



Sudan University of Science and Technology

College of Graduate Studies



**Antimicrobial Susceptibility of (*Rumexvesicarua*) Extractand
Commonly Used Antibiotics against Aerobic Bacteria Isolated from
Wound Infection of Diabetic Patients in Khartoum State**

حساسية مستخلص نبات (الحميض) والمضادات الحيوية شائعة الاستخدام ضد البكتيريا الهوائية
المعزولة من جروح مرضى السكري في ولاية الخرطوم

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بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

الآيَة

وَخَفِضْ لَهُمَا جَنَاحَ الذُّلِّ مِنَ الرَّحْمَةِ وَقُلْ رَبِّ ارْحَمْهُمَا كَمَا رَبَّيَانِي صَغِيرًا (24)

(صدق الله العظيم)

سورة الإسراء الآية (24)

Dedication

I dedicate this thesis to:

My mother, my daughter, my sisters, my brothers, my friends and all those who have helped with their advice and efforts.

Acknowledgment

All thank to ALMIGHTY ALLAH for giving me strength and courage to complete this work and made all the things possible. Then thanks to my supervisor **Dr. HishamNouraldayerAltayeb Mohammed** who encouraged me and gave me new ideas thoughts my work.

Finally I'd like also to thank all the microbiology staff in Sudan University.

ABSTRACT

This is a cross sectional study, aimed to identify the aerobic bacterial pathogens associated with diabetic wounds in Khartoum State and testing the antimicrobial susceptibility of the most commonly used antibiotics in treatment of wound diabetic person comparison with the activity of plant extract, in the period between February to August, 2016. Fifty wound swab samples were collected and cultured in MacConkey and Blood agar medium.

After identification, the isolates were subjected to antimicrobial sensitivity using Amikacin, Cotrimoxazol, Imipenam, Ciprofloxacin for Gram negative bacteria, and Erythromycin, Tetracycline, Penicillin and Gentamicin for Gram positive bacteria. Then, the isolated organisms were also subjected to plant fruits extract (*Rumex vesicarius*) to test their antimicrobial activity.

The results revealed that *Staphylococcus aureus* and *Pseudomonas aeruginosa* were the predominant pathogens in diabetic wound infections. The Amikacin and Imipenam showed 100% activity against isolated bacteria when compared with other standard antibiotics, the aqueous extracts of *Rumex vesicarius* gave a high percentage of activity against *Proteus mirabilis* (28%) and there was no any activity against *Escherichia coli*.

Further studies in *Rumex vesicarius* in other parts of plant must be done, to detect if they have any antimicrobial activity.

ملخص الدراسة

هذه عبارة عن دراسة مقطعية، والتي تهدف إلى التعرف على مسببات الأمراض البكتيرية الهوائية المرتبطة بجروح مرضى السكري في ولاية الخرطوم واختبار الحساسية للمضادات الحيوية الشائعة في علاج جروح مرضى السكري ضد أنواع البكتيريا الهوائية و المقارنة مع مستخلص ثمره الحميض، في الفترة ما بين فبراير الى اغسطس 2016. تم جمع خمسون عينة وتزرعها في ماكونكي وأجار الدم بعد التعرف على مسببات الأمراض البكتيرية المرتبطة بجروح السكري تم اختبار حساسيتها للمضادات الحيوية باستخدام المضادات الآتية الأمبيسلين، كوتر ايموكسازول، إيميبينيم، سيبروفلوكساسين للبكتيريا الموجبة والاريثروميسين، التتراسيكلين، البنسلين، الجنتاميسين للبكتيريا السالبة للجرام. عرضت البكتيريا المعزولة أيضا لمستخلص ثمره الحميض لاختبار نشاطهم ضد الميكروبات.

وقد تبين أن أكبر اصابه كانت بأنواع البكتيريا الهوائية من المكورات العنقودية الذهبية تليها الزائفة الزنجارية وهما أكثر انواع البكتيريا السائدة في التهابات جرح السكري. أميكاسين/ إيميبينيم افضل المضادات الحيوية فعالية بنسبه 100% مقارنة مع المضادات الحيوية القياسية الأخرى، وكان مستخلص الحميض الذي أعطى أعلى نسبة فعالية ضد بكتيريا المكورات العنقودية الذهبية 28% و لم يعطي أي فعالية على القولونية.

يجب عمل مزيد من الدراسات الواسعة في الحميض في الاجزاء الاخرى من النبات لاكتشافها إذا كان لها أي نشاط كمضاد للميكروبات.

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LIST OF ABBREVIATIONS

MIC	Minimum inhibitory concentration
IDDM	Insulin dependent diabetes mellitus
NIDDM	Non-Insulin dependent diabetes mellitus
DM	Diabetes mellitus
MHA	Muller-Hinton agar
KIA	Kliger Iron Agar
CFU	Colony forming unit
WHO	World Health Organization
NCCLS	National culture collection laboratories

CHAPTER ONE
INTRODUCTION AND OBJECTIVES

INTRODUCTION

1.1. Introduction

Diabetes mellitus is a condition in which the pancreas no longer produces enough insulin or cells stop responding to the insulin that is produced. There is a general consensus among clinicians that diabetic patients are at increased risk of developing infection (Braces, 2007).

This special vulnerability has been attributed to impaired leukocyte function associated vascular diseases, poor glucose control and altered host response (McMahon and Bistrian, 1995).

Once infection occurs, it is difficult to treat since the clinical course of the infection is more fulminant and severe, and possess a greater threat to the glycemic status of the patient (Louie *et al.*, 1993).

The development of wounds is a serious complication for patients with diabetes. Numerous factors related to diabetes can impair wound healing, including wound hypoxia (inadequate oxygen delivered to the wound) infection, nutrition deficiencies, and the disease itself (Lavery *et al.*, 2007).

Fluctuating blood sugar and hypoxia from poor circulation may impair the ability of white blood cells to destroy pathogenic bacteria and fungi, increasing infection risk (Stadelmann *et al.*, 1998).

There are several well accepted predisposing factors that place patients with diabetes at high risk for a lower extremity amputation. Foot ulcers are much feared complications of diabetes, with recent

studies suggesting that lifetime risk of developing. Foot ulcer in diabetic patient may be as high as 25% (Singh *et al.*, 2005).

Fifteen percent of people with diabetes will develop a foot ulcer at some time during their life, and 85% of major leg amputations begin with a foot ulcer (Ramani *et al.*, 1991).

Since the discovery of antibiotics and their use as chemotherapeutic agents, there was a belief in the medical fraternity that this would lead to the eradication of infectious diseases. However diseases and disease agents that were once thought to have been controlled by antibiotics are returning in new forms resistant to antibiotic therapies (Levy and Marshall, 2004). Incidents of epidemics due to such drug resistant microorganisms are now a common global problem posing enormous public health concerns.

At present, most clinical isolates of *S. aureus* are multiple drug resistant (resistant to three or more of agents such as ciprofloxacin, erythromycin, clindamycin, gentamicin, trimethoprim/sulphamethoxazole, linezolid, and vancomycin (Styers *et al.*, 2006). When infections become resistant to first choice or first line antimicrobials, treatment has to be switched to second- or third-line drugs, which are nearly always expensive. In many poor countries, the high cost of such replacement drugs is prohibitive, with the result that some diseases can no longer be treated in areas where resistance to first-line drugs is widespread (WHO, 2002).

Plants have traditionally provided a source of hope for novel drug compounds, as plant herbal mixtures have made large contributions to human health and well-being. Owing to their popular use as remedies for many infectious diseases, searches for substances with antimicrobial activity in plants are frequent (Beton *et al.*, 2006; Shibata *et al.*, 2005).

1.2. Rationale

Diabetic foot wounds are a major complication of diabetes resulting in a substantial morbidity and mortality. The risk of lower-extremity amputation is increased eight-fold in patients once an ulcer develops. Foot disorders such as ulceration, infection, and gangrene are the leading causes of hospitalization in patients with diabetes mellitus (Boulton, 2002). An ankle is lost due to diabetes somewhere in the world every 30 second, a more important fact is that up to 85% of all amputations in diabetes should be preventable (Boulton *et al.*, 2005). Early detection of causative pathogens could help in selection of appropriate treatment of these ulcers, and this may prevent amputations. Infected diabetic foot ulcers may be complicated by septicemia and may even result in the death of the patient.

Intensive use of antibiotics is often followed by the development of resistant strains. Because of this drug resistance, the search for new antibiotics continues unabated. The interest in the study of medicinal plants as source of pharmacologically active compounds has increased worldwide. Usually in Sudan, plants are one of the main medicinal source to treat infectious diseases so this study aimed to screen the bacterial pathogens present in diabetic wound and to determine their antibiotic sensitivity pattern in comparison with plant extract from (*Rumex vesicaria*).

1.3. Objectives

1.3.1. General objective

Phenotypic identification of aerobic bacteria that cause wound infection among diabetic patients and testing their antibiotic susceptibility against commonly used antibiotics in comparison to their sensitivity against plant extract (*Rumexvesicaria*)

1.3.2. Specific objectives

1. To determine the frequencies of aerobic bacteria species that cause complication in diabetic patient wounds
2. To determine the antimicrobial activity of most commonly used antibiotic against diabetic wound infection causative bacteria
3. To test the antimicrobial activity of local plant(*Rumexvesicaria*) against diabetic wound infection causative bacteria.

CHAPTER TWO

LITRETURE REVIEW

2.1. Diabetes mellitus:

Is a group of metabolic diseases characterized by elevated blood glucose levels (Hyperglycemia) resulting from defects in insulin secretion, insulin action or both. Insulin is a hormone manufactured by the beta cells of the pancreas, which is required to utilize glucose from digested food as an energy source. Chronic hyperglycemia is associated with microvascular and macrovascular complications that can lead to visual impairment, blindness, kidney disease, nerve damage, amputations, heart disease, and stroke. In 1997 an estimated 4.5% of the US population had diabetes.

Direct and indirect health care expenses were estimated at \$98 billion. (American Diabetes Association, 1998)

The type of diabetes is based on the presumed etiology. This table provide information about the two most common types of diabetes: type 1 and type 2 diabetes (see Table 1).

TABLE 1 Characteristics of the Common Types of Diabetes

	Type 1(IDDM)	Type 2(NIDDM)
Age	Childhood	Pubertal
Onset	Acute ; severe	Mild-severe; often

		insidious
Insulin secretion	Very low	Variable
Insulin sensitivity	Normal	Decreased
Insulin dependence	Permanent	Temporary; may occur later
Racial/ethnic groups at increased risk	All (low in Asians)	African, Americans, Hispanics, Native Americans, Asian/Pacific Islanders
Genetics	Polygenic	Polygenic
Proportion of those with diabetes	80%	10%-20%
Association: obesity	No	Strong
Acanthosisnigricans	No	Yes
Autoimmune etiology	Yes	No

(Stang and Story, 2005)

In type 1 diabetes, the body does not produce insulin, and daily insulin injections are required. Over 700,000 people have type 1 diabetes; this is 5-10% of all cases of diabetes mellitus in USA.

Type 1 diabetes is usually diagnosed during childhood or early adolescence and it affects about 1 in every 600 children (Stang and Story, 2005).

Type 2 diabetes is the result of failure to produce sufficient insulin and insulin resistance. Elevated blood glucose levels are managed with reduced food intake, increased physical activity, and eventually oral medications or insulin. Type 2 diabetes is believed to affect more than 15 million adult, 50% of whom are undiagnosed. It is typically diagnosed during adulthood. However with the increasing incidence of childhood

obesity and concurrent insulin resistance, the number of children diagnosed with type 2 diabetes has also increased worldwide (Rosenbloom *et al.*, 1999)

2.2. Etiology of contributing factors:

2.2.1. Type 1 Diabetes

- Caused by the immune destruction of the beta cells of the pancreas.
- Antibodies to islet of Langerhans cells and insulin are present at diagnosis.
- Insulin secretion gradually diminishes.
- May present at any age, but most common in childhood and adolescence.
- Insulin by injection is necessary for survival.
- Contributing factors:
 - Genetic predisposition
 - Environmental triggers (infection or other stress)

2.2.2. Type 2 Diabetes

- Caused by insulin resistance in the liver and skeletal muscle, increased glucose production in the liver, over production of free fatty acids by fat cells and relative insulin deficiency.
- Insulin secretion decreases with gradual beta cell failure.
- Reductions in blood glucose levels can often be achieved with changes in food intake and physical activity patterns. Oral medication and/or insulin injections are eventually required.
- Contributing factors:
 - Obesity
 - Age (onset of puberty is associated with increased insulin resistance)
 - Lack of physical activity

- Genetic predisposition
- Racial/ethnic background (African American, Native American, Hispanic and Asian/Pacific Islander)
- Conditions associated with insulin resistance, (e.g., polycystic ovary syndrome (Monket *al.*, 1996).

2.3. Complications:-

It is important to be tested for diabetes if one is at risk. If left untreated or improperly managed, diabetes can result in a variety of complications, including heart attack, stroke, kidney failure, blindness, problems with erection (impotence) and amputation.

2.3.1. Acute Complications

Acute complications arise from uncontrolled high blood sugars (hyperglycemia) and low blood sugars (hypoglycemia) caused by a mismatching of available insulin and need. Some acute complications require immediate medical attention.

These emergencies include:

- Hypoglycemia
- Hyperglycemic Hyperosmolar State (HHS)
- Diabetic Ketoacidosis (DKA)

2.3.2. Chronic Complications

Chronic complications tend to arise over years or decades. Often, there is damage before there are symptoms so routine screening is recommended to catch and treat problems before they occur or get worse (Kennedy, 2006).

Problems include:

- Vision loss or blindness
- Kidney damage or failure
- Nerve pain and damage
- Heart and blood vessel disease
- High blood pressure
- Dental problems
- Hand problems
- Foot problems

2.4. Causes of Diabetic Wounds

The main concern with diabetic wounds is poor or delayed healing. Healing problems are caused by the peripheral arterial diseases and peripheral neuropathy that can occur with diabetes, wherein the small blood vessels in different parts of the body, especially in the extremities (hands and feet), grow narrower and reduce the blood circulation to those areas. A lack of circulation in the extremities can result in a reduced supply of oxygen and nutrients to the body tissue and nerves, which is necessary for healing. Over time, nerves in these areas may become damaged, decreasing the sensation of pain, temperature and touch, making patients vulnerable to injury.

2.5. Types of Diabetic Wounds

For a diabetic patient, every wound is a health concern and requires immediate attention. The most common two types are wounds of external origin and wounds of internal origin. Due to peripheral neuropathy, wounds of external origin, such as skin cuts, burns, bumps and bruises, may often go unnoticed by the diabetic patient. If external wounds go

unnoticed for some time, delayed treatment can put the patient at risk for further complications. Wounds of internal origin, such as skin ulcers, ingrown toenails or calluses, can lead to the breakdown of skin and surrounding tissue, increasing the risk of bacterial infections.

2.6. Signs and Symptoms of Diabetic Wounds

Diabetic wounds may present with the following signs and symptoms:

Chronic pain or completely painless, signs of inflammation (swelling, redness, heat, pain and loss of function), signs of infection (pus drainage, discharge, bad odor and dead tissue), new numbness and dullness (signs of nerve damage), fever and/or chills (signs of progressively worsening infection that can be limb-threatening or even life-threatening)

2.7. Potential wound pathogens

The majority of micro-organisms are less than 0.1mm in diameter and can therefore only be seen under a microscope. They can be categorized into different groups, such as bacteria, fungi, protozoa and viruses, depending on their structure and metabolic capabilities.

Diabetic patients with foot ulcers are subjected to several factors that may be associated with multidrug resistant microorganism's carriage, such as inappropriate antibiotic treatment chronic course of the wound and frequent hospital admission (Kandemiret *al.*, 2007)

2.8. Bacterial wound infection

2.8.1. *Staphylococcus aureus*

2.8.1.1. General characteristics

Staphylococci are Gram-positive cocci that form clusters and produce the enzyme catalase. The genus consists of several species. *Staphylococcus aureus* is the most virulent species, produces the enzyme coagulase, whereas all the other, less virulent species are collectively named coagulase-negative *Staphylococci*(Irving *et al.*, 2005).

2.8.1.2 Classification

Staphylococci are catalase positive whereas streptococci are catalase negative. *S.aureus* (b-hemolytic and coagulase positive) is distinguished from the coagulase negative *Staphylococci*, which are nonhemolytic.

2.8.1.3. Pathogenesis

Staphylococci possess carbohydrate capsules and produce numerous cell-wall-bound and secreted proteins that are designed to help the organism survive in various environments and invade the host. *S. aureus* produces several potent toxins which are responsible for the wide range of clinical syndromes caused(Irving *et al.*, 2005).

2.8.1.4. Epidemiology:

Most people carry *Staphylococci* on their skin and in their anterior nasal nares. The bacteria also colonize most animals and are transmitted by direct or indirect contact. Methicillin resistant *S. aureus*(MRSA) strains colonize hospital in patients and may cause epidemic outbreaks of disease among high-risk patients, such as those with post-operative wounds(Irving *et al.*, 2005).

2.8.1.5. Clinical infections:

S. aureus causes serious infections of the skin, soft tissues, bone, lung, heart, brain or blood. Coagulase-negative *Staphylococci* species are mainly associated with infected medical implants, although they can cause invasive disease in the immunocompromised host(Irving *et al.*, 2005).

2.8.1.6. Laboratory diagnosis:

Catalase-positive, Gram-positive cocci are labeled *S. aureus* if they are positive for coagulase and DNAase enzymes. Coagulase-negative Staphylococci are negative in the latter two tests. The antibiotic susceptibilities of all isolates are determined (Irving *et al.*, 2005). Strains associated with serious illnesses or outbreaks of infection (e.g. MRSA) are further characterized by typing and DNA fingerprinting (Irving *et al.*, 2005).

2.8.1.7. Treatment:

The majority of *Staphylococci* are resistant to penicillin, but sensitive to some of its derivatives, including methicillin and flucloxacillin. Alternative antibiotics for resistant organisms (e.g. MRSA) include vancomycin, erythromycin and gentamicin. Some strains become resistant to multiple antibiotics (Irving *et al.*, 2005).

2.8.1.8. Prevention:

Disrupt transmission by hand washing, use of effective disinfectants and reduce *Staphylococcus* on sheets and clothing (drying at $>70^{\circ}\text{C}$ or dry cleaning) reduce carriage use brief, high-dose perioperative antibiotics (Johnson *et al.*, 2002).

2.8.2. *Streptococcus pyogenes*

2.8.2.1. General characteristics

Streptococcus pyogenes is an aerobic, Gram-positive extracellular bacterium. (Cunningham 2008; Collins and Kennedy, 1983). Is a non-motile, non-spore-forming cocci that are less than 2 μm in length and that form chains and large colonies greater than 0.5 mm in size. (Murray *et al.*, 2007; Kilian 1998). It has a β -hemolytic growth pattern on blood agar and there are over 60 different strains of the bacterium (Bessen, 2009; Bocket *et al.*, 2000).

2.8.2.2. Classification

In 1928, Rebecca Lancefield published a method for serotyping *S. pyogenes* based on its M protein, a virulence factor displayed on its surface (Lancefield,1928).Later, in 1946, Lancefield described the serologic classification of *S. pyogenes* isolates based on their surface T antigen(Lancefield,1946).

Four of the 20 T antigens have been revealed to be pili, which are used by bacteria to attach to host cells(Mora *et al.*, 2005).

Over 220 M serotypes and about 20 T serotypes are known. This is why the plural term group *A Streptococci* (referring to the serotypes) and the singular term group *A Streptococcus* (referring to the single species) are both commonly encountered.

2.8.2.3 Pathogenesis

This bacterium is responsible for a wide array of infections (Cohen *et al.*, 2005;Carapetis *et al.*, 2005). It can cause streptococcal sore throat which is characterized by fever, enlarged tonsils, tonsillar exudate, sensitive cervical lymph nodes and malaise (Vincent *et al.*, 2004; Brocket *et al.*, 2000). If untreated, can last 7-10 days(Vincent *et al.*, 2004).

Scarlet fever (pink-red rash and fever) as well as impetigo, infection of the superficial layers of skin and pneumonia are also caused by this bacterium. (Murray *et al.*, 2007; Brock *et al.*, 2000; Cohen *et al* 2005 andFleming and Hunt, 2006).

Septicemia, otitis media, mastitis, sepsis, cellulitis, erysipelas, myositis, osteomyelitis, septic arthritis, meningitis, endocarditis, pericarditis, and neonatal infections are all less common infections due to *S. pyogenes*(Murray *et al.*, 2007; Brock *et al.*, 2002; Cohen *et al* 2005).

Streptococcal toxic shock syndrome, acute rheumatic fever (joint inflammation, carditis and CNS complications), post-streptococcal glomerulonephritis (inflammation, hematuria, fever, edema, hypertension, urinary sediment abnormalities and severe kidney pain) and necrotizing fasciitis (rapid and progressive infection of subcutaneous tissue, massive systematic inflammation, hemorrhagic bullae, crepitus and tissue destruction) are some of the more serious complications involving *S. pyogenes* infections (Cunningham, 2008; Brock *et al.*, 2000; Vincent *et al.*, 2004).

There are at least 517,000 deaths globally each year due to severe *S. pyogenes* infections and rheumatic fever disease alone causes 233,000 deaths (Carapetis *et al.*, 2005).

1,800 invasive *S. pyogenes* disease-related deaths are reported in the USA yearly where necrotizing fasciitis kills about 30% of patients and streptococcal toxic shock syndrome has a mortality rate of 30-70% (Cunningham 2008; Stevens 1995; Torralba and Quismorio, 2009).

2.8.2.4. Epidemiology

Different clinical manifestations of this bacterium are common in different parts of the world. Streptococcal pharyngitis is predominant in temperate areas and peaks in late winter and early spring (Bessen, 2009; Stevens 1995).

It is estimated that 616 million cases of pharyngitis caused by *S. pyogenes* world-wide each year (Bessen 2009; Carapetis *et al.*, 2005). 15-20% of school-aged children has *S. pyogenes* in its carrier form in their throats and are more at risk of having the disease (Bessen 2009; Vincent *et al.*, 2004). Impetigo is more common with children in warm humid climates and there are 111 million reported cases world-wide each year

(Bessen, 2009). There are 115.6 million cases of rheumatic heart disease yearly and at least 18.1 million cases of invasive infections, predominantly in older people (Murray *et al.*, 2007; Carapetis *et al.*, 2005).

Post-streptococcal glomerulonephritis is seasonal and is more common in children, young adults and males. Crowding and poor hygiene increases the chance of an outbreak of GAS infections (Cunningham, 2008).

2.8.2.5 Clinical infection Strep Pharyngitis

Strep Pharyngitis are known as strep throat, this disease can have symptoms similar to viral sore throats. Symptoms usually involve a low-grade or moderate fever, swollen lymph nodes, and a beefy, red throat with draining white patches. Children may also complain of headache and stomach ache (Cunningham, 2008).

Rheumatic Fever

Rheumatic fever is an uncommon, inflammatory complication of strep throat. It most often affects the skin, joints, heart, and the nervous system. Symptoms are quite variable. Fever, joint pain and a rash are most common. Chest pain or the feeling of heart flutter may occur.

Impetigo

Impetigo is a common superficial skin infection in young children, which spreads through direct contact with an infected person. Small pustules and reddened skin appear most commonly on the arms, hands, and face, although the lesions can occur anywhere on the body. The itchy sores ooze and form yellow crusts.

Scarlet Fever

Scarlet fever is an uncommon illness that produces a sore throat, fever, headache, and muscle aches. A rash caused by a strep bacterial toxin appears first on the neck and chest, then begins to spread. The pinkish-red rash has the feel of fine sandpaper and turns white with pressure. The papillae on the tongue become inflamed in what is called a “strawberry tongue. As the rash heals, it begins to peel and flake (Fleming and Hunt, 2006).

Toxic Shock Syndrome

Toxic shock syndrome is an uncommon but serious illness occurring in association with a primary infection somewhere in the body, usually the skin. Fever, confusion, very low blood pressure, kidney and liver failure, difficulty in breathing and bleeding problems develop within 48 hours of the onset of illness. The disease has a mortality rate of 30-70% (Murray *et al.*, 2007).

2.8.3. Enterobacteriaceae:

Enterobacteriaceae (also known as enterobacteria or coliforms) include numerous genera and species with several properties in common. They are Gram-negative bacilli, tolerate bile salts, grow under aerobic or anaerobic conditions, ferment glucose and produce catalase but not oxidase enzymes. *Escherichia coli* is the most common, and best known, species of this family. Other medically important enterobacterial genera include *Citrobacter*, *Enterobacter*, *Hafnia*, *Klebsiella*, *Morganella*, *Proteus*, *Providencia*, *Salmonella*, *Serratia*, *Shigella* and *Yersinia* (Irving *et al.*, 2005).

2.8.3.1. Antigen structure:

The most important antigens of the Enterobacteriaceae are: **O antigens**: these are specific polysaccharide chains in the lipopolysaccharide

complex of the outer membrane and the H antigens: flagella antigens consisting of protein' (Irving *et al.*, 2005).

K antigens: Linear polymers of the outer membrane built up of a repeated series of carbohydrate units (sometimes proteins as well). Which may cover the cell densely and render them O inagglutinable (Irving *et al.*, 2005).

F antigens: Antigens of protein attachment fimbriae (Irving *et al.*, 2005).

2.8.3.2. Pathogenesis:

Most Enterobacteriaceae members are gut commensals, but they are also pathogens. Some are more pathogenic than others (Irving *et al.*, 2005).

Virulence factors include capsules, endotoxins, motility elements, pili, numerous exotoxins and interactive surface molecules (Irving *et al.*, 2005).

2.8.3.3. Epidemiology:

Enterobacteria exist in very large numbers in the small and large intestine of humans and animals. Hospital environments usually have resident Enterobacteriaceae that are resistant to multiple antibiotics (Irving *et al.*, 2005).

2.8.3.4. Clinical infections:

Enterobacteriaceae cause a wide range of clinical conditions. Common ones include urinary tract infection, septicemia, gastroenteritis and hospital-acquired infections (Irving *et al.*, 2005).

2.8.3.5. Laboratory diagnosis:

Appropriate specimens are processed in the laboratory where Gram stain, colonial morphology, serology and biochemical reactions will all be used to identify the organism to species level, dependent on the seriousness of the condition and the clinical value of the information (Irving *et al.*, 2005).

Bacteria will be tested for their susceptibility to a range of antibiotics. Antibiotic choices depend on sensitivity of the isolate. Commonly used agents include amoxicillin, cephalosporins, gentamicin, and ciprofloxacin. Hospital-resident. Enterobacteriaceae can accumulate resistance against many of these antibiotics(Irving *et al.*, 2005).

Prevention and control: Generally, keeping good personal and food hygiene

2.8.3.6 *Escherichia coli*:

Escherichia coli is the best known and most important species of the genus *Escherichia*, one of the most prevalent members of Enterobacteriaceae, and the most common opportunistic pathogen that lives in human and animal gut Strains of *E. coli* are diverse, as confirmed by genome sequencing, and cause diverse clinical syndromes, including wound infection, septicemia and in neonates, severe meningitis (Irving *et al.*, 2005).

These include entero-pathogenic *E.coli*(EPEC), *E.coli*(ETEC), entero-invasive *E.coli*(EIEC), entero-aggregative *E.coli*(EAEC) and enterotoxigenic verocytotoxin-producing *E. coli* O157 (VTEC). They demonstrate different phenotypic characteristics and virulence capabilities.

VTEC strains live in animal gut and can cause major outbreaks of hemorrhagic colitis and hemolytic uremic syndrome which can be fatal(Irving *et al.*, 2005).

2.8.4. Opportunistic Enterobacteriaceae

2.8.4.1. *Klebsiella* spp

Klebsiella bacteria tend to be rounder and thicker than other members of the Enterobacteriaceae family. They typically occur as straight rods with

rounded or slightly pointed ends. They can be found singly, in pairs, or in short chains. Diplobacillary forms are commonly found *in vivo* (Ristucciaand Cunha, 1984).

They have no specific growth requirements and grow well on standard laboratory media, but grow best between 35 and 37 °C and at pH 7.2. The species are facultative anaerobes, and most strains can survive with citrate and glucose as their sole carbon sources and ammonia as their sole nitrogen source (Ristuccia andcunha, 1984).

Members of the genus produce a prominent capsule, or slime layer, which can be used for serologic identification, but molecular serotyping may replace this method (Brisse *et al.*, 2004) *K. pneumonia*, *K. oxymora* and other Lactose-positive, non-Motilestrains have a polysaccharide capsule, cause approximately 10% of nosocomial Infections. *Klebsiellaozaenae*, is the causative pathogen in ozena; atrophy of nasal mucosa. *Klebsiellarhinoscleromatis* the causative pathogen in rhinoscleroma granuloma in the nose and pharynx(Irving *et al.*, 2005).

2.8.4.2. *Proteus spp*:

P.mirabilis, *P.vulgaris*, Lactose-negative, highly motile, wanders on surface of nutrient agar (swarming). O antigens OX-2, OX-19, and OX-K from *P.vulgaris* is identical to rickettsiae antigens. For this reason, antibodies to rickettsiae were formerly identified using these strains by the (Weil-Felix agglutination test) (Irving *et al.*, 2005).

2.8.5. Other organisms

2.8.5.1 *Actinomycesisraelii*

Gram –positive, anaerobic, filamentous bacterium that causes a variety of soft tissue infections. It is a part of the normal flora of gingival crevices and the female genital tract, under condition of tissue damage (lower tissue oxygenation), invades soft tissue and bone and may disseminate (rarely) to the brain (Johnson *et al.*, 2002).

2.9. Treatment of Diabetic Wounds

Once a diagnosis of wound infection has been confirmed and antibiotic sensitivities identified, appropriate management regimens should be considered, with a high priority given to reducing the risk of cross infection. It is important to treat the patient as a whole and not the infection alone, so management strategies must be based on data derived from an holistic assessment of the needs of the individual. In addition to antibiotic therapy, there are two main generic groups of wound management products that have the potential to reduce the bacterial burden in the wound, these are compounds containing silver or iodine (Lawrence 1996).

The best treatment is prevention, since medical treatment for diabetic wounds provides limited help. If a wound occurs, treatment can include: keeping all wounds clean and properly dressed, administering antibiotics (for infected wounds or as a preventive measure for wounds at risk of getting infected), and surgical debridement (the dead or infected tissue is removed to allow the healthy tissue to heal and regenerate). Referral to a podiatrist or a wound care center (for patients with calluses, corns, hammertoes, bunions, toenail problems or chronic non-healing ulcers), and limb amputation (to save as much of a limb as possible when there is a serious infection).

2.9.1. Prevention

Prevention of diabetic wounds is critical for diabetic patients to ensure a normal and active healthy life. It is important to remember that diabetic wounds can be disabling and life threatening in some cases. Prevention should begin with: controlling diabetes by following the doctor's recommendations for treatment and lifestyle modifications that include a healthy diet, regular exercise, cessation of smoking and regular monitoring of blood glucose levels

2.10. Botanical Medicine

They are plants (or substances that come from plants) that are used to treat or prevent disease. Plants have been used in this way in all cultures from pre-history on. Research shows that many botanical medicines offer health benefits, often without some of the risks or side effects of pharmaceutical drugs. However: Botanicals are not always without risk, should be aware of possible adverse reactions. Botanicals are not always cheap, so you want to make sure you are getting the right, quality product.

In short, learning about botanical medicines can help to get the most benefit while reducing the risks (Building and Minneapolis, 2013)

2.10.1. *Rumex vesicarius*

2.10.1.1. Origin and geographic distribution

In Africa *Rumex vesicarius* occurs in drier regions from Mauritania and Mali east to Sudan, Ethiopia and Somalia; outside Africa from the Mediterranean east to India. It has occasionally been tried with success in more humid areas, e.g. in Tanzania. In other areas, e.g. Australia, it has

become a difficult to eradicate weed after introduction. (Backer and Bakhuizen *et al.*, 1963)

2.10.1.2. Botany

Annual or perennial, shrubby, rhizomatous herb up to 50 cm tall, strongly branched from the base, with young herbaceous green stems turning brown and woody when older. Flowers bisexual or male, enlarging to 2 cm in fruit and then with conspicuous red reticulate venation, fruit a trigonous nut 3–5 mm long, brown (Backer and Bakhuizen *et al.*, 1963).

2.10.1.3. Uses

In several parts of the Sahara and Sahel regions *Rumexvesicarius* is eaten as a vegetable, e.g. in Mauritania, Mali and Sudan. In India it is considered a famine food and the leaves are first boiled. It is grazed by livestock. *Rumexvesicarius* has many important medicinal uses such as treatment of tumors, hepatic diseases, bad digestion, can be used also to reduce biliary disorders and control cholesterol levels (Batanouny, 1999)

Extract of roots was found to be the most effective against *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Staphylococcus aureus* and *Streptococcus pyogenes* (Mostafa *et al.*, 2011).

Sunitha *et al.* (2011) evaluate anthelmintic prospective of crude benzene, ethanol and aqueous extracts on aerial parts of *Rumexvesicarius* Linn. Four concentrations (20, 40, 80 and 100 mg/ml) of benzene, ethanol and aqueous extracts of aerial parts of *Rumexvesicarius* Linn were investigated for in vitro anthelmintic activity employing Indian adult earthworms (*Pheretima posthuma*), (which involved) determination of

time paralysis (P) and the time of death (D) of the worms. Albendazole (20 mg/ml) was included as standard reference and ethanol: water (1:9) as control. All extracts of showed significant anthelmintic activity.

CHAPTER THREE

3. Materials and Methods

3.1. Study design

Descriptive cross-sectional study.

3.2. Study area

This study was conducted in Zinam Specialist Hospital and Diabetes and Endocrinology Internal Medicine Specialist hospital in Khartoum.

3.3. Study population

This study was performed on patients having wound infection associated with diabetes mellitus in Khartoum State locality.

3.4. Ethical consideration

Ethical approval to conduct this study was obtained from the college of Graduate Studies, Sudan University of Science and Technology. Permission was obtained from the hospitals in each of the centers that was included in the study. Verbal consent was taken from the patients to participate in this study.

3.5. Sampling

Fifty wound swabs were collected from wound diabetic patients during the study period from Febto June2016.

3.6. Characterization of Bacterial Isolates:

Wound samples were collected using sterile cotton swabs after the wound was washed by sterile normal saline. The swab specimen was wetted by nutrient broth before inoculated then inoculated on blood and MacConkey agar plates under aseptic technique. The streaked plates were incubated at 37°C for 24 hr. Identification of isolates were done based on colony morphology, Gram staining and biochemical tests (Koneman *et al.*,2005).

3.7. Media used in culturing the organisms

3.7.1. Blood agar

Blood agar(Appendix 1) is used to grow fastidious organisms (species that do not grow easily) requiring a rich media providing many nutrients and growth factors that are largely supplied by blood. It is also a differential media in allowing the detection of hemolysis (destroying the RBC) by cytolytic toxins secreted by some bacteria, such as certain

strains of *Bacillus*, *Streptococcus*, *Enterococcus*, *Staphylococcus*, and *Aerococcus*.

3.7.2. MacConkey agar (MAC)

MacConkey Agar (Appendix 1) is a selective and differential medium designed to isolate and differentiate bacteria based on their ability to ferment lactose.

3.8. Culturing of the specimens

Wound swabs were cultured on MacConkey agar and blood agar. Two plates were inoculated and incubated aerobically for 24 h at 37°C.

3.8.1. Identification

The identification of bacteria was based on different criteria (Cheesbrough, 2005).

3.8.1.1. Colonial Morphology

Small or large, low convex, non mucoid or mucoid, moist, transparent, irregular or regular, hemolytic colonies.

3.8.1.2. Smear preparation

Single pure colony was transferred from blood agar plates onto clean dry slide using a sterile loop, the smear was prepared and then fixed by heat.

3.8.1.3. Gram stain method

The smear was covered with crystal violet stain for 30-60 seconds. The stain was rapidly washed by tap water and tipped off the slide. Stained smear then was covered with iodine for 60-90 seconds. Iodine was immediately washed off by tap water and the smear was decolorized with ethanol. Safranin was added to the smear for 1 minute. The red stain was

then washed off with tap water and smear preparations were subsequently air-dried and microscopically examined using high – resolution objectives power (X100) under oil.

3.8.2. Identification of bacteria

Identification of bacteria was carried out using different biochemical tests.

3.8.2.1. For Gram negative bacteria

3.8.2.1.1. Oxidase test:

A piece of filter paper was placed in a clean petri-dish and 3 drops of freshly prepared oxidase reagent was added. A colony of the tested organism was removed using a piece of wooden stick and smeared on the filter paper if then is a purple colour within 10 seconds will be considered positive (Cheesbrough 2005).

3.8.2.1.2. Indole test:

A sterile loop was used to inoculate the tested organism into 2 ml peptone water, the tube was incubated at 37°C for 24 hrs 0.5 ml of Kovac's reagent (4 (p) – dimethylaminobenzaldehyde) was added, it was shaken gently and examined for red color ring within 10 mins

3.8.2.1.3. Citrate utilization test:

The test organism was inoculated into 2 ml of Simmon citrate medium using sterile straight wire. Then the medium was incubated at 37°C for 24 hrs. Blue color change that mean a positive result.

3.8.2.1.4. Urease production test:

The test organism was inoculated into slope surface of Christensen's urea medium using sterile straight wire, the medium was incubated at 37 °C for 24 hrs, and pink color change that mean a positive result.

3.8.2.1.5. Kligler Iron Agar (KIA)

The tested organism was inoculated in KIA medium, using a straight wire loop, agar butt was stabbed, the opening was closed and then the top slope was streaked (as zigzag). The medium was incubated at 37°C for 24 hrs,

glucose fermentation, lactose fermentation, H₂S production, gas production was looked for (Cheesbrough 2005).

3.8.2.2. For Gram positive bacteria

3.8.2.2.1 Catalase test:

Two ml of 3% hydrogen peroxide solution was poured into a test tube, then by using a sterile wooden stick a colony of the tested organism was removed and immersed in the solution, if immediate active bubbling was observed this will indicate for positive result (Cheesbrough, 2005).

3.8.2.2.2. Coagulase test (slide method): A drop of normal saline was placed on a slide, and a colony of the tested organism was emulsified to make a milky suspension, then a drop of undiluted plasma was added, mixed gently. Clumping of the organism within 10 seconds was observed (Cheesbrough 2005).

3.8.3. Susceptibility testing techniques

Kirby–Bauer antibiotic testing (KB testing or disc diffusion antibiotic sensitivity testing

In this test, antimicrobial discs were placed on an agar plate where a bacterium was cultured and then incubated. If an antibiotic stops the bacteria from growing or kills the bacteria, there will be an area around the wafer where the bacteria have not grown enough to be visible. This is called a zone of inhibition.

The size of this zone depends on how effective the antibiotic is at stopping the growth of the bacterium. A stronger antibiotic will create a larger zone, because a lower concentration of the antibiotic is enough to stop growth

3.9. Antibiotics and plant extract

Antibiotic susceptibility test was measured by agar diffusion method (disc test) to determine diameter of inhibition zones measured by (mm) by using Mueller-Hinton Agar.

The following antibiotic were used:

Antibiotic disc used for Gram negative bacteria:

Ampicillin 10 mc g/disc

Cot-rimoxazol25mc g/disc

Imipenam 10mcg/disc

Ciprofloxacin5 mc g/disc

Antibiotic disc used for Gram positivebacteria:

Erythromycin 15mc g/disc

Tetracycline 10mc g/disc

Penicillin 10mc g/disc

Gentamicin 10 mc g/disc

3.9.1. Plant materials

The plant *Rumex vesicarius* fruits were obtained from Industrial Research and Consultancy Centre (IRCC) farm, Khartoum State, Sudan.

3.9.1.1. Methods of plants preparation

The collected fruits parts of the plant were washed and dried under the shade. Fruits parts of the plant were soaked and extracted with ethanol alcohol 95% using Soxhlet apparatus. The solvent was evaporated on a rotary evaporator under reduced pressure and dried by oven.

3.9.2. Bacterial strains

Identified organisms colonies were stored in nutrient agar slope and were stored in the refrigerator at 4°C until used.

3.9.4. Obtaining Pure Culture of Bacteria by Streak Plate Method:

Subculture from nutrient agar slope was streaked across in MacConkey agar-solidified surface medium. The plates were then incubated under favorable conditions at 37°C. The key to this method is that, by streaking, a dilution gradient (a decreasing concentration of bacterial cells) was established across the surface of the plates, so that well isolated colony arises from a single bacterium and represents clone of a pure culture, which was then incubated at 37 °C for 24 hours to obtain pure colonies.

3.9.5. Antibacterial Testing of plants:

Antibacterial testing of plant (Appendix 1). The antibacterial activity and plant extract was tested by well-agar diffusion method. Use Mueller Hinton agar media and McFarland standard for testing the sensitivity. Six wells (Three wells for each plant) (4 mm in diameter) were bored in the

agar using a sterile cork borer and the agar discs were removed. 50 μ l from plants extract were placed into a well with a pipette and the plate was held for 2 hours at refrigerator for diffusion of extract into agar. Subsequently, the plate was incubated at 37°C for 24 hours. After incubation the averages of diameter of inhibition zones were measured and interpreted in terms of the commonly used terms: sensitive, and resistant. Extract resulting in more than 18 mm growth inhibition zones are considered to possess relatively high antimicrobial activity, and those resulting 14-18 mm are of intermediate activity, and those resulting in zone below 14 mm are inactive (Cruickshank *et al.*, 1975). According to the interpretation of results discussed, the extracts were arranged into groups according to their activity.

A three-fold serial dilution of each extract was prepared in DMSO (diluent) to achieve a decreasing range of extract concentrations from 100 mg/ml_50mg/ml_25mg/ml DMSO as a negative control.

CHAPTER FOUR

RESULTS

4.1.Epidemiological finding

4.1.1. Gender

Fifty wound swab specimens were collected from diabetic males and females patients with wound infection. Out of 50 patients 9 (18%) patients were males and 41 (82%) patients were females. (Table 2).

table2. Gender and frequency

Gender	Frequency	Percent (%)
Males	41	82
Females	9	18
Total	50	100

4.1.2. Age group

Age groups enrolled in this study were from 20-98 years. (Table 3)

Table 3. Distribution of enrolled patients according to gender

Age	Frequency	Percent (%)
20-40	4	8
41-60	23	46
61-80	20	40
81-100	3	6
Total	50	100

4.2. Bacterial growth

4.2.1. Growth of bacteria on MacConkey Agar

The MacConkey agar medium after inoculated under aseptic condition and incubated overnight at 37 °C, gave characteristic colonies that ferment lactose or not and different morphology large red surround by turbid zone (*E.coli*), translucent colorless colonies surround by yellow zone (*Proteus spp*), large mucoid colonies (*Klebsiella spp*), pale colored colonies (*P.aeruginosa*) according to the species

4.2.2. Growth on blood agar

Inoculation of specimen under aseptic condition and after incubate overnight at 37 °C, gave different characteristic colonies according to hemolysis of RBS and colour of colony

4.3. Bacteria isolated from the diabetic wound infection

From the table it is evident that *Staphylococcus aureus* (30%) is the predominant bacterium that is isolated from the diabetic wound followed by *Pseudomonas aeruginosa* (22%) and low evident *Escherichia coli* (8%).

Table 4. The aerobic bacteria that isolated from the diabetic patients

Name of bacteria	Number of patient	%
<i>Staphylococcus aureus</i>	15	30
<i>Pseudomonas aeruginosa</i>	11	22
<i>Proteus vulgaris</i>	4	8
<i>Proteus mirabilis</i>	7	14
<i>Klebsiella pneumoniae</i>	9	18
<i>Escherichia coli</i>	4	8
Total	50	100

4.4. Susceptibility of the organisms:

This activity of the plant extract and antibiotics for *S. aureus* is shown in (table 5)

Table 5. Percentage of antibiotics susceptibility test of four antibiotics and two plant extracts against *Staphylococcus aureus*

Antibacterial agent	Conc	Percent of (S)	Percent of (R)
Erythromycin	15mc g/disc	60	40
Tetracycline	10mc g/disc	66.6	33.4
Gentamicin	10mc g/disc	46.6	53.4
Penicillin	10mc g/disc	0	100
<i>Rumex vesicarius</i>	100%	20	80

Key:

S: sensitive.

R: resistance. **Appendix 1**

The activity of plant extract and antibiotic on other isolated organisms is show in table 6. Only *Proteus* species and *Pseudomonas* were slightly sensitive to *Rumexvesicarus*.

Table 6. Percentage of antibiotic susceptibility test of four antibiotic and plant extract against other isolated bacteria

Bacteria	Antibacterial agent									
	CIP %		COT %		IMP %		AMC %		Rumex %	
	R	S	R	S	I N	S	I N	S	R	S
<i>Proteus mirabilis</i>	57.2	42.8	71.4	28.6	1	99	1	99	71.4	28.6
<i>Klebsiella pneumoniae</i>	33.4	66.6	66.6	33.4	1	99	0	100	100	0
<i>Pseudomonas aeruginosa</i>	27.3	72.7	100	0	0	100	1	99	100	0
<i>Proteus vulgaris</i>	25	75	75	25	0	100	0	100	75	25
<i>E. coli</i>	50	50	100	0	0	100	0	100	100	0
<i>p. value</i>	0.41		0.12		0.05		0.05		0.11	

Key:

CIP= Ciprofloxacin COT= Cotrimoxazole IMP= Imipenam AMC= Amikacin

Rumex= Rumexvesicarus

CHAPTER FIVE

DISCUSSION

This study was conducted in order to identify the bacterial pathogens associated with diabetic wounds and determining their susceptibility to antibiotic and plant extract. The isolated bacteria from the diabetic wound were as follows: *Staphylococcus aureus* 15(30%), *Pseudomonas aeruginosa* 11 (22%), *Klebsiella pneumoniae* 9 (18%), *Proteus mirabilis* 7 (14%), *Proteus vulgaris* 4 (8%), *Escherichia coli* 4 (8%).

These findings were partially agreed with those of Lycos, (2007). Who reported the isolation of *propionic bacterium granulosum* (as a predominant anaerobic bacteria in diabetic wounds (17cases 19.54%),

following by *staphylococcus saprophytics* as aerobic bacteria (12 cases 13.79%). While *Pseudomonas aeruginosa* was a predominant pathogens isolated from non-diabetic wound infection. The ability of *S. aureus* to cause diabetic foot infection is defined by numerous virulence factors among which secreted toxins play an important role in colonization, persistence, are evasion of the immune system and dissemination (Vandeneschet *al.*, 2012).

All strains of *Staphylococcus aureus* were found to be 100% resistant to penicillin because of the ability of the bacteria to produce beta-lactamase enzyme for drug resistance (Kluytmans *et al.*, 1997; Deurenberget *al.*, 2007). An estimated 80% of all *S. aureus* isolates are penicillin-resistant. Most isolates were found to be 20% sensitive to Fruit *Rumex*.

Most isolates were found to be 70-100% resistant to fruit *Rumex vesicarius* these findings were in consistent with those of (Pandurajuet *al.*, 2009) showing the potential usefulness of *Rumex vesicarius* in the treatment of pathogenic diseases. Effective antibiotic is Amikacin and Imipnem (broad-spectrum beta-lactam) showed high degree of sensitivity (100%), Imipenem was also reported to be the most effective agents against bacteria isolated in diabetic foot infection inconsistent with Gadepalliet *al.* (2006); Abdulrasaket *al.*, (2005). All strains were found to be resistant to the most antibiotics and their susceptibility was found to be 100% resistant, (p.value < 0.05 significant).

Conclusion

- *Staphylococcus aureus* was the most common pathogens identified in this study that is causing wound infection.

- The fruit extract of *Rumex vesicarius* showed low effectiveness against the bacterial pathogen isolated from wound infection of diabetic patient's comparison with other effective antibiotic used.
- Amikacin and Imipenem were the most effective antibiotics

Recommendations

1. Further extensive studies in *Rumex vesicarius* could be done to show their effectiveness on the other parts of plant.
2. Further extensive studies on diabetic mellitus to identify the risk and designing control measures.
3. The amount of sample must be increased to detect the amount of resistance and sensitivity to plant extract.
4. Further studies in *Rumex vesicarius* may be adopted by another extraction technique.

References

Abdulrasak, A., Z. Bitarl, A. AL-Shamali and L.A. Mobasher, (2005). Bacteriological study of diabetic foot infections. *J. Diabetes Complications*, 138-141.

American Diabetes Association (1998) 'Economic consequences of .diabetes mellitus in the U.S. in 1997' *Diabetes Care*; 21(2):296-309.

Backer, C. A. and R. C. Bakhuizen van den Brink jr., (1963) - Flora of Java (Spermatophytes only). 1 Noordhoff Groningen: 648 pp.

- Batanouny, K.H.** (1999). Wild medicinal plants in Egypt. Academy of Scientific Research and Technology, Egypt and International Union for Conservation (IUCN), Switzerland; . pp. 166- 167.
- Bessen, D. E.** (2009). Population biology of the human restricted pathogen, *Streptococcus pyogenes*. *Infection, Genetics and Evolution : Journal of Molecular Epidemiology and Evolutionary Genetics in Infectious Diseases*, **9**(4), 581-593.
- Betoni JEC, Mantovani RP, Barbosa LN, Di-Stasi LC, and Fernandes A** (2006). Synergism between plant extract and antimicrobial drugs used on Staphylococcus aureus diseases. *Mem. Inst. Oswaldo Cruz*.
- Boulton, A.J.**, (2002). The diabetic foot: a global view. *Diabetes Metab. Re.s Rev.* **16**(suppl 1):S2 S5.
- Boulton, A.J.M., L. Vileikyte, G. Ragnarson- Tennvall and Apelqvist, J.** (2005). The global burden of diabetic foot disease. *Lancet* . **366**:1719 1724.
- Braces A** (2007). Infection of the diabetic foot. Available at <http://www.Bracesandsupports.com./diabetes in 28-4-2007>.
- Brisse, Sylvain; S Issenhuth-Jeanjean; P AD and Grimont** (2004). "Molecular Serotyping of Klebsiella Species Isolates by Restriction of the Amplified Capsular Antigen Gene Cluster". *Journal of clinical Microbiology* **42** (8): 3388–3398.
- Brock, T. D., Madigan, M. T., Martinko, J. M., and Parker, J.** (2000). *Biology of Microorganisms* (9th ed.). New Jersey, USA: Prentice-Hall, Inc.

Building MM and Minneapolis, MN (eds) (2013) *Botanical Medicine*,
Carapetis, J. R., Steer, A. C., Mulholland, E. K., and Weber, M.
(2005). The global burden of group A streptococcal diseases. *The Lancet Infectious Diseases*, **5**(11), 685-694.

Cheesbrough, M (2005) *Laboratory Practice in Tropical Countries* part
22^{ed} edition. 62-70.

Cohen, R., Aujard, Y., Bidet, P., Bourrillon, A., Bingen, E., Foucaud, P., Francois, M., Garnier, J. M., Gendrel, D., Guillot, M., Hau, I., Olivier, C., Quinet, B., and Raymond, J. (2005). Streptococcus pyogenes an emerging pathogen. [Le streptocoque du groupe A. Un pathogene majeur pour la prochaine decennie?] *Archives De Pediatrie : Organe Officiel De La Societe Francaise De Pediatrie*, **12**(7), 1065-1067.

Collins, C. H., and Kennedy, D. A. (Eds.). (1983). *Laboratory-acquired Infections* (4th ed.). Oxford: Butterworth-Heinemann.

Cruikshank, R., (1975). *Medical Microbiology: A Guide to Diagnosis and Control of Infection*. 1st. Edinburgh and London: E and S Livingston, Ltd, pp: **888**.

Cruikshank, R.; J.P. Duguid; B. P. Marmion and Swain. R. H.
(1975), *Medical Microbiology* (Eds), Churchill Livingstone (pub)
Edinburgh, 12.Ed.

Cunningham, M. W. (2008). Pathogenesis of group A streptococcal infections and their sequelae. *Advances in Experimental Medicine and Biology*, **609**, 29-42

Deurenberg, RH, Vink, C, Kalenic, S, Friedrich, AW, Bruggeman, CA, and Stobberingh, EE. (2007) the molecular evolution of methicillin-

resistant *Staphylococcus aureus*. *Clinical Microbiology and Infection*. **13**:222-235.

Fleming D & Hunt D (Ed.). (2006). *Biological Safety Principles and Practices* (4th ed.). Washington: ASM Press.

Gadepalli, R., B. Dhawan, V. Sreenivas, A. Kapil, A.C. Ammini and R. Chaudhry, (2006). A Clinical microbiological study of Diabetic foot ulcers in an Indian tertiary care hospital *Diabetic Care*, **29**: 1727-1731.

<http://www.takingcharge.csh.umn.edu/explore-healing-ractices/botanical-medicine>

Irving, W.; Boswell, T and Ala'Aldeen, D; (2005) Section C Human pathogens: Gram-positive bacteria *Medical Microbiology* 2^{ed} edition 113-116; Gram-negative bacteria. 133-156.

Iwu MW, Duncan AR, and Okunji CO (1999). New antimicrobials of plant origin. Janick J (ed.), *Perspectives on new crops and new uses*, pp. 457-462.

Johanson, Arthur, G.Ziegler, Richard, J. Lukasewycz, Omelan A. Hawley, Louise B(2002). *BRS Microbiology & Immunology* 4th Edition.

Kamel, C. (2000). A novel look at a classic approach of plant extracts. *Feed Mix* **8**:16-17.

Kandemir, O., E. AkbaySahin. A. Millan and R. Gen (2007). Risk factor for infection of the dia foot with multi-antibiotic resistant microorganisms. *J. Infect*, **54**: 439-445.

Kannan I, Premavathy RK, Cecilia S, Jayalakshmi M, Sruthi Priyadarsini S, and Shantha S. (2014) Isolation and antibiotic susceptibility of bacteria from foot infections in the patients with diabetes

mellitus type I and type II in the district of Kancheepuram, Tamil Nadu, India. *Int J Res Med Sci*;457_61.

Kennedy M, (2006_2017) complication of diabetic
<http://dtc.ucsf.edu/living-with-diabetes/complications>

Kilian, M. (1998). *Streptococcus* and *Lactobacillus*. In A. Balows, & B. I. Duerden (Eds.), Topley & Wilson's microbiology and microbial infections (9th ed., pp. 633-668). London: Arnold.

Kluytmans J, van Belkum A, Verbrugh H (July 1997). "Nasal carriage of *Staphylococcus aureus*: epidemiology, underlying mechanisms, and associated risks". *Clin. Microbiol. Rev.* **10** (3): 505–20.

Koneman, W.K., S.D. Allen, W.M. Janda, P.C. Schreckenberger, G.W. Procop, G.L. Woods and W.C. Winn,(2005). *Jr.* Philadelphia Color Atlas and Textbook of Diagnostic Microbiology, 6thed. th Lippincott-Raven Publisher, pp: 624-662).

Lalitha, M. K. 2004 "Manual on antimicrobial susceptibility testing." *Performance standards for antimicrobial testing: Twelfth Informational Supplement* 56238 : 454-456.

Lancefield RC(1928)."*The antigenic complex of Streptococcus hemolyticus*". *J Exp Med* **47** (1): 9–10.

Lancefield RC, and Dole VP(1946)."*The properties of T antigen extracted from group A hemolytic streptococci*". *J Exp Med* **84** (5): 449–71.

Lavery LA, Reinkhart YM, Dulph LB, and Gohensen MY (2007). Preventing diabetic foot ulcer recurrence in high-risk patients. *Diabetes care*, **30**: 14-20.

Lawrence JM; Contreras, R; Chen, W; Sacks. DA; (1996), Trends in Prevention of preexisting Diabetes and Gestational Diabetes mellitus among racially, ethnically diverse population of pregnant women diabetes.

- Levy SB, and Marshall B** (2004). Antibacterial resistance worldwide: causes, challenges and responses. *Nat. Med.* 10: S122-S129.
- Louie A, Baltch AL, and Smith RB** (1993). Gram negative bacterial surveillance in diabetic patients. *Infect. Med.*, **10**(2): 33-45. Lycos Inc. (2007). Ulcer: Diabetic foot ulcers. Available at [http://www.LycosRetriever.com/ulcer diabetic foot./html](http://www.LycosRetriever.com/ulcer%20diabetic%20foot./html).
- McMahon MM, and Bistrain BR** (1995). Host defences and susceptibility to infection in patients with diabetes mellitus. *Infect. Dis.Clin. North Am.*, 9: 1-7.
- Monk A, Pearson J, Hollander P, Bergenstal RM** (1996). Managing type II diabetes: your invitation to a healthier lifestyle. Minneapolis, MN: IDC Publishing.
- Mora M, Bensi G, Capo S, Falugi F, Zingaretti C, Manetti AG, Maggi T, Taddei AR, Grandi G, and Telford JL** (2005). "Group A *Streptococcus* produce pilus-like structures containing protective antigens and Lancefield T antigens". *Proc Natl Acad Sci USA***102** (43): 15641–6.
- Mostafa, H.A.M, Elbakry, A.A. and Eman, A. ALAM,** (2011), uses of *rumex vesicarius* *International Journal of Pharmacy and Pharmaceutical Sciences* 2,109-110.
- Murray, P. R., Baron, E. J., Jorgensen, J. H., Landry, M. L., and Pfaller, M. A.** (Eds.). (2007). *Manual of Clinical Microbiology* (9th ed.). Washington: ASM Press.
- Panduraju,T.Rao,P.R.S.Kumar,V.S,**(2009)characteristics of diabetes mellitus *International Journal of Pharmacy and Technology*,1, 21-25.
- Prasai, T.; Yami, K.D.;and Joshi D.R.** (2008).Microbial Load on Paper/Polymer Currency and Coins. *Nepal Journal of Science and Technology***9**:105-109.

- Ramani, A., R. Ramani, P.G. Shivananda and Kundaje,G.N.** (1991). Bacteriology of diabetic foot ulcers. *Indian. J. Pathol. Microbiol.* **34**: 81-87.
- Ristuccia, Patricia A; and Cunha Burke A** (1984). "Klebsiella". *Topics in Clinical Microbiology* **5** (7): 343–348. JSTOR30144997.
- Rosenbloom AL, Joe JR, Young RS, Winter WE** (1999). Emerging epidemic of type 2 diabetes in youth. *Diabetes Care*; **22**(2):345-354.
- Shibata H, Kondo K, Katsuyama R, Kawazoe K, Sato Y, Murakami K, Takaishi Y, Arakaki N, and Higuti T** (2005). Alkyl Gallates, Intensifiers of β -Lactam Susceptibility in Methicillin-Resistant *Staphylococcus aureus* *Antimicrob. Agents Chemother.* **49**(2): 549-555.
- Singh, N., D.G. Armstrong and Lipsky, B.A.** (2005). Preventing foot ulcers in patients with diabetes. *JAMA.* **293**; 217-228.
- Stadelmann WK, Digenis AG, and Tobin GR** (1998). Impediments to wound healing. *Am. J. Surg.*, **176** (2Asuppl): 395-475.
- Stang J, and Story M** (eds)(2005) *Guidelines for Adolescent Nutrition Services* **167**.
- Stang J, Story M** (eds) (2005) *Guidelines for Adolescent Nutrition Services* 167 http://www.epi.umn.edu/let/pubs/adol_book.shtm.
- Stevens, D. L.** (1995). Streptococcal toxic-shock syndrome: spectrum of disease, pathogenesis, and new concepts in treatment. *Emerging Infectious Diseases*, **1**(3), 69-78.
- Styers D, Sheehan DJ, Hogan P, and Sahm DF** (2006). Laboratory-based surveillance of current antimicrobial resistance patterns and trends among *Staphylococcus aureus*: 2005 status in the United States. *Ann. Clin. Microb. Antimicrob.* **5**: 2.

Sunitha Ch, David Banjii, Sandhyas and Saikumar (2011), *Asian J. Plant Sci. Res.*, Pharmacognostic Studies on *Rumex vesicarius*, **1** (1):102-115 <http://www.prota4u.org/protav8.asp?en=1&p=Rumex+vesicarius+L>

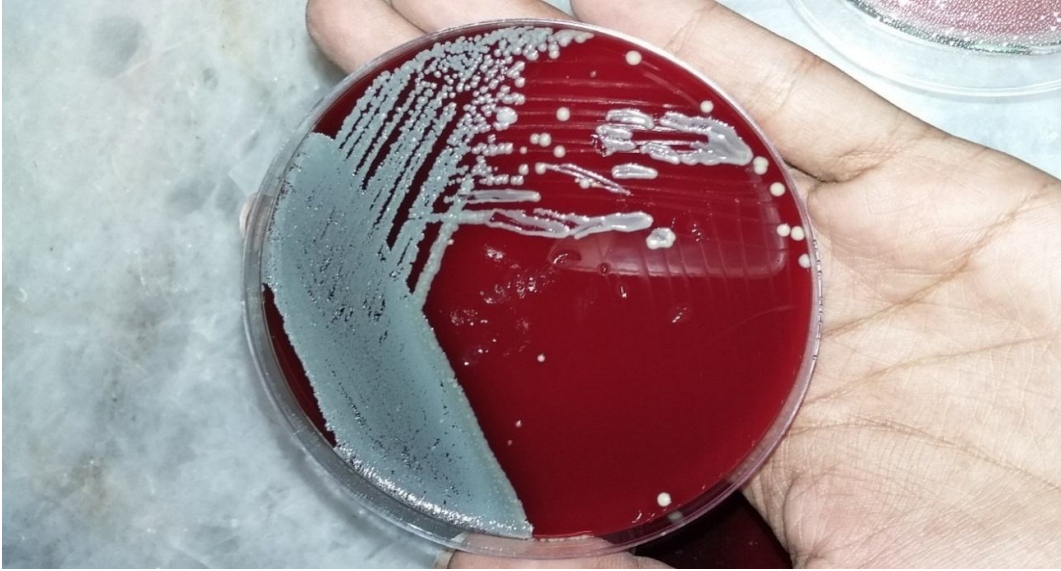
Torralba, K. D., and Quismorio, F. P., Jr. (2009). Soft tissue infections. *Rheumatic Diseases Clinics of North America*, **35**(1), 45-62.

Vandenesch, F; Lina, G.; and Henry, T. (2012) *Staphylococcus aureus* hemolysins, bi-component leukocidins, and cytolytic peptides: A redundant arsenal of membrane-damaging virulence factors? *Front. Cell. Infect. Microbiol.* **2**, 12.

Vincent, M. T., Celestin, N., and Hussain, A. N. (2004). Pharyngitis. *American Family Physician*, **69**(6), 1465-1470.

World Health Organization (WHO) (2002). Antimicrobial resistance. Fact sheet No. 194.

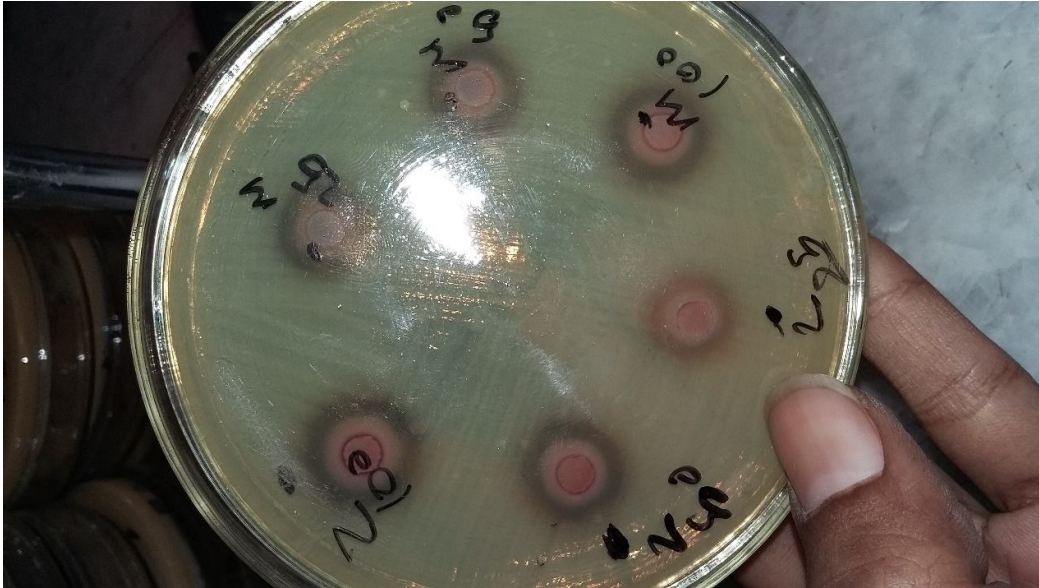
Appendix I



Blood ager culture (large colonies) and non hemolytic



macConkey agar culture lactose ferment



Antimicrobial Susceptibility of Plants Extract (Rumex vesicaria and Botanical garden)



Antimicrobial Susceptibility of Plants Extract (Rumex vesicaria and Botanical garden)

Appendix II

Preparation of reagents and culture media

1. Blood agar base

Blood agar base is recommended as base to which blood may be added for use in the isolation and cultivation of fastidious pathogenic microorganisms.

Compositions

Ingredients	Gms/L
Beef heart, infusion (beef extract).....	5000
Tryptose	10
Sodium chloride	5
Final pH	7.3

Directions

Suspend 40 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterile by Autoclave at 15 lbs pressure (121°C) for 15 min. Cool to 50°C and aseptically add 5% v/v sterile defibrinated blood. Mix well and pour into sterile petridishes.

2. Crystal violet Grams stain

To make 1 liter

Crystal violet.....	20 g
Ammonium oxalate	9 g
Ethanol or methanol, absolute	95ml
Distilled water	to 1 liter

3. Weight the crystal violet on a piece of clean paper. Transferred to a brown bottle pre marked to hold one litter
4. Add the absolute ethanol or methanol and mix until the dye is completely dissolved.
5. Weight the ammonium oxalate and dissolve in about 200 ml of distilled water. Add the stain, make up to one litter with distilled water and mixed well.

6. Label the bottle and store it at room temperature. The stain is stable for several months.

3. Kligler Iron Agar (KIA)

KIA reactions are based on the fermentation of lactose and glucose (dextrose) and the production of hydrogen sulphide.

Compositions

Ingredients	Gms/L
Peptic digest of animal tissue.....	15
Yeast extract.....	3
Beef extract.....	3
Peptose peptone	5
Dextrose	1
Lactose	10
Ferrous sulphate	0.20
Sodium chloride	5
Sodium thiosulphate.....	.0.3
Phenol red.....	..0.042
Agar.....	15
Final pH (at 25°C).....	7.4

Directions

Suspend 57.5 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterile by Autoclave at 15 lbs pressure (121°C) for 15 min. mix and pour. Set as slope with butt.

5. Lugol's iodine solution

To make one litter

Potassium iodine solution	20 g
Iodine	10 g
Distilled water	to 1 litter

1. Weight the potassium iodine, and transfer to brown bottle pre marked to hold 1 litter.

2. Add about quarter of the volume of water, and mix until the potassium iodine solution is completely dissolved.
3. Weight the iodine, and add to potassium iodide solution. Mix until the iodine is dissolved.
4. Make up to 1 litter distilled water, mix well. Label the bottle and marked toxic. Store at dark place

6. Mac Conkey Agar medium

Mac Conkey Agar medium is a differential medium to distinguish between bacteria by neutral red indicator which changes colour when acid is produced following fermentation of lactose sugar.

Composition

Ingredients	Gms/L
Peptic digest of animal tissue.....	17
Protease peptone.....	3
Lactose.....	10
Bile salts	1.5
Sodium chloride	5
Neutral red.....	.03
Agar.....	15
Final pH (at 25°C).....	7.2

Directions

Suspend 51.53 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterile by Autoclave at 15 ibs pressure (121°C) for 15 min. mix and pour.

7. McFarland Standard Turbidity tube 0.5:

Ingredients

Conc. Sulphuric acid	1 ml
Dehydrated barium chloride	0.5g
Distilled water	99 ml

Prepare 1% V/V of sulphuric acid solution by adding 1 ml of concentrated sulphuric acid to 99 ml of DW and mix. Prepare 1% w/v solution of barium

chloride by dissolve 0.5g of dehydrated barium chloride in 50 ml of distilled water. Add 0.6 ml of sulphuric acid then mix well.

8. Muller Hinton agar

Muller Hinton agar is used for testing susceptibility of common and rapidly growing bacteria using antimicrobial disc, it manufactured to contain low level of thymine, thymidine, calcium and magnesium.

Compositions

Ingredients	Gms/L
Casein acid hydrolysate	17
Beef heart infusion	2
Starch soluble	1.5
Agar.....	17
Final pH (at 25°C).....	7.3

Directions

Suspend 38 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterile by Autoclave at 15 lbs pressure (121°C) for 15 min. mix and pour.

9. Nutrient agar

Nutrient agar is used for cultivation of less fastidious organisms, can be enriched with blood or other biological fluids.

Compositions

Ingredients	Gms/L
Peptone	10
Beef extract	10
Sodium chloride	5
Yeast xtract.....	1.5
Agar.....	15
Final pH (at 25°C).....	7.3

Directions

Suspend 28 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterile by Autoclave at 15 lbs pressure (121°C) for 15 min. mix and pour.

1. Oxidase Reagent

Prepare fresh before use.

To make 10 ml:

Tetramethyle-*p*-phenylenediamine dihydrochloride0.1 g

Distilled water10ml

Dissolve the chemical in water. The reagent is not stable.

2. Peptone water

Used for culturing organisms to proceed indole test in the presence of Kovac's or Ehrlich's reagent that reacts with the indole to produce a red coloured compound.

Compositions

Ingredients	Gms/L
Peptic digest of animal tissue.....	10
Sodium chloride	5
Final pH(at 25°C).....	7.2

Directions

Suspend 15 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterile by Autoclave at 15 lbs pressure (121°C) for 15 min. mix and pour.

3. Simmons citrate Agar

This test is used to assist in the identification of enterobacteria. The test is based on the ability of an organism to use citrate as its only source of carbon.

Compositions

Ingredients	Gms/L
Magnesium sulphate	0.20
Ammonium dihydrogen phosphate	1
Dipotassium phosphate	1
Sodium citrate	2

Sodium chloride	5
Bromothymol blue	0.08
Agar.....	15
Final pH (at 25°C).....	6.8

Directions

Suspend 24.28 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterile by Autoclave at 15 lbs pressure (121°C) for 15 min. mix and pour. Set as slope.

4. Urea Agar Base (Christensen)

Testing for Urease enzyme activity is important in differentiating enterobacteria. Especially for *proteus spp*

Compositions

Ingredients	Gms/L
Peptic digest of animal tissue.....	1
Dextrose	1
Disodium phosphate	1.20
Monopotassium phosphate	0.80
Sodium chloride	5
Phenol red.....	0.012
Agar.....	15
Final pH (at 25°C).....	6.8

Directions

Suspend 24 grams in 950 ml distilled water. Heat to boiling to dissolve the medium completely. Sterile by Autoclave at 10 lbs pressure (115°C) for 20 min. Cool to 50°C and aseptically add 50 ml of sterile 40% of urea solution (FD048) and mix.