



بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ



Republic of Sudan

Ministry of Higher Education and Scientific Research

University of Shendi

Faculty of Graduate Studies and Scientific Research

**Antimicrobial Activity of *Acacia nilotica* Extracts
Against Bacteria Isolated from Wound Infection**

A thesis submitted in partial fulfillment of the degree of MSc. In Medical
Laboratory Science (Microbiology).

By

Abdel Hafeez Gasmel Seid Fadelmola

B. Sc of Medical Microbiology Omdurman Islamic University (2008)

Supervisor

Dr. Ahmed Mohammed Ahmed

August – 2018

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

الآية

قال تعالى:

((وَهُوَ الَّذِي أَنْزَلَ مِنَ السَّمَاءِ مَاءً فَأَخْرَجْنَا بِهِ نَبَاتَ كُلِّ شَيْءٍ فَأَخْرَجْنَا مِنْهُ خَضِرًا نُخْرِجُ مِنْهُ حَبًّا مُتَرَاكِبًا وَمِنَ النَّخْلِ مِنْ طَلْعِهَا قِنْوَانٌ دَانِيَةٌ وَجَنَّاتٍ مِنْ أَعْنَابٍ وَالزَّيْتُونَ وَالرُّمَّانَ مُشْتَبِهًا وَغَيْرَ مُتَشَابِهٍ ۗ انظُرُوا إِلَى ثَمَرِهِ إِذَا أَثْمَرَ وَيَنْعِهِ ۗ إِنَّ فِي ذَٰلِكُمْ لَآيَاتٍ لِقَوْمٍ يُؤْمِنُونَ))

سورة الأنعام الآية 99

Dedication

I dedicate this work to ...

Soul of my dear Father ...

Who dedicated his life for the sake of our comfort?

And keep us happy...

To ... the melted candle which illuminate the way for us.....

My mother

To my all friends

Acknowledgment

First of all my thanks to ALMIGHTY ALLAH for giving me health and strength to accomplish this work.

Secondly special thanks to my director and supervisor

Dr. Ahmed Mohammed Ahmed

for his encouragement, follow up and guiding and directing.

My great thanks to all staff of Medical and Aromatic Plant Research Institute, Department of Microbiology for their help.

My thanks and gratitude's must also go to *Us. Khalid saed* who generosity offered me unlimited help in this study.

My thanks and appreciation is also extended to mainly *Mohammed Almahy*.

Last, but not least I should thank and appreciate any one who helped us directly or indirectly in the preparation and revision of this study during the research work or writing and analyzing the manuscript. My best regards to all without any exceptions.

Abstract

Background : The recent recognition of emerging infectious diseases has heightened appreciation of the importance of epidemiologic information. In addition, some well-known pathogens have assumed new epidemiologic importance by virtue of acquired antimicrobial resistance. Some plants are known as medicinal because they contain active substances that cause certain reactions, from relating to cure of disease on human.

Objectives: this study aim to study the antibacterial activity of the different concentrations of aqueous extract of the medicinal plants *A.nilotica* sub spp *Nilotica pods* using agar diffusion method.

Methodology: Fifty-five wound swabs were collected from patients with infected wounds who attended to Shendi Teaching and Almek-nimir University Hospitals during period from March to August 2018.

according age Out of 55 specimens, 31 samples were taken from patients of with mean age + <20 age group, from 21-40 age group, from 41-60 age group, 9 from 61-80 agegroup and 3 from >80 age group. The age grouped in to fivegrouped majority samples taken from age group 21-40 Year.

Results: From this study it was found that wound infection had high frequency in patients of age 21-40 years. *S.aureus* was major isolates, and no significant association was found between wound infection and history of disease. And according to gender Out of 55 specimens, 35(64%) were males whereas 20(36%) were females.

The aqueous extract of *A.nilotica pods* (Family-Fabaceae) were screened for their antimicrobial activity against clinical isolates of *S.aureus* (29), *E.coli*(2), *K.pneumoniae*(15), *Ps.aeruginosa*(2), and *Proteus spp*(1).

The aqueous extract pods of *A.nilotica* exhibited high activity against *S.aureus*.

The antibacterial activity of reference drug Gentamicin is determined against the isolates bacteria and their activities were compared to the activity of plants extract.

At this study the mean inhibition zone diameter of microorganism isolated increases with the increase in extract concentration. *A.nilotica* extract was tested using different concentration (100, 50, 25 and 12.5) *E.coli* mean of inhibition zone according to concentration (13.5,12.5,10.5 and 3.5)(P= 0.010) *S.epidermidis* (16.6,14.6, 13.3and 11.3) (P=0.003), *S.aureus* (16.8,14.4,12.9 and 11.1) (P=0.005), *Ps. Aerginosa* (19.0, 16, 15.0 and 11) (P=0.023) *K. pneumonia* (9.7,8.8,8.5and 8.2)(P=0.044) which are statistically significant except *P. vulgris*. Which have mean inhibition zone close to each other (12.0,11,11and 10) (P=0.051).

The aqueous extract of *A.nilotica* sub spp. exhibited high antibacterial activity against *S.auerus* and moderately activity against *E.coli*..

The results of antimicrobial activity of crude extract was compared with the positive control (Standard drugs) the bacteria is sensitive to extract in relation to positive control (Gentamicin) the aqueous extract exhibits maximum relative percentage inhibition against *S.epidermidis* (68.8%) and minimum relative percentage inhibition against *Klebsiellapneumoniae*. (10.7%).

المستخلص

المقدمة: في الآونة الأخيرة أصبح انتشار معدل الأمراض الخمجية ذات أهمية بالغة خاصة في مجال علم الوبائيات. بالإضافة إلى ذلك بعض العوامل الممرضة المعروفة أصبحت ذات أهمية وبائية نتيجة لانتشار الأنواع المقاومة للمضادات الحيوية. بعض أنواع النباتات عرفت بخواصها الطبية لاحتوائها على مواد كيميائية فعالة قادرة على قتل الميكروبات الممرضة للإنسان.

الأهداف: هدف هذه الدراسة كان دراسة النشاط المضاد للبكتريا لتراكيز مختلفة للمستخلص المائي لأحد النباتات الطبية وهو نبات السنط باستخدام طريقة اختبار الانتشار في الأشجار.

منهجية البحث : أخذت خمسة وخمسون مسحة من الجروح من مستشفى شندي التعليمي ومستشفى المك النمر الجامعي في الفترة من مارس إلى أغسطس 2018م.

النتائج: وجدت في هذه الدراسة أن التهاب الجروح في الفئات العمرية أقل من 20، ومن 21 إلى 40 ' ومن 41 إلى 60 واكبر من 80 أن أكثر تكررا في الفئات العمرية من 21-40سنه والمكورات العنقودية الذهبية كانت أكثر تكررا.

ووفقا للجنس كان عدد الرجال 35 بمعدل (64%) وكان عدد النساء 20 بمعدل (36%).

وقد وجد انه لا توجد علاقة بين تاريخ المرض والتهاب الجروح.

اختبرت النشاطات المضادة للبكتريا لمستخلص الماء لنبات السنط ضد البكتريا المعزولة وهي (المكورات العنقودية الذهبية (27) والكلبسيلا الرئويه (15) والزائفة الزنجارية (2) والاشريكية القولونية (2) والمتقلبة الاعتيادية (1).

المستخلص المائي لنبات السنط قضى على واحد أو أكثر من البكتريا المختبرة (المكورات العنقودية الذهبية والكلبسيلا الرئويه والزائفة الزنجارية والاشريكية القولونية والمتقلبة الاعتيادية)

حدد النشاط المضاد للبكتيريا بالجنتاميسين ضد البكتيريا المعزولة وقورن نشاطها مع نشاط المستخلص النباتي.

المستخلص المائي لنبات السنط اجري على البكتيريا المعزولة باستخدامه على عدة تراكيز (100،50،25 و 12.5) وقد أظهرت التحاليل الإحصائية أن قيمة P ذات دلالة إحصائية بين معدل حجم نطاق التثبيط و تركيز المستخلص المائي لكل من المكورات العنقودية الذهبية, (P=0.005), (16.8,14.4,12.9 and 11.1) والمكورات البشروية (P=0.003) (16.6,14.6, 13.3,) (والكلبسيلا الرئوية (P=0.044) (9.7,8.8,8.5and 8.2) والزائفة الزنجارية (19.0, 11and16,15.0, (P=0.023) والاشريكيه القولونية (12.0,11,11and 10) (P=0.010) والمتقلبة الاعتيادية (13.5,12.5,10.5 and 3.5) (P=0.051).

قورنت نتائج معدل التثبيط للمستخلص المائي لنبات السنط مع مضاد حيوي لتحديد معدل التثبيط النسبي و وجد أن اكبر معدل لتثبيط النسبي ظهر على البكتيريا البشروية بنسبة 68.6 % و اقل معدل ضد بكتيريا البروتيس بنسبة 10.1%.

Table of contents

Topic	Page
الآية	I
Dedication	II
Acknowledgment	III
Abstract	IV
Abstract Arabic	VII
List of contents	VIII
List of tables	XI
CHAPTER ONE(INTRODUCTION)	
1.1. Introduction	1
1.2. Rational	2
1.3. Objectives	3
CHAPTER TWO 2. LITERATURE REVIEW	
2.1.plant extract as antimicrobial agent	4
2.2. Bonticaletho pharmacological properties of	5
Acacia nilotica	6
2.2.1. Acacia nilotica	6
2.2.1.1. Taxonomical classification	6
2.1.1.2. Distribution	6
2.1.1.3. Botanical description	6
2.1.1.4. Chemical constituents	6
2.1.1.5. Ethopharmacological Studies	7
2.1.1.6. Medical uses	8
2.2.2. Principle of infective therapy	9
2.2.3. In vitro antimicrobial activity	9
2.2.4. Antimicrobial resistance	10
2.2.5. Measurement of antibacterial activity	10
2.2.6. Dilution method	11
2.2.7. Infection of skin, wound and soft tissue	12
2.2.8. Bacterial pathogen causing wound infection	12
2.2.9. General characteristics of isolated bacteria	13
2.2.9.1. Staphylococcus aureus	13
2.2.9.2. Klebsiella pneumonia	13
2.2.9.3. Pseudomonas aeurginosa	14
2.2.9.4. Escherichia coli	14
2.2.9.5. Proteus spp	14

CHAPTER THREE	
3. MATERIANS AND METHODS	
3.1. Type of study	15
3.2. Study area	15
3.3. Study population	15
3.4. Inclusion criteria	15
3.5. Exclusion criteria	15
3.6. Sampling	15
3.7. Sample size	15
3.8. Data collection	15
3.9. Methodology	16
3.9.1. Collection of specimens	16
3.9.2. Culture of specimens	16
3.9.3. Cultural characteristics	16
3.9.4. Purification and preservation of isolates	16
3.10. Microscopic examination	17
3.11. Identification of bacteria	17
3.12. Biochemical identification	17
3.12.1. Catalase test	17
3.12.2. Coagulase test	17
3.12.3. DNase test	17
3.12.4. Mannitol fermentation test	18
3.12.5. Oxidase test	18
3.12.6. Indole production test	18
3.12.7. Citrate utilization test	18
3.12.8. Urease test	18
3.13. Extraction of medical plants	19
3.13.1. Collection and preparation of plant samples	19
3.13.2. Preparation of aqueous extract	19
3.13.3. Aqueous extraction	19
3.13.4. Preparation of bacterial suspension	19
3.14. 3.16. Data analysis	20
CHAPTER FOUR	
4. Results	21
CHAPTER FIVE	
Discussion, Conclusion and recommendation	
5.1. Discussion	28

5.2. Conclusion	30
5.3. Recommendation	31
References	32
Appendix 1 Questionnaire	38
Appendix 2 Photography	39

List of Tables

No	Title	Page NO
4.1	Frequency and percentage of sampling according to age group Age group Frequency Percentage	22
4.2	Frequency and percentage of sampling according to gender	22
4.3	Frequency and Percentage of bacterial growth.	23
4.4	Gram stain for isolated bacteria	23
4.5	Characters and biochemical tested of <i>S.aureus</i> .	24
4.6	Characteristics and biochemical properties of tested Gram negative bacteria	24
4.7	Frequency and percentage of isolated bacteria	25
4.8	The mean zone and stander deviation of growth inhibition diameter zone (mdiz) in millimeter (mm) of isolated bacteria according to extract concentration	25
4.9	Shows isolated susceptibility to control	26
4.10	Show the relative percentage inhibitions extract compared to Gentamicin	26
4.11	Antibacterial activity of <i>Acacia nilotca</i> (<i>Algarad</i>)aqueous extracts against isolated bacteria in different concentrations	27

Chapter One

Introduction

Rationale

Objectives

CHAPTER ONE

1. INTRODUCTION

1.1 Introduction

The recent recognition of emerging infectious diseases has heightened appreciation of the importance of epidemiologic information. In addition, some well-known pathogens have assumed new epidemiologic importance by virtue of acquired antimicrobial resistance (e.g., *Penicillin-resistant Pneumococci*, *Vancomycin-resistant Enterococci*, and multi-resistant *Mycobacterium tuberculosis*). (RAY G.C, RYAN J.K, 2004).

The ability to direct therapy specifically at a disease-causing infectious agent is unique to the management of infectious diseases. Its initial success depends on exploiting differences between our own makeup and metabolism and that of the microorganism in question the mode of action of antimicrobials on bacteria (RAY G.C, RYAN J.K, 2004).

According to World Health Organization (WHO) plants are source of compounds that have the ability to combat disease, anti-microbial, anti viral and anti-fungal activities (Abeysinghe, 2010).

Some plants are known as medicinal because they contain active substances that cause certain reactions, from relating to cure of disease on human (Silva *et al.*, 1994).

Some antibiotics have become almost obsolete because of drug resistance consequently new drugs must be sought for, so herbal treatment is one possible way to treat diseases caused by multi drug resistant bacteria. The use of plant extracts and phytochemicals with known antibacterial properties may be of immense importance in therapeutic treatments. In the past few years, number of studies has been conducted in different countries to prove such efficiency (Indranil *et al.*, 2006).

Acacia nilotica is a multipurpose plant, it was used for treatment of various diseases; the plant contains a profile of a variety of bioactive components (Singh *et al.*, 2009).

1.2 Rational:

Utilization of plants for wound healing purposes is getting popular as they are believed to be beneficial and free of side effect. Although conventional antimicrobial drugs are available but increase resistant to this drug can result in treatment failure.

The *A.nilotica* possesses antibacterial activity because it is used in rural medical care for treatment of many diseases such a sore throat, cold, bronchitis, pneumonia and diarrhea and the previous studies reported that the *A.nilotica* has antibacterial effect.

To verify the claimed activity of this plants use to treat wound infections, this study was designed to answer this question.

1.3. Objectives

1.3.1. General objective:

To estimate the effectiveness of *Acacia nilotica* extracts against Bacteria isolated from wound infection.

1.3.2. Specific objectives:

- 1- To isolate and identify the bacteria cause wound infections.
- 2- To find out the anti-bacterial activity of *Acacia nilotica* on the bacterial causing wound infection.
- 3- To determine the minimum inhibitory concentration (MIC).

Chapter TWO

Literature Review

CHAPTER TWO

2. LITERATURE REVIEW

2.1. The medical plants and their traditional uses

Medicinal plants have been used as source of medicine in virtually all culture. During the last decade, the use of traditional medicine (TM) has expanded globally and gaining popularity. It has continued to be used not only for primary health care of the poor in developing countries, but also in countries where conventional medicine is predominant in national health care system (**Hailuet al, 2005**).

Virtually all cultures around the globe have relied historically, and continue to rely on medicinal plants for primary health care. There is currently a worldwide upsurge in the use of herbal preparation and the active ingredients isolated from medicinal plants in health care up to 40% of modern drugs are derived from natural source, using either natural substance or synthesized version (**Jasim and Naji, 2003**).

In the early 19th century, when chemical analysis first became available, scientists began to extract and modify the active ingredients from plants. Later, chemists began making their own version of plants compounds and over time, the use of herbal medicines declined in favor of drugs. Almost one fourth of pharmaceutical drugs are derived from plant. Herbal medicine is used to treat many conditions, such as asthma, eczema, premenstrual syndrome, rheumatoid arthritis, migraine, menopausal symptoms, chronic fatigue, irritable bowel syndrome and cancer (**Steven and Ehrlich, 2011**).

In Africa and other developing countries, these traditional medicines derived from plants have continued to form the basis of rural medical care. This is due to the fact that this medicine are easy to get and available in cheap prices (**Mohamed, 2012**).

In Iranian traditional medicine “ITM” the use of plants in treatment of burns, dermatophytes and infectious disease or as antiseptic and anti inflammatory was common (**Ghahrman and Attar et al., 1998**).

Extract of 13 Brazilian medicinal plants were screened by Holtez et al (2002) for antimicrobial activity against bacteria and yeast, of these 10 plants extract showed varied levels of antimicrobial activity. Total of 82 Indian medicinal plants traditionally used in medicines were subjected to preliminary antibacterial screening against several pathogenic microorganism by Ahmed et al (1998) the result indicate that 56 exhibited antimicrobial activity.

Extract of 111 Sudanese medicinal plants were subjected to preliminary antibacterial activity by Almagboul (1992) out of 573 extracts screened, 433(76%) exhibited antibacterial activity. Basno (2009) showed that the antibacterial activity of the extracts of *Acacia nilotica* assay against *Streptococcus viridians*, *Sheila sonnei*, *S.aureus*, *E.coli* and *Bacillus subtilis*, the result showed antibacterial activity against all above said organisms, but *Bacillus subtilis* most susceptible to the plant extract. Ethanol and petroleum ether extract of *acacia nilotica* by Deshpande (2013) showed that highest antibacterial activity against (*S.aureus*, *E.coli*. *Proteus mirabilis*, *P.vulgaris*, *P. mirabilis*, *Salmonella paratyphi* and *Klb.pneumoniae*). One study was done by Rahman et al (2014) to screen the antimicrobial activity of *Acacia nilotica* and was found to give the most potent antimicrobial extract. The antibacterial activity of *Acacia nilotica* methanolic extracts against wound infection bacterial isolates *S. aureus*, *E.coli*. And *P. aeruginosa* screened by Abass and Elhag (2015).

The result showed high activity 100% of methanolic extract.

2.2. Botanical ethno- pharmacological properties of acacia nilotica

Different parts of selected *acacia nilotica* were recognized as component of the traditional medicine in Sudan. They were arranged with their family, scientific and common name, distribution, botanical description, chemical constituents, antimicrobial activity and medical uses.

2.2.1. *Acacia nilotica*

2.2.1.1. Taxonomical classification

Kingdom: Plantae

Subkingdom: Tracheobinota

Division: Magnoliophyta

Class: Magnoliposida

Subclass: Rosidae

Order: Fabales

Family: *Fabaceae*

Genus: *Acacia*

Spices: *nilotica*

Adansonii(*Malviya et al., 2011*).

Vernacular names: UnaniTibbi: Aqaqia , English: Indian gum Arabic, Black babool, Arabic: Ummughilan, Hidi: Kikar, Kannada : Jaali,Gobbi , Latin : *Acacia Arabica*, Kashmiri : Sac , Punjabi : Kikkar , Bengali :Babla

2.2.1.2. Distribution: The species is wide spread in Africa and Asia, and occur in Australia and Kenya. Indian gum Arabic tree is found in well watered Sahelian and Sudanian savannas to the southern Arabian Peninsula, East Africa and in the Gambia, the Sudan, Togo, Ghana, and Nigeria. It widely cultivated in the Indian (*Malviyaet al., 2011*).

2.2.1.3. Botanical description

Acacia nilotica is a single stemmed plant, grows to 15-18 m in height and 2-3 m in diameter. Pods and seeds: pods are 7-15 cm long green and tomentose (when immature) or greenish black (when mature). Seeds are 8-12 per pods, compressed, ovoid, dark brown shining with hard testa(*Malviyaet al., 2011*).

2.2.1.4. Chemical constituents

Acacia species contains secondary metabolites including amines and alkaloids, cyanogenic glycosides, cyclitols, fatty acids and seed oils, fluoroacetate, gums, non protein amino acids, terpenes, flavonoids and condensed tannins (**Seigler, 2003**).

The mature seeds contain crude protein, crude fibre, crude fat, carbohydrates, potassium, phosphorus, magnesium, iron and manganese occurred in high concentration and it richer source of cysteine, methionine, threonine, lysine and tryptophan. Fruit also contains mucilage and saponin(Pande, 1981 and Siddhurajuet *al.*, 1996).

2.2.1.5. Ethopharmacological Studies

Several research worker have reported different biological activities of *A.niloticain* various in vitro and in vivo test model, these have been highlighted in following:

Anti hypertensive activity: Gilaniet *al* (1999) determine that methanolic extract of *acacia nilotica* pods possess decrease in arterial blood pressure at dose (3- 30mg/kg).

Anti mutagenic activity: Aroraet *al* (2003) concluded that acetone extract of *acacia nilotica* exhibited anti mutagenic activity.

Antibacterial activity: Saini (2008) examined antibacterial activity of *acacia nilotica*.

Anti fungal activity: Mahesh and Satish (2008) have concluded that antifungal activity of methanolic and aqueous extract of *Acacia nilotica*.

Anti viral activity: Singh and Singh (1972) evaluated crude extract of leave of plant that showed in vitro anti viral activity to Turnip mosaic virus.

Anti microbial activity: Khan (2009) explores the antimicrobial activity of crude ethanolic extract of five plants against multi drug resistant strains.

Anti diabetic: hypoglycemic effect) activity:

Wadoodet *al* (1989) evaluated the *acacia nilotica* indica fed for one week was found the exhibit hypoglycemic effect (blood sugar lowered by 25.05%) in normal rat.

Anti oxidant activity: Agrawal (2010) explored methanolic extract of plant have anti oxidant activity.

Anti diarrheal: Agunuaet *al* (2005) decribed that medicinal plants (*A.nilotica*) use in diarroeal treatment in Kadune State, Nigeria were investigated.

Anti plasmodium activity: aqueous root extract of *Acacia nilotica* was analyzed for anti plasmodial activity in mice.

Anti infertility and Abortifacient activity: Nathet *al* (1992) reported that aqueous or 90% ethanol extract of plants were studied in rat orally dose for 10 days the effect on foetal *Moringa* were 100% abortive while *A. nilotica* appeared to lack teratologic potential at the doses tested.

Lipid profile and platelet aggregation and Hyperglycaemic: Asad *et al* (2011) investigated the *A. nilotica* leaf streptocin, induced diabetic rats. The results showed significant differences ($P < 0.05$) in blood glucose, serum insulin, platelet aggregation and triglyceride level as compared to diabetic controlled rats.

Galactagogue activity: Eline *et al* (2004) said that aqueous extract of *A. nilotica* can stimulate milk production in lactating women.

2.2.1.6. Medical uses

Several research workers reported different biological activities of *Acacia Arabia* in vitro and in vivo test models:

Babul plant is therapeutically used as: anti cancer, anti tumors, antiscorbutic, astringent, anti-oxidant, natriuretic, antispasmodic, diuretic, intestinal pain and diarrhea, nerve stimulant, cold, congestion, cough, dysentery, fever, hemorrhages, leucorrhoea, ophthalmia and sclerosis. Seed has antimalarial activities, antihypertensive and antispasmodic activities. Leaves & pods are an excellent fodder with anti-inflammatory properties, Bark it is used in the treatment of hemorrhage, cold, diarrhea, tuberculosis and leprosy. Roots it is used as an aphrodisiac and the flower for treating syphilis lesions. Gum obtained from the tree is pharmaceutically used as suspending and emulsifying agent and in preparation of many formulations (Malviya *et al.*, 2011).

The *Acacia nilotica* has been used to treat sore throat, cold, bronchitis, pneumonia, ophthalmia, diarrhea, dysentery, leprosy, venereal disease and hemorrhage. Existing literature reported that the *Acacia nilotica* has demonstrated considerable antibacterial & antifungal (Abdelnabiet *et al.*, 1992).

2.2.2. Principle of infective therapy

This has been of interest in the investigation of natural material as a source of new antibacterial agent. Different extracts from traditional plants have been tested. Many reports showed the effectiveness of traditional herbs against microorganisms, as result, plant is one of bedrocks for modern medicine to attain new principle (**Evans *et al.*, 2002**).

Natural products have been approved as new antibacterial drugs; there is an urgent need to identify novel substance, which is active towards highly resistant pathogens (**Recio, 1989**).

Hence evaluation of natural products to find new, safe and effective active compounds to rotate or substitute with invalidated ones is one of the scientific strategies to combat drug resistance pathogens (**WHO, 2002**).

The term antibiotics strictly refer to naturally occurring product of one organism that is inhibitory to others according to these definition chemical compounds such as sulphonamides, quinolones and nitrofurans i.e. chemotherapeutic agents. Some antibiotics can be manufactured synthetically while others are the products of chemical manipulation of naturally occurring semi-synthetic antibiotics (**Greenwood *et al.*, 1992**). Bacteriostatic is having the property of inhibiting bacterial multiplication. Action differ from bactericidal only is being irreversible, that is killed organisms can no longer reproduce, even after being removed from contact with the agent. Selective toxicity is an ideal antimicrobial agent that exhibit selective toxicity. This term implies that a drug is harmful to the parasite without being harmful to the host. The chemotherapeutic index (selective toxicity), compare the maximum dose that can be tolerated by the host without causing death, with minimum dose that cures the particular infection (**Praneeet *et al.*, 1999**).

2.2.3. In vitro antimicrobial activity:

Antimicrobial activity is measured in vitro in order to determine: The potency of antimicrobial agent in solution. Sensitivity tests are also used to evaluate new

antimicrobial agent by testing them against a large number of organisms (**Bae and Byun, 1987**).

To evaluate the sensitivity of a given microorganism to a known concentration of the drug.

2.2.4. Antimicrobial resistance:

There are four major mechanisms that mediate bacterial resistance to drugs:

1-Bacteria produce enzymes that inactivate the drug, example B-lactmases can inactivate penicillins and cephalosporins by cleaving the B-lactum ring of the drug.

2-Bacteria synthesize modified targets against which the drug has no effect, e.g: a mutant protein in the 30s ribosomal subunit can result in resistance to streptomycin and a methylated 23s rRNA can result in resistance to erythromycin. (Monica Cheesbrough , 1991)

3-Bacteria decrease their permeability so that effective intracellular concentration of the drug is not achieved, e.g: change in porins can reduce the amount of penicillin entering the bacterium.

4-Bacteria actively export drugs using a multidrug resistance pump (MDR pump),the MDR pump imports protons and exports a variety of foreign molecules including certain antibiotics, such as quinolones. (Monica Cheesbrough , 1991)

2.2.5. Measurement of antibacterial activity

Determination of these quantities may be under taken by dilution method, using appropriate standard test organism. This method can be employed to estimate either the potency of antibiotic in sample or the sensitivity of a microorganism.

The aim of agar dilution method is to determine the lowest concentration of assayed antimicrobial agent minimum inhibitory concentration (MIC) that under defined test conditions inhibits the visible growth of the bacterium being investigated. MIC value is used to determine the susceptibilities of bacteria to

drugs and to evaluate the activity of new antimicrobial agents. In agar dilution method, the medium is inoculated with test organism and the samples to be tested are mixed. The inhibition zones are dependent upon both the dispersion of the agent in the medium and the degree of susceptibility of the organism. The speed of growth and the size of inoculums can influence to marked degree the size of inhibitory zones. **(Peter and Plorde, 1963., Kavanagh, 1972).**

2.2.7. Infection of skin, wound and soft tissue

Skin and soft tissue infection are infection involving the non –skeletal tissue. Most skin infection result from a break in the skin such as surgery, decubitus, ulcer, cuts, punctures, animal or insect bites, thorn and needle picks or burns. When a whole is created on the skin, microorganisms usually the opportunistic ones invade the holes and multiply leading to delay in the healing process and finally infectious condition.

The spectrum of infection range from a symptomatic colonization to bacteremia and death **(Nesteret *al*, 2004).**

Colonization by opportunistic bacteria which begin immediately after birth is usually lifelong and may lead to infectious condition whenever the skin is perforated. Some of microorganisms frequently isolated in skin and wound infection include *Staphylococci*, *Streptococci*, *pseudomonas*, *Bacilli* and *E.coli*. These bacteria have greater resistance and virulent capabilities including formation of bio-films on colonized surface **(Grenetet *al*, 2004).**

Eradication of these pathogens has been shown to result in rapid wound healing. Complications from burns, surgical wound, skin and soft tissue arise from colonization of such sites by some bacteria and fungi. These complications can be avoided by proper sanitation and good hygienic practices **(Pfalleret *al*, 2001; Muhammed and Muhammad, 2005).**

Wounds infection by bacteria and resistance to common antibiotics are the common post-surgical and medical challenges. Wounds bacterial contamination are the common hospital acquired infections causing more than 80% of the mortality. The most common bacterial genera infecting wounds are *Enterococci*,

Escherichia, Pseudomonas, Klebsiella, Enterobacter, Proteus and *Acinetobacter*. Wounds infection have been a recognized as the most critical problem especially in the presence of foreign materials that increases the risk of serious infection even with relatively small bacterial infection. Nosocomial infection is usually higher in burn patients that correlate with other factors like nature of burn injury, age of patient, extent of **injury** and burn depth. Other microbial factors such as type, number of organisms, enzymes, toxins production, colonization of the burn wound site, systemic dissemination of the colonizing organisms, have a strong effect on severity of bacterial wound infection .As well as, widespread using of vast groups of antibiotics together with the length of time causes a significant development of antibiotic resistance to wound infecting bacteria, that subsequently increase the complications and costs of treatment (**Anguzu et.al 2007**).

Wound infections remain a major source of postoperative morbidity, accounting for about a quarter of the total number of nosocomial infections. Today, many of these infections are first recognized in the outpatient clinic or in the patient's home due to the large number of operations done in the outpatient setting. This leads to errors in establishing the true incidence of their occurrence but undoubtedly decreases the overall real cost and length of hospital stay. The pathogens implicated in the development of wound infections remain largely the human microorganisms from the exogenous environment and the endogenous organ microflora. Many perioperative factors have been identified that increase the incidence of the development of postoperative wound infection. Avoidance of these factors as well as the appropriate use of perioperative antibiotic prophylaxis has decreased the incidence of wound infection (Nichols et,al 2010).

2.2.8. Bacterial pathogens causing wound infection

According to Cheesbrough (2005), the bacteria belong to wound pathogens include:

A. Gram positive bacteria

- *Staphylococcus aureus*.

- *Streptococcus pyogenes*.
- *Enterococcus faecalis*.
- *Bacillus anthracis*.
- *Bacillus cereus*.
- *Corynebacterium diphtheria*.
- *Colistridiumperfringens type A*.

B. Gram negative bacteria

- *Escherichia coli*.
- *Proteus mirabilis*.
- *Klebsiella pneumonia*.
- *Pseudomonas aeruginosa*.
- *Aeromonashydrophilia*.

2.2.9. General characteristics of tested bacteria

2.2.9.1. *Staphylococcus aureus*

It is Gram positive, aerobic and also grow an aerobically but less well. Temperature range for growth (10-42°C), with optimum of (35-37°C).

It grows on blood agar producing creamy to yellowish colonies, occasionally white with diameter 1-2 mm. In Macconkey's agar it produces smaller colonies (0.1-0.5mm). It ferment 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).

2.2.9.2 *Klebsiella pneumonia*

Tend to be slightly shorter and thicker than other enterobacteria and straight rods, its capsular material is produced in greater amounts in media rich in carbohydrate. *K.pneumoniae* is non-motile, it is facultative an aerobe, but growth under aerobic conditions is rare. It ferment glucose and produce urease enzyme, but negative for methyl red.

It causes urinary tract infection, severe bronchopneumonia and woundinfections (Greenwood *et al.*, 1998).

2.2.9.3 *Pseudomonas aeruginosa*

It is Gram negative, rod shaped, non sporing and motile. It can found in intestinal tract, water, soil and sewage. It includes water soluble pigments (pyocyanin + pyoverdin). It grows over a wide temperature range (4-42°C) with optimum of (35-37°C). On Macconkey's agar, it produces pale color colonies and in Kligler iron agar media, it produces red slope, but no gas is formed and no H₂S is produced. It is oxidase positive. It can cause purulent infection of wounds, burns, urinary tract infection, respiratory tract infection; especially in patients with fibrosis and bed sore disease (Cheesbrough.,2005).

2.2.9.4. *Escherichia coli*

Is Gram negative, usually motile rod, some strains are capsulated, it is aerobic and facultative anaerobic, and it produces 1-4 mm in diameter colonies on blood agar after over night incubation at 35-37°C. The colonies may appear mucoid and some strains are haemolytic. On Macconkey agar most of strains produce lactose fermenting colonies. Some EPEC are late or non lactose fermenter (Cheesbrough., 2005).

2.2.9.5. *Proteus spp*

Proteus species are part of Enterobacteriaceae family of Gram negative, usually motile rod, aerobic and facultative anaerobic, non sporing and non capsulated. Cause superficial skin infection eg wound infection and burn infection, urinary tract infection, Bacteremia and deep seated infections eg: meningitis, endocarditis, septic arthritis and shock (Cheesbrough., 2005).

Chapter Three

Materials and Methodology

CHAPTER THREE

3. MATERIAL AND METHODOLOGY

3.1. Study design

This is prospective, descriptive hospital base study.

3.2. Study area

All Patients coming to Almak Nimer University and Shendi Teaching Hospitals with wound infections were including in this study.

3.3. Study population

All Patients coming to Almak Nimer and Shendi Teaching Hospitals with wound infections were including in this study from March to August 2018.

3.4. Inclusion criteria

wound infection patients.

3.5. Exclusion criteria

Patients under antimicrobial treatment.

3.6. Samples collection

After take consent, patients at risk to become Under aseptic conditions, wound swabs were collected using sterile cotton swabs moistened in normal saline. The samples were transported in transport medium immediately to the laboratory for investigation.

3.7. Sample size

A total (n=55) were collected.

3.8. Data collection

Data were collected from the patients using structural questionnaire containing all study variable such as Personal data. (**AppendixI**).

Calculation of relative percentage of inhibition

Relative percentage inhibition = $100 \times (x - y) / (z - y)$

x: total area of inhibition of the test extract.

y: total area of inhibition of the solvent.

z: total area of inhibition of the standard drug.

The total area of the inhibition was calculated by using $\text{area} = \pi r^2$; where, r = radius of zone of inhibition.

π value = 3.14

X: Total area of inhibition of the test extract = 3.14 x (radius of zone inhibition of acacia nilotica extract in mm)².

Y: Total area of inhibition of the solvent = 3.14 x (radius of zone inhibition of water in mm)².

Z: Total area of inhibition of the standard drug = 3.14 x (radius of zone inhibition of Gentamicin in mm)².

3.9. Methodology

3.9.1 Collection of specimens

Under aseptic conditions, wound swabs were collected using sterile cotton swabs moistened in normal saline. The samples were transported in transport medium immediately to the laboratory for investigation.

3.9.2. Culture of specimens

Collected swabs were cultured on Blood agar (HImedia) and Macconkey's agar (HImedia) plates using standard sterile loop. All plates were incubated aerobically at 37°C for 24 hrs.

3.9.3. Cultural characteristics

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

3.9.4. Purification and preservation of isolates

Purification was done by repeated sub-culturing of typical and well isolated colonies on nutrient agar (HImedia). The resulting growth was checked for purity using Gram's staining procedure. Obtained pure cultures were presented through inoculation into nutrient agar slope and stored at 4°C after 24hrs incubation at 37°C.

3.10. Microscopic examination

Smears were made from sub cultured colonies, fixed by gentle heating and stained using Gram's staining technique by Barrow and Feltham (1993), and then examined microscopically under oil immersion.

3.11. Identification of bacteria

The purified isolates were identified according by Barrow and Feltham (1993). This includes staining reaction, organism morphology growth characteristics, haemolysis on blood agar, and lactose fermentation on Macconkey's agar (HImedia) media, motility and biochemical characteristics.

3.12. Biochemical identification

3.12.1. Catalase test

The test was carried out as describe by Barrow and Feltham (1993). 0.5ml of 3% H₂O₂ was placed on clean tubes, and one colony of the tested culture from nutrient agar (HImedia) was picked with a wooden stick and added to the tubes. A positive reaction was indicated by production of air bubbles.

3.12.2. Coagulase test

The test was used to identify *S.aureus* which was coagulase positive from other Staphylococci species which were coagulase negative. Coagulase causes plasma to clot by converting fibrinogen to fibrin. On clean slide place drop of distilled water and emulsify a colony of tested organism then add loop full of plasma on the suspensions and mixed gently the results was clumping of organisms within 10 seconds (Cheesbrough, 2005).

3.12.3.DNAase test

The test was used to differentiate *S.aureus*(positive) from other *Staphylococci* species (negative). The tested organism was culture on a medium which contain DNA, after overnight incubation the colonies were tested by flooding the plate witha weak hydrochloric acid (Hcl). The acid precipitates un hydrolyzed DNA. DNase produced colonies were surrounded by clear area indicating DNA hydrolysis(Cheesbrough, 2005).

3.12.4. Mannitol fermentation test

This medium was used to differentiate *S.aureus* from other *Staphylococci* species. A portion of colony was inoculated on mannitol salt agar containing 75 g/l sodium chloride and incubated aerobically at 37°C for 18-24 hrs. *S.aureus* ferment mannitol producing yellow colonies (Cheesbrough, 2005).

3.12.5. Oxidase test

The technique was described by Barrow and Felham (1993). Strips of filter paper was soaked in 1% solution of tetra -methyl-p-phenylenediamine dihydrochloride and dried in hot air oven and then placed on clean glass slide bacterial colony by sterile glass rod and rubbed on filter paper strip. If purple color developed with 5-10 seconds, the reaction was considered positive.

3.12.6. Indol production test

Indol production was carried out as described by Barrow and Felham (1993). The tested organism was inoculated into pepton water and incubated at 37°C for 48 hrs. 1 ml of kovac's reagent was run down along the side of the test tube. Appearance of a pink color in the reagent layer within a minute indicated positive reaction.

3.12.7. Citrate utilization test

It was done according to Cheesbrough (2005). Simmon's citrate medium was inoculated with the tested organism and incubated at 37°C for up to 48 hours; utilization of citrate was recognized by a bluish color.

3.12.8. Urease test

It was done according to Cheesbrough (2005). The slant surface of urea agar medium was streaked with the tested microorganism and incubated at 37°C for 24- 48 hrs. The development of a pink color was indicative of production of NH₃ Negative and weak tests were left for a week before being considered as negative.

3.13. Extraction of medical plants

3.13.1. Collection and preparation of plant samples

They were authenticated by protocol of Medicinal and Aromatic plant Research Institute (MAPRI). The dried *Acacia nilotica* were cleaned from dust and grass; 100 grams of each plant sample were separately crushed to a powder from using sterilized mortar and pestle.

3.13.1. Preparation of the aqueous extract

Extraction was carried out according to method described by Sukhdev *et al.*,(2008). Fifty gram of each plant sample was soaked in 500 ml hot distilled water, and left till cooled down with continuous stirring at room temperature. Extract was then filtered and freezed in a deep freezer. Freezed at - 20°C extract was dried using freeze dryer -40°C till powdered extract obtained. Yield percentage was calculated.

3.13.4. Preparation of bacterial suspension

One ml aliquots a 24hrs broth culture of the test organisms were aseptically distributed on the nutrient agar slopes and incubated at 37°C for 24hrs. The bacterial growth was harvested and washed off with 100 ml sterile normal saline, to produce suspension containing about (10⁸-10⁹) colony forming units/ml. The inocula density was compared with Macfarland standard solution of BaSO₄ (0.1ml of 1% BaCl₂ + 9.9ml of 1% H₂SO₄). The suspension was stored in the refrigerator at 4°C until used (Monica Cheesbrough, 2005).

3.13.5. In vitro testing for antibacterial activity

The cup plate agar diffusion method according to (Kavangh, 1972). Was adapted to assess the antibacterial activity of prepare extracted. One ml of the standardized bacterial stock suspension (10⁸-10⁹) colony forming unit/ml was thoroughly mixed with 100ml of Muller-Hinton agar (HiMedia, India) which was maintained at 45°C and 20ml aliquots of the inoculated Muller-Hinton agar were distributed into sterile plates. The agar was left to set and in each of these plates, 4 cups (7 mm in diameter) were cut using a sterile cork borer (No.1) and agar disc were removed. Alternate cups filled with 0.1ml samples of each of the

extracts using automatic micro titerpipette, and allowed to diffuse at room temperature for 2hrs. The plates were then incubated in the upright position at 37°C for 24hrs. Two replicates were carried for each extracts against each of the test organisms. After incubation the diameters of the resultant growth inhibition zones were measured and the mean values were tabulated.

3.14. Determination of Minimum Inhibition Concentration (MIC) by agar plate dilution method

The principle of agar plate dilution was the inhibition of the growth on the surface of the agar by the plant extracts incorporated into the medium. Plates were prepared in series of decreasing concentrations of the plant extraction in the following order 100, 50, 25, 12.5 mg/ml. the bottom of each plate was marked off in to 4 segments. The tested organisms were grown in broth over night to contain 10⁸ organisms per ml. loop-full of diluted culture was spotted with a standard loop which delivers 0.001ml on the surface of each segment and then incubated at 37°C for 24 hours. The end point (MIC) was the least concentration of antimicrobial agent that completely inhibits the growth. Results were reported as the MIC in mg/ml.

3.16. Data analysis

Data were entered, check and analyzed using Microsoft Excel 2007 and SPSS (Statistical Package of Social Science) soft program version 11.5.

Chapter Four

Results

Chapter Four

4. Results

The current study was carried out to screen the antibacterial activity of *Acacia nilotica* sub spp *nilotica* aqueous extract was used against wound infection bacterial according age Out of 55 specimens from March to August 2018.

15 samples were taken from patients of with mean age less than 20years old age group, 20 patients from 21-40 years old age group, 10 patients from 41-60 years old age group, 7 patients from 61-80 years old age group and 3 patients from more than 80 years old age group.

The ages grouped in to five groups majority samples taken from age group 21-40 years old. And according to gender Out of 55 specimens, (57%) were males where as (43%) were females.

4.1. Frequency and percentage of sampling according to age group

Sample was taken according to age and gender (shows in Table 1 and 2)

Table 4.1: Shows distribution and percentage of sampling according to age group

Age group	Frequency	Percent
< 20	15	27
21 – 40	20	36
41 – 60	10	18
61 – 80	7	13
>80	3	6
Total	55	100

Table 4.2: Shows sample distribution according to gender

Gender	Frequency	Percent
Male	35	64
Female	20	36
Total	55	100

4.2. Bacteriological Result

Table 4.3: Shows bacterial growth distribution .

Result of culture	Frequency	Percent
Growth	49	89
No growth	6	11
Total	55	100

4.3. Gram Stain for isolated bacteria species

Out of the 49 positive cultures for bacterial growth 32 were Gram positive (65%) and 17 were gram negative Shows distribution (35%) are shown in (table 4).

Table 4.4. bacterial isolated in relation to Gram satin

Gram reaction	Frequency	Percent
Gram positive	32	65
Gram negative	17	35
Total	49	100

Table 4.5 Shows distribution of isolated bacteria.

Isolated Bacteria	Frequency	Percent
<i>E.coli</i>	2	4.1
<i>K. pneumoniae</i>	12	24.5
<i>Ps. aeruginosa</i>	2	4.1
<i>P. vulgris</i>	1	2.0
<i>S. aureus</i>	29	59.2
<i>S.epidermidis</i>	3	6.1
Total	49	100

Table 4.6. Shows the mean of inhibition zone of bacteria

Concentration Organisms	100%		50%		25%		12.5%	
	Mean	Std.	Mean	Std.	Mean	Std.	Mean	Std.
<i>E.coli</i>	13.5	9.1	12.5	7.7	10.5	4.9	9.5	3.5
<i>K. pneumoniae</i>	9.7	4.5	8.8	3.3	8.5	2.8	8.2	2.4
<i>P. aeruginosa</i>	19.0	1.4	16	2.8	15.0	4.2	11	1.4
<i>Proteus</i>	12.0	.	11	.	11	.	10	.
<i>S. aureus</i>	16.8	2.6	14.4	2.4	12.9	2.8	11.1	1.7
<i>s.epidermidis</i>	16.6	1.1	14.6	1.1	13.3	1.1	11.3	1.1

Interpretation of the result

MDIZ mean diameter of growth inhibition zone in (mm) , If MDIZ.

- > 7 sensitive.
- < 7 Resistant

Table 4.7: Shows antibacterial activity of *Acacia nilotica* against Gram negative bacteria isolated.

<i>Gram negative bacteria</i>	100%		50%		25%		12.5%	
	Mean	Std.	Mean	Std.	Mean	Std.	Mean	Std.
<i>E.coli</i>	13.5	9.1	12.5	7.7	10.5	4.9	9.5	3.5
<i>K. pneumoniae</i>	9.7	4.5	8.8	3.3	8.5	2.8	8.2	2.4
<i>P. aeruginosa</i>	19.0	1.4	16	2.8	15.0	4.2	11	1.4
<i>Proteus</i>	12.0	.	11	.	11	.	10	.

Interpretation:

Mean inhibition zone (mm) of Gram negative bacteria 19.0 ± 8.2

Table 4.8: Shows Antibacterial activity of *Acacia nilotica* against Gram positive bacteria isolated.

<i>Gram positive bacteria</i>	100%		50%		25%		12.5%	
	Mean	Std.	Mean	Std.	Mean	Std.	Mean	Std.
<i>S. aureus</i>	16.8	2.6	14.4	2.4	12.9	2.8	11.1	1.7
<i>s.epidermidis</i>	16.6	1.1	14.6	1.1	13.3	1.1	11.3	1.1

Interpretation:

Mean inhibition zone (mm) of Gram positive bacteria 16.8 ± 11.1

Table 4.9: Shows isolated susceptibility to control

Test organisms	Inhibition zone diameter (mm)	
	Positive control (Gentamicin 10 mcg)	Negative control (Distill water)
<i>Escherchia coli.</i>	18	0
<i>Klebsiellapneumoniae.</i>	24	0
<i>Staphylococcus aureus.</i>	29	0
<i>Staphylococcus epidermidis.</i>	20	0
<i>Proteus vulgaris</i>	23	0
<i>P. aeruginosa .</i>	28.5	0

Table 4.10: Show the relative percentage inhibitions extract compared to Gentamicin.

Test organisms	Relative percentage inhibition (%)
<i>Escherchia coli.</i>	5.6
<i>Klebsiella pneumoniae.</i>	10.7
<i>Staphylococcus aureus.</i>	33.5
<i>Staphylococcus epidermidis.</i>	68.8
<i>Proteus vulgaris</i>	27.2
<i>P.aeruginosa</i>	44.4

Table (4.11). Shows antibacterial activity of *Acacia nilotica* (Algarad) aqueous extracts against isolated bacteria in different concentrations:

concentration	<i>K. pneumoniae</i>		<i>S. aureus</i>		<i>s.epidermidis</i>		<i>E.coli</i>		<i>P. aeruginosa</i>		<i>Proteus Vulgaris</i>	
	S	R	S	R	S	R	S	R	S	R	S	R
100%	9.7	0	16.8	0	16.6	0	13.5	0	19	0	12	0
50%	8.8	0	14.4	0	14.6	0	12.5	0	16	0	11	0
25%	8.5	0	12.9	0	13.3	0	10.5	0	15	0	11	0
12.5%	8.2	0	11.1	0	11.3	0	9.5	0	11	0	10	0
P-value	0.044		0.005		0.003		0.010		0.023		0.051	

Chapter Five

Discussion

Conclusion

Recommendations

Chapter Five

5. Discussions

5.1. Discussions

The recent recognition of emerging infectious diseases has heightened appreciation of the importance of epidemiologic information. In addition, some well-known pathogens have assumed new epidemiologic importance by virtue of acquired antimicrobial resistance. Some plants are known as medicinal because they contain active substances that cause certain reactions, from relating to cure of disease on human.

The current study was carried out to screen the antibacterial activity of *Acacia nilotica* sub *sppnilotica* aqueous extract was used against wound infection bacteria according to age.

55 wound swab samples were collected from patients, 15 samples were taken from patients of age less than 20 years old, 20 samples from age 21-40 years old, 10 samples from age 41-60 years old, 7 samples from age 61-80 years old and 3 samples from age more than 80 years old.

The majority of samples taken from age group 21-40 years. And according to gender Out of 55 specimens, 35/55 (57%) were males where as 20/55 (43%) were females.

Forty nine swab samples were showing growth 49/55(89%) where 6 /55 samples (11%) showing no growth.

Out of the 49 positive cultures for bacterial growth 32 samples were Gram positive cocci (65%) and 17 samples (35%) were Gram negative bacilli.

Isolated bacteria are *S. aureus* 29/49 (59.2 %,) *K. pneumonia* 12/49 (24.5%) *S. epidermidis* 3/49 (6.1%) *E. coli* of percentage 2/49 (4.1%), *Ps. Aerginosa* 2/49 (4.1%), *P. vulgris* 1/49 (2.0%).

The mean of inhibition zone (mm) of Gram negative bacteria is 19.0 ± 8.2 and the mean of inhibition zone (mm) of Gram positive bacteria is 16.8 ± 11.1 .

The result showed high activity of the aqueous extract of *A. nilotica* sub spp *Nilotica*. *A. nilotica* is against isolated bacteria. This result was agreed with the study reported by El-kamli and El- karim (2009).

At this study the mean inhibition zone diameter of microorganism isolated increases with the increase in extract concentration. *A. nilotica* extract was tested using different concentration (100, 50, 25 and 12.5) for *E. coli* mean of inhibition zone according to extract concentration were (13.5,12.5,10.5 and 3.5) with $P=0.010$, for *S. epidermidis* (16.6,14.6,13.3 and 11.3) ($P=0.003$), *S. aureus* (16.8,14.4,12.9 and 11.1) ($P=0.005$), *Ps. aeruginosa* (19.0 ,16,15.0 and 11) ($P=0.023$) *K. pneumonia* (9.7,8.8,8.5 and 8.2) ($P=0.044$) which are statistically significant except *P. vulgaris*. Which have mean inhibition zone close to each other (12.0, 11,11 and 10) ($P=0.051$). This result was in agreement with study reported by Suleiman, (2013).

The aqueous extract of *A. nilotica* sub spp. exhibited high antibacterial activity against *Ps. aeruginosa* and moderately activity against *K. pneumonia*. This was consistent with study reported by Saini 2008 and Ahmed *et al* 2007.

The results of antimicrobial activity of crude extract was compared with the positive control (Standard drugs) the bacteria is sensitive to extract in relation to positive control (Gentamicin) the aqueous extract exhibits maximum relative percentage inhibition against *S. epidermidis* (68.8%) and minimum relative percentage inhibition against *Klebsiella pneumonia*. (10.7%).

5.2 Conclusion

- The current study showed that *Acacia nilotica* plants showed potent antibacterial activity.
- *Acacia nilotica* had highly activity against some bacteria, which justify their traditional use as treatment of wound infection.
- The efficiency of the antibacterial activity of extract was found to increase by increasing the concentration for certain levels.

5.3. Recommendations

1. Based on this study and result, it is recommended that to isolate and the active ingredients in the compound extracts responsible for the antibacterial activity using gas chromatography.
2. Determination of the minimum inhibitory concentration (MIC) for active ingredient of each bacteria causing wound infection.
3. Study of the toxicity of the active ingredients.

References

Appendixes

REFERENCES

- Abass Z and Elhag W. (2015).**In vitro antibacterial activity of *acacia nilotica* methanolic extract against wound infection pathogen, *Am J Res Commun*, 3(2): 111-21.
- Abd El-babi UM, Resinger E, Reinthaler FF, Still F and Eibel, UGJ (1992).**Antimicrobial activity of *acacia nilotica wild*.Exdel var nilotica. *J. Ethnopharmacol*, 77-79.
- Abeyasinghe PD (2010).** Antimicrobial activity of some medicinal mangroves against antibiotic resistant pathogenic bacteria.*Indian. J. Pharm Sci*, 167,
- Agrawal S, Kulkarni GT, Sharma VN (2010).** A Comparative Study on the Antioxidant Activity of Methanolic Extract of *Acacia nilotica* and *Berberis chitria*. *Adv in Nat Appl Sci* 4(1)78-84.
- Agunua A, Yusuf S, Andrewa GO, Zezi AU, Abdurahmana EM (2005).** Evaluation of five medical plants used in diarrhea treatment in Nigeria. *J. Ethnopharmacol*; 101: 27-30.
- Ahmad I, Mehmood Z, Mohammad F (1998).** Screening of some Indian medicinal plants for their antimicrobial properties. *J. Ethopharmacol*. 74(2):113-23.
- Ahmed A M, Gumaa SA, Abdelmageed MAM, Almagboul AZ (2007).** In Vitro antimicrobial activity of some Sudanese plants Used in Traditional Medicine. Omdurman. *J. Pharm. Sci*. 1(3): 232-396.
- Al Magboul AZ (1992).** Antimicrobial and phytochemical investigations of *Vernonia amygdalina* and other Sudanese medicinal plants. Ph.D. Thesis. University of Khartoum, Sudan
- Arora S, Kaur K, Kaur S (2003).** Indian medical plants as a reservoir of protective phytochemicals.*TeratoCarci Muta Suppl*; 1: 295-300.
- Asad M, Aslam M, Munir TA, Nadeem A (2011).** Effect of *Acacia nilotica* leaves Extract on Hyperglycaemia, Lipid profile and Platelet Aggregation in Strptzotocin Induced Diabetic Rats. *J Ayub Med Coll Abbottabad*; 23(2).

Bae K and Byun J (1987).Screening of leaves of higher plants for antibacterial action.*Kor. J. Pharmacol*, 18 (1):1-4.

Barrow GF and Feltham RKA (1993).Cowan and Steel's manual for identification of medical bacteria, 3rd edition, University press, Cambridge.

Basno A (2009). Phytochemical and antibacterial investigation of bark extracts of *Acacia nilotica*, *J. Med. Plants. Res.* 3(2): 082-085.

BP.(2002). Screening of some plants used in Brazilian folk medicine for the treatment of infectious diseases.*Mem Inst Oswaldo Cruz.* 97(7): 1027-31.

Cheesbrough M (2005). District Laboratory Practice in tropical countries, 2nd edition part 2.

Deshpande SN (2013). Preliminary phytochemical Analysis and In Vitro Investigation of Antibacterial activity of *Acacia nilotica* against clinical isolates.*J. Pharmacogn and Phytochem.* 1(5): 23-27.

Eline MB, Ouedraogo ZL, Heide D (2004). Effect of aqueous extract of *Acacia nilotica* ssp *adansonii* on milk production and prolactin release in the rat.*J ENDOCRIN*; 182:257-266.

EL-Kamali, H and EL-Karim, E (2009).Evaluation of antibacterial activity of some Medicinal plants used in Sudanese Traditional Medicine for Treatment of wound infection, *Acad. J. Plants. Sci*, Sudan, 2(4):246-251.

Evans CE, Banso A and Samuel OA (2002).Efficacy of some nupe medicinal plants against *Salmonella typhi* an in vitro study.*J. Ethnopharmacol*, 80.21-24.

Gahraman A and Attar F (1998).Biological diversity of Iranian plant species, Tehran uni, press, Iran.

Gilani AH, Shaheen F, Zaman M, Janbaz KH, Shah BH, Akhtar MS (1999).Studies on Antihypertensive and Antispasmodic activities of Methanolic Extract of *Acacia nilotica* pods.*Phyto ther Res* B665-69.

Greenwood D, Slach R and Penthere J (1992).Medical microbiology 4th edition, Churchill, Livingstone, New York, Edinburgh, London, Madrid and Tokyo.

Greenwood D, Slack R and Penthere J (1998).Medical microbiology 4th edition Churchill, Livingstone, New York, Edinburgh, London, Madrid and Tokyo.

Grenet K, Guillemot D, Jarlier V, Moteanu B, Duboudier S, Ruimy, R Armand-Leeure L, Bau P and Andremont A (2004). Antibacterial resistance, Wayampis Amerindians, French Guyana. *Eerg. Infect. Dis.* 10(6) 1150-1153.

Hailu T, Endris M, Kaleab A and Tsige GM (2005).Antibacterial activities of some selected traditional Ethiopian medicinal plants used in treatment of skin disorders. *J. Ethnopharmacol* 100(1-2):168-175.

Holetz FB, Pessini GL, Sanches NR, Cortez DA, Nakamura CV, Filho

Indranil B, Soroj K, Chatterjee S, Chatterjee G C (2006).Antibacterial Potentiality of Argemone Mexicana solvent extract *against some pathogenic bacteria.**Mem, Inst.* 101(6).

Indranil B, Soroj K, Chatterjee S, Chatterjee G C (2006). Antibacterial Potentiality of Argemone Mexicana solvent extract against some pathogenic bacteria.*Mem, Inst.* 101(6).

Ivanova, D, Gerova T, Chervenkov and T.Yankova (2005).Polyphenols and antioxidant capacity of Bulgarian *Med.plants.**J. Ethnopharmacol.*96:145-150.

Jasim S AA, Najji MA (2003). Novel antiviral agents a medicinal plant prespective.*J. app. microbiol* 95(3)412-427.

Kavanagh F (1972). Analytical microbiology, Vol, 11, Academic press (Pub) New York and London.

Khan R (2009).Antimicrobial Activity of Five Herbal Extracts Against Multi Drug Resistant (MDR) Strains of Bacteria and Fungus of Clinical Origin. *Molecules*; 14(2): 586-597.

Mahesh B and Satish S (2008).Antimicrobial Activity of Some Important Medicinal Plant against plant and Human Pathogens. *W. J. Agri. Sci.* 4(S):839-843.

- Malviya S, Rawat S, Kharia A and Verma M (2011).** Medicinal attributes of *Acacia nilotica* linn- A comprehensive review on Ethnopharmacological claims, *Int. J. Pharm. Sci*, Suresh Gyan Vihar University, Jaipur (Rajasthan), India, 2(6): 830-837.
- Mohamed N A (2012).** Italic antifungal activity screening test of extracted parts of *Citrullus colocynthis* on pathogenic dermatophytes clinical isolates. M.Sc. Thesis, Sudan University for Science and Technology.
- Muhammad HS and Muhammed S (2005).** The antibacterial properties of *Passiflora foetida* L- a common exotic medicinal plant. *AFR.J. Biotechnol* 6(22): 2650-2653.
- Nath D, Sethi N, Singh RK, Jain AK (1992).** Commonly used Indian abortifacient plant plants with special reference to their teratologic effects in rats. *J Ethnopharma* 36: 147-154.
- Nesteret A, Roberts p and Nester (2004).** Microbiology Human perspective 4th edition McGrawhill Inc. pp.109-121.
- Pande M B (1981).** Note on the nutritive value of babul (*Acacia nilotica* L) seeds (extracted). *Ind. J. Anim. Sci.* 1981, 51(1):107-108.
- Peters R G and Plorde T J (1963).** The usefulness of in vitro sensitivity tests in antibiotic therapy. *Ann Rev, Med.* 14, 41-56.
- Pfaller M, Jones RN, Biedenbach A (2001).** Antimicrobial resistance trends in medical centers using carbanens, report of 1991 and 2000 results from the MYSTIC programme (USA). *Diagn. Micro. Infect. Dis.* (41): 177-182.
- Pranee J, Paranee K, Yuwadee W, Penchom P, Potjane S, Saraya ML S, Orawan R (1999).** Anticough and antimicrobial activities of *Psidium guajava* Linn. Leaf extract. *J. Ethnopharmacol*, 67, 2, 203-212.
- Rahman A, Shakoor A, Zaib G, Mumtaz A, Ihtesham Y and Napar A (2014).** Comparative antibacterial activity of *Acacia nilotica* L. Leave extract against pathogenic bacteria and fungi, *J. Med. Plants. Res.* Vol. 8(29), pp.975-982.

- RAY G.C, RYAN J.K (2004)** – *Sherris Medical Microbiology and introduction to infectious diseases*- 4th edition – USA –page 867-871.
- Recio M C (1989)**. A review of some antimicrobial compounds isolated from medical plants reported in literature 1978-1988. *Phytother.Res.* 3 117-125.
- Ruttoh E K (2009)**. Antimicrobial efficacy of selected plants used by traditional medical practitioners in Keiyo, Kenya. Unpublished Msc Thesis – Kenyatta University. 78-87.
- Saini M L (2008)**. Comparative Pharmacognostical and antimicrobial studies of *Acacia* species (*Mimosaceae*).*J. Med. Plants. Res.*2 (12):378-386.
- Satish S, Raghavendra MP, Raveesha KA (2008)**. Evaluation of antibacterial potential of some plants against human pathogenic bacteria.*Ad.Bio Res.* 2(3-4):44-48.
- Seigler D S (2003)**. Phytochemistry of *Acacia*-sensu lato *Biochem.Syst. Ecology.*, 31(8):845-873.
- Siddhuraju P, Vijayaku Mari K, Janardhanan K (1996)**. Chemical composition and nutritional evaluation of an underexploited legume, *Acacia nilotica* (L) Del. *Pharma nutrition.* 57(3):358-391.
- Siddiqui MB, Husain W (2009)**. Traditional treatment of diarrhea and dysentery through herbal drug in rural India *Fitoterpia J. Pharmacogn and Phytochem* 62:325-529.
- Silva junior, A.A; Vizotto, Z.J.; Giorgi, E.; Macedo, S.G and Matalca, K.Z. (1994)**.Antimicrobial activity of *psidium guajava* and *juglans regia* leaf extracts to acne-developing organism, *Am .J. Chins. Med.* 33, 197-205.
- Singh BN, Singh BR, Singh RL, Prakash D, Sarma BK, Singh HB (2009)**.*Antioxidant and antiquorum sensing activities of green pod of Acacia nilotica L. Food Chem.Toxicol.*47:778-786.
- Singh BN, Singh BR, Singh RL, Prakash D, Sarma BK, Singh HB (2009)**. Antioxidant and antiquorum sensing activities of green pod of *Acacia nilotica* L. *Food Chem.Toxicol*, 47:778-786.

- Singh R, Singh R (1972).** Screening of some plant extract for antiviral properties. *Techno* (Sindri); 9: 415-416.
- Steven, D. and Ehrlich, N.M.D (2011).** Solutions Acupuncture, a private practice specializing in complementary and alternative medicine, phoenix, AZ. Review provided by *VeriMed Healthcare Network*.
- Sukhdev, S.H., Suman, P.S., Gennaro, L. and Dev, S.R. (2008).** Extraction technologies of medicinal and aromatic plants. United Nations Industrial Development Organization and the International Center for Science and High Technology. Pp 116.
- Suleiman, S.M. (2013).** Antibacterial activity of Guava (*Psidium guajava*) leaves and Safflower (*Carthamus tinctorius*) seeds extracts against wound infection bacteria. M.Sc. Thesis, Sudan University for Science and Technology.
- Wadood A, Wadood N, Shah SA (1989).** Effect of *Acacia Arabica* and *Arallum edulis* on blood glucose levels of normal and Alloxan diabetic rabbits. *J. Pak. Med. Assoc*; 38(1):33-38.
- World Health Organization (2002).** WHO Publication on strategy for traditional medicine.
- Anguzu JR, Olila D (2007).** Drug Sensitivity Patterns of Bacterial Isolates from Septic Post-Operative Wounds in a Regional Referral Hospital in Uganda. *Afr Health Sci* 7: 148-154.

Appendix I

Shendi University

College of post Graduate Studies & Research's

Questionnaire about Antimicrobial activity of *Acacia nilotica* extracts against Bacteria isolated from wound infection

Sample number:

1- Name:.....

2- Age: a<15 (.....) b- 15 – 24 (...). c- 25 – 35 (...). d- 36 – 45 (.....) e-
46 and more (.....)

3- Residence :

a- City (.....)

b- Village (.....)

4- Site of infections.....

5- type of wound

A-traumatic (.....) b- post-operative (.....) c- others (.....)

6- Infection Duration:

past history of recent hospitalization yes (.....) No (.....)

7- Hospital admission a- in patient (.....) b- out patient (.....)

8- Treatment:

a- Treated with antibiotic (.....).

b- Not treated (.....).

9- Past History of wound infection:

b- Yes (.....)

b- No (.....)

10- History of disease:

a-Diabetes (.....) b- Hypertension (.....) c- Other (.....) d- None (.....)

Appendix II



Pods of *A. nilotica*



Babool Gum



***A. nilotica* flowers..**



Pods of *A. nilotica* sub spp *nilotica* ..

- *This photography from internet*



Activity of aqueous extract of: *A. Anilotica* against *S.aureus*.



Activity of aqueous extract of: *A. Anilotica* against *MRSA*.