Escherichia coli* 16mm in diameter 100mg/ml. antimicrobial activity (Table 9)



#### **4.6. Compared between activity of ethanolic extracts of *Cymbopogon proximus*, *Azadirachta indica* and *Guiera senegalensis* against each isolated bacteria strains**

Compared between *S. aureus* and three plants were shown in table 7. There was significant difference effect of antimicrobial plants (*p. value* ,006), and compared between difference mean shown in table 8. The *Cymbopogon proximus* and *Azadirachta indica* were significant effect compared with *Guiera senegalensis*. Followed compared *Ps. Aeruginosa* with the three plants were shown in table 7. There was insignificant difference effect (*p. value*,055)

But compared between difference mean were shown in table 8. The *Cymbopogon proximus* it's was best antimicrobial compared with *Azadirachta indica* and *Guiera senegalensis*. *E. coli*, *P. mirabilis* and *K. pneumonia* isolated compare with plants were shown in table 7. There was insignificant difference effect of antimicrobial plants, and compared between difference mean of plants were shown in table 8. There was insignificant difference effect.

**Table 7: Compared between activity of ethanolic extracts of *Cymbopogon proximus*, *Azadirachta indica* and *Guiera senegalensis* against each isolated bacteria**

Isolated bacteria and number	Mean inhibition zone including well diameter in mm at 100mg/ml			<i>P. value</i>
	<i>Cymbopogon proximus</i>	<i>Azadirachta indica</i>	<i>Guiera senegalensis</i>	
<i>S. aureus</i> (22)	18.72	17.63	12.4	0.006
<i>Ps. Aeruginosa</i> (11)	18.27	16.64	15.64	0.055
<i>P. mirabilis</i> (5)	18.80	14.60	13.60	0.935
<i>E. coli</i> (5)	17.40	16.00	13.8	0.799
<i>K. pneumonia</i> (5)	19.00	14.46	13.40	0.938

**Table 8: Multiple comparison of antimicrobial**

Isolates bacteria	Antimicrobial		Difference mean	P.value
<i>S.aureus</i>	<i>C.proximus</i>	<i>A.indica</i>	,947	,527
		<i>G.senglenises</i>	4,737	,002
	<i>A. indica</i>	<i>C.proximus</i>	,947	,527
		<i>G.senglenises</i>	3,789	,014
	<i>G.senglenises</i>	<i>C.proximus</i>	-4,737	,002
		<i>G.senglenises</i>	-3,789	,014
<i>Ps.aeruginosa</i>	<i>C.proximus</i>	<i>A.indica</i>	2,273	,044
		<i>G.senglenises</i>	2,455	,031
	<i>A.indica</i>	<i>C.proximus</i>	-2,273	,044
		<i>G.senglenises</i>	,182	,868
	<i>G.senglenises</i>	<i>C.proximus</i>	-2,455	,031
		<i>A.indica</i>	-,182	,868
<i>P.mirabilis</i>	<i>C.proximus</i>	<i>A.indica</i>	1,200	,767
		<i>G.senglenises</i>	,100	,981
	<i>A.indica</i>	<i>C.proximus</i>	-1,200	,767
		<i>G.senglenises</i>	-1,300	,762
	<i>G.senglenises</i>	<i>C.proximus</i>	,100	,981
		<i>A.indica</i>	1,300	,762
<i>E.coli</i>	<i>C.proximus</i>	<i>A.indica</i>	,800	,874
		<i>G.senglenises</i>	,3.200	,528
	<i>A.indica</i>	<i>C.proximus</i>	,800	,874
		<i>G.senglenises</i>	2,400	,634
	<i>G.senglenises</i>	<i>C.proximus</i>	-3,200	,528
		<i>A.indica</i>	-2,400	,634
<i>K.pneumonia</i>	<i>C.proximus</i>	<i>A.indica</i>	1,200	,767
		<i>G.senglenises</i>	-,100	,981
	<i>A.indica</i>	<i>C.proximus</i>	-1,200	,767
		<i>G.senglenises</i>	-1,300	,762
	<i>G.senglenises</i>	<i>C.proximus</i>	,100	,981
		<i>A.indica</i>	1,300	,762

**Table 9: Minimum Inhibition Concentration of Ethanol extract of *Cymbopogon proximus* and *Azadirachta indica* against bacteria**

Compared between MIC of extracts *Cymbopogon proximus* and *Azadirachta indica* for each isolated as shown in table 8. There was in significant effect between isolated bacteria and extracts plants (*p.value* 0.132)

Bacteria Name Strains	Minimum Inhibition Concentration mg\ml	
	<i>Cymbopogon Proximus</i>	<i>Azadirachta Indica</i>
<i>S. aureus</i>	12.5	12.5
<i>Ps. Aerginosa</i>	6.25	25
<i>Proteus mirabilis</i>	12.5	12.5
<i>E. coli</i>	25	25
<i>K. peumoniae</i>	25	12.5

## 5. DISCUSSION

### 5.1. Discussion:

Several drugs of plant, mineral and animal origin are described in the Ayurveda for their wound healing properties, some of these plants have activity in different pharmacological models and patient (Tuhin, 2003)

This prevalence study was carried out on wound infection of various age, the highest percentage of isolates were from adults (20-40 age). Most of patients were in health immunity, they wound's infection evolve to become chronic infection. It has been established evidence that bacteria acquired virulence and risk factor, which helps bacteria to be resistant for many antibiotics.

The current study was carried out to screen the antibacterial activity of *Cymbopogon proximus*, *Azadirachta indica* and *Guiera senegalensis*, Ethanolic extract against wound infection bacterial isolates *S.aureus*, *Ps.aeruginosa*, *Proteus spp*, *E.coli* and *K.pneumonia*. The results showed high activity of ehtanolic extract of *Cymbopogon proximus*, these results completely agreed with that obtained by (Samy, and Selim, 2011) and (Opeyemi *et al* .,2015).

The *Azadirachta indica* exhibited high antibacterial activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa* isolated from some diabetes mellitus patients. It was agreed with reported by ( Sukanya *et al.*, 2009),( Subapriya *et al.*, 2005) and ( Hemraj and Jindal, 2012). While ethanolic extract of *Guiera sengalensis* exhibited less antibacterial activity of concentration 100% against isolates unless increase extract's concentration, these result agreed with previous reported by (Harami *et al.*, 2004). A number of compound derived from plants often showed considerable activity against Gram positive bacteria but not against Gram

negative species. Gram negative bacteria have an effective permeability barrier, comprised of outer member, which restrict the penetration of amphipathic compound and multidrug resistance pump that extrude toxin across this is barrier. It is possible that the apparent ineffectiveness of plant antibacterial is largely due to the permeability barrier (Tegoes *et al*; 2012).

The diameter of growth inhibition zone of clinical isolates of bacteria were increased with increased in extract concentration of *Cymbopogon proximus*, It's result agreed with reported ( Gaber *et al.*, 2006). However,

*Azadirachta indica* revealed the best diameter of growth inhibition zone for *Staphylococcus aureus* in concentration 100% and decreased concentration extract, this result agreed with that obtained by Wendy (2011).

The result agreed with (Samy and Selim, 2011), who reported the activity component of *C. proximus* consider as strongly inhibition bacteria growth: piperitone, elemol,  $\alpha$  eudesmol,  $\beta$  eudesmol and limonene.

The activity of Alneem and Algobesh are mainly due to the secondary metabolites, as it is reported the active antimicrobial compound for *A. indica* and *G. senegalensis* leaves alkaloids, glycosides, flavonoids, saponins, tannins and phenolic compound (Nandita and Feoma, 2002 and Kubmarawa *et al.*, 2007).

## **5.2. Conclusion:**

Ethanollic extracts of *Cymbopogon proximus*, *Azadiracht indica* and *Guiera senegalensis* possessed antibacterial activity against same bacteria that showed resistant to a number of antibiotic, which justify their traditional herbs use for treatment various infection cause by resistant pathogenic bacteria.

The ethanolic extract showed high activity against gram positive than gram negative.

The efficiency of the antibacterial of ethanolic extract was found to increase by increasing of concentration.

## **5.3. Recommendations:**

Based of study result, it recommended that to:

- isolate and purify the active ingredient in the extract responsible for antibacterial activity.
- Determination of minimum inhibition concentration (MICS) for the active ingredients of each bacteria including in this study.
- Determination of the toxicity of the active ingredient.
- To avoid bacteria resistant to antibiotic, patients have to complete all days of using antibiotics.
- More research is required to understand the mode of actions of these plants.
- Manufacture these extracts at pharmacological form such as topical ointment

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## **Appendix (1)**

### **Material**

#### **A- Equipment**

Autoclave

Bunsen burner.

1- Cork borer.

3- Freezer dryer

4- Hot air

5- Incubator

6- Light microscope with oil immersion lens.

7- Rack.

8- Refrigerator.

9- Soxhlet apparatus (round bottom, reflex, condenser).

10- Straight loops with handle

11- Water bath

12- Wire loops with handle

#### **B- Glassware**

1- Petri dishes (plates).

2- Flask with different size.

3- Measuring cylinder.

5- Funnels.

6- Spoons

7- Sterile containers (bijou bottles).

8- Test tubes.

9- Slides.

### **C- Disposable material**

1- Disposable syringes.

2- Wound swab

3- Filter papers.

### **D- Culture media**

Different culture media were used for inoculation, isolation, and identification of organisms. These include

#### **1- Nutrient ager**

Typical formula in g/L

#### **Contents**

Peptone.....5.0

Meat extract.....3.0

Agar.....15.0

pH 7.0± 0.2

#### **Preparation**

Suspend 23g of powder in of D.W and heat to boiling. Dispenses into containers and sterilize in the autoclave at 121°C for 15 minutes.

## 2- DNase agar

Typical formula g/l

### Contents

Tryptose.....	20
Deoxyribonucleic acid.....	2
Sodium choride.....	5
Agar.....	12

p H 7.0±0.2

### Preparation

Suspend 3.9g in 1L of D.W Bring to boil to dissolve completely. Sterilize by autoclave at 121°C for 15 minutes. Cool to 50°C and pour into the sterile Petri dishes. Dry the surface of the medium before inoculation.

3- Media for biochemical reactions (Kligler iron agar, simmon's agar, christenensin urea media, media containing tryptophan, semi solid agar medium)

### Simmons citrate medium

Typical formula in g/L

### Contents

Magnesium sulfate.....	0.20
Monoammonium phosphate.....	1.00
Dipostassium phosphate.....	1.00
Sodium citrate.....	2.00

Sodium chloride.....	5.00
Bromothymol blue.....	0.08
Agar.....	15.00

p H 6.8±0.2

### **Preparation**

Dissolve 24g of powder in 1L of D.W. Bring to the boil. Dispense in tubes and sterilize by autoclaving at 121°C minutes. Solidify with the

Long slant.

### **Kligler Iron Agar**

Typical formula g/L

### **Contents**

Balanced peptone.....	20.0
Lactose.....	10.0
Sodium chloride.....	1.0
Ferric ammonium.....	0.5
Sodium thiosulphate.....	0.025
Agar.....	12.0

p H 6.9±0.2

### **Preparation**

Dissolve 49g powder in 1L Soak for 10 min. Swirl to mix bring to boil. Distribute into tubes and sterilize by autoclave at 121°C for 15 minutes. Let's the medium set as slopes about 3cm deeps.

## **Christensen Urea Medium**

Typical formula g/L

### **Contents**

Gelatin peptone.....	1000
Dextrose.....	1.00
Sodium chloride.....	5.000
Monopotassium phosphate.....	2.000
Phenol red.....	0.012
Agar.....	15.000

p H 7.0±0.2

### **Preparation**

Suspend 24g in 950 ml of D.W and bring to bring to the boil. Sterilized by autoclave at 121°C for min. Let it cool to 50-55°C. Added 50ml of urea sterile solution 40% (Ref. 06-083) and mix well. Distribute aseptically in tubes and let them solidify stanted.

Peptone water

Typical formula g/L

### **Contents**

Peptic digest of animal.....	10.00
Sodium chloride.....	5.00
Phenol red.....	0.02

p H 6.8±0.2

## Preparation

1-Suspend 15.0 g of powder in 100 ml D.W. Add the test carbohydrate in desired quantity and dissolve completely.

2- Dispense in tube with or without inverted Durham's tubes and sterilize by autoclaving at 121°C for 15 Ib pressure (121°C) for 15 minutes.

## 4- Mueller Hinton agar

Typical formula g/L

### Contents

Casein acid hydrolysate.....	17.50
Beef heart infusion.....	2.00
Starch, soluble.....	1.50
Agar.....	17.00

pH (at 25°C) 7.3±0.1

### Preparation

Suspend 38g of powder in 1000ml D.W mix well and heat to boiling to dissolve the medium completely. Sterilize by autoclave at 121°C for 15 minutes

To make about 35 blood agar plates:

Nutritious agar.....	500ml
Sterile defibrinated blood.....	25ml

### Preparation

1- Prepare the agar medium as instructed by the manufacturer. Sterilize by autoclaving at 121 °C for 15 minutes. Transfer to a 50°C water

2- When the agar has cooled to 50°C, add aseptically the sterile blood and mix gently but well. Avoid forming air bubbles

3- Dispense aseptically in 15 ml amount in sterile petri dishes as described subunit

4- Date the medium and give it a batch number.

5- Store the plate at 2-8 °C, preferably in sealed plastic bags to prevent loss of moisture

pH of medium: 7.2-7.6 at room temperature.

## **6- Mannitol salt agar**

### **Contents**

Peptone, *Lab- lemco* powder, mannitol, sodium chloride, phenol red, agar

### **Preparation**

1- Prepare the medium as instructed by the manufacturer. Sterilize by autoclaving at 121°C for 15minutes.

2- When the medium has cooled to 50-55°C, mix well, and dispense it aseptically in sterile petri dishes. Date the medium and give it a batch number.

3-Store the plates at 2-8°C preferably in plastic bags to prevent loss of moisture.

pH. of medium: 7.3-7.7 at room temperature.

## **7- Nutrient broth**

### **Contents**

Peptone, *Lab-Lemco* powder, yeast extract, sodium Chloride.

### **Preparation**

- 1- Prepare as instructed by the manufacture. Sterilized by autoclaving at 121°C for 15 minutes
- 2- Dispense aseptically in the required amounts. Date the medium and give it a batch number,
- 3- Store in a cool dark place.  $\text{pH}$  of medium: 7.2-7.6 at room temperature.

### **E- Chemicals and reagents**

- 1- Ethanol.
- 2- Sodium chloride (normal saline).
- 3- Methanol.
- 4- Oxidase reagent.
- 5- McFarland's turbidity standard.
- 6- Kovc's reagent.

### **Kovac's reagent**

#### **Contents**

To prepare 20 ml:

4-dimethylaminobenaldehyde.....	1g
Isoamylalcohol(3-methyl-1-butanol) .....	15 ml
Concentrated hypochloric acid.....	5 ml



## **Preparation**

Weight the dimethylaminobenzaldehyde, dissolve in the isoamylalcohol.

Added concentrated hydrochloric acid and mix well. Transfer to clean brown bottle and at 2-8°C

## **Oxidase reagent**

### **Contents**

To prepare 10 ml:

Tetramethyl-p-phenylenediaminedihydrochloride.....0.1g  
Distill water.....10ml

### **Preparation**

Dissolve the chemical in the D.W. this reagent should be prepared immediately before use because is unstable.

## **McFarland turbidity standard**

### **Contents**

Concentrated sulphuric acid..... 1ml  
Dihydrate barium chloride.....0.5g  
Distilled water.....150ml

### **Preparation**

1- Prepare 1% (v/v) solution of sulphuric acid by adding 1ml of concentrated sulphuric acid to 99ml of water and mix well.

2- Prepare 1.175% (w/v) solution of barium chloride by dissolving 2.35g.

3- Add .5ml of barium chloride solution to 99.5 ml of sulphuric acid solution and mix.

## Appendix 2

Sudan University of Science and Technology College  
Of Graduate Studies

Antimicrobial Activity of *Cymbopogon proximus*, *Azadirachta indica* and  
*Guiera senegalensis* Medicinal Plants Against Multi Drugs Resistant  
Bacteria Isolates from Wound Infection

Data collection form

Name.....

Sex.....

Age.....

History of disease.....

Under antibiotics treatment

Yes

No

### **Character and biochemical tested of *Staphylococcus aureus***

The biochemical tests for both Gram positive and Gram negative stain were shown in 10

**Table 10: Characters and biochemical tested of *S. aureus*:**

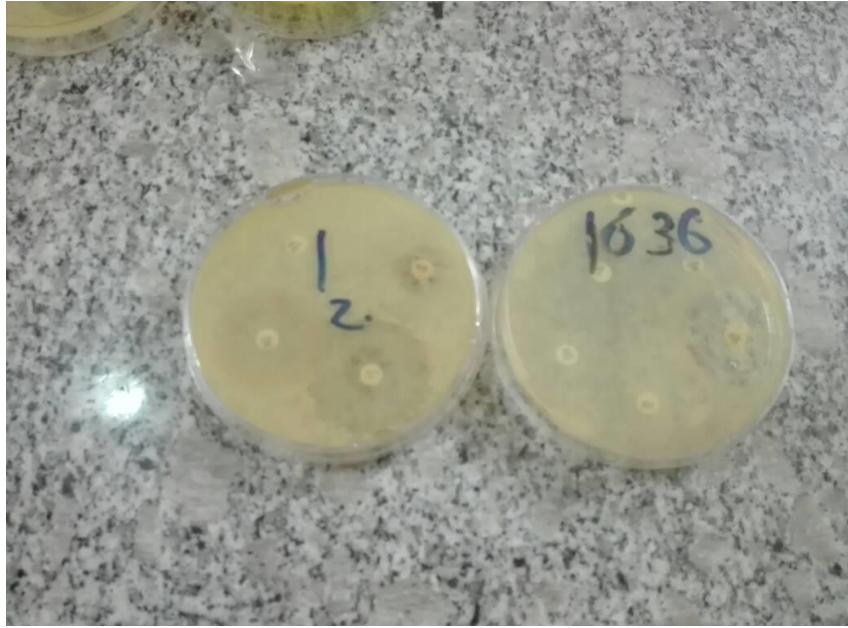
<b>Character</b>	<i>S. aureus</i>
MacConkey's agar	Yellow colonies
Blood agar	White creamy colonies
Hemolysis	Beta – hemolysis
Catalase	+
DNase	+
Coagulase	+
Mannitol fermentation	+

**Table 11. Characteristics and biochemical properties of tested Gram-negative bacteria:**

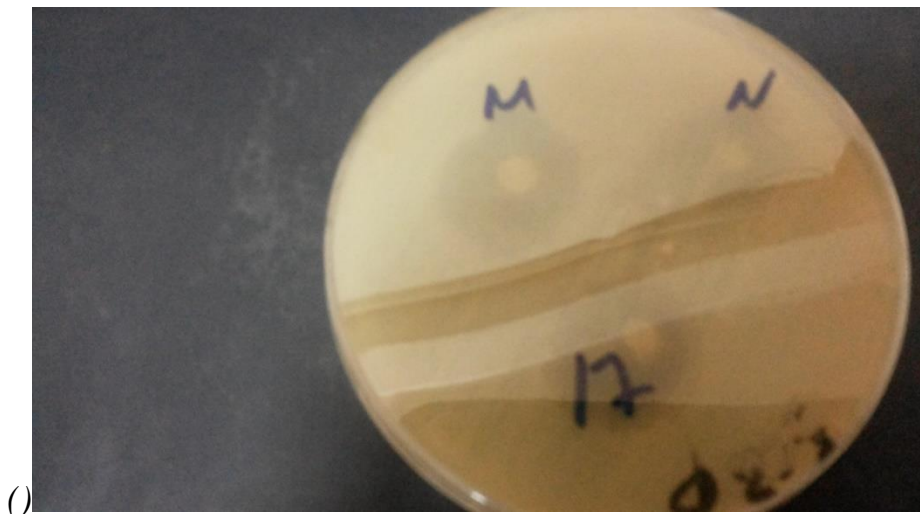
<b>Character</b>	<i>P. aeruginosa</i>	<i>Proteus. Spp</i>	<i>E. coli</i>	<i>K. Pneumonia</i>	<i>C. spp</i>
MacConkey's Agar	Yellow	Yellow	Red	Red	Yellow
Blood agar	Large Haemolytic Colonies	Fishy odor and swarming	Large white, non-hemolytic colonies	Large mucoid colonies	
Shape	Rod	Rod	Rod	Rod	Rod
Oxidase test	+	-	-	-	-
Catalase test	+	+	+	+	+
Citrate test	+	+/-	-	+	+
Ureas test	-	+	-	+	-
KIA Slop/butt	R/R	R/Y	Y/Y	Y/Y	R/Y
Gas production	-	+	+	+	+
H <sub>2</sub> S production	-	+	-	-	-
Lactose fermentation	-	-	+	+	-
Indole test	-	+	-	+	-

**Key:**

(-) negative, (+) positive, Y(Yellow), R (Red), KIA (Kligler Iron Agar)



**Plate 1: Susceptibility test for *S. aureus***



**Plate 2: Activity of ethanol extract of *Cymbopogon proixmus* (M), *Azadirachta indica* (N) and *Grewia senegslensis* (17) against *S. aureus***



**Plate 3: Activity of ethanol extract of *Cymbopogon proixmus* (M), *Azadirachta indica* (N) and *Grewia senegslensis* (17) against *K. pneumoniae***

