Sudan University of Science and Technology

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In vitro Antibacterial activity of Acacia nilotica (Algarad) methanolic and aqueous extracts against wound pathogens

نشاط مستخلص السنط الميثانولي والمائي على البكتريا المعزوله من الجروح

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الايـــــــــــــــــه

قال تعالى :

(وَهُوَ الَّذِي أَنزَلَ مِنَ السَّمَاءِ مَاءً فَأَخْرَجْنَا بِهِ نَبَاتَ كُلِّ شَيْءٍ فَأَخْرَجْنَا مِنْهُ خَضِرًا نُّخْرِجُ مِنْهُ حَبًّا مُّتَرَاكِبًا وَمِنَ النَّخْلِ مِن طَلْعِهَا قِنْوَانٌ دَانِيَةٌ وَجَنَّاتٍ مِّنْ أَعْنَابٍ وَالزَّيْتُونَ وَالرُّمَّانَ مُشْتَبِهًا وَغَيْرَ مُتَشَابِهٍ انظُرُوا إِلَى ثَمَرِهِ إِذَا أَثْمَرَ وَيَنْعِهِ إِنَّ فِي ذَلِكُمْ لَآيَاتٍ لِّقَوْمٍ يُؤْمِنُونَ).

صدق الله العظيم

سورة الانعام الاية 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 Prof. Yousif Fadlalla Hamed Elnil, for his encouragement, follow up and guiding and directing.

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ABSTRACT

Aimed to this study was to study the antibacterial activity of the different concentrations of methanolic and aqueous extract of the medicinal plants A.nilotica sub spp nilotica and sub spp adansonii pods using agar dilution method.

Hundred wound swabs were collected from patients with infected wounds who attended to Soba Teaching Hospital and Alban Gadeed Teaching Hospital during period from March to October 2015.

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 (P value 0.872).

The methanol and aqueous extract of A.nilotica pods (Family-Fabaceae) were screened for their antimicrobial activity against Standard bacteria; One Gram positive (S.aureus, ATCC25923) and Four Gram negative (K.pneumonae ATCC53657, P.aeurginosa ATCC 27853, E.coli ATCC 25922 and Proteus spp ATCC 6380). These extracts were also screened against clinical isolates of S.aureus, MRSA, E.coli, K.pneumonae, P.areuginosa, and Proteus spp.

Methanol extract of A.nilotica sub spp nilotica and adansonii were more active against one or more of six bacteria.

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

The antibacterial activity of 4 reference drugs: Gentamicin, Tetracycline, Ampicillin and Erythromycin were determined against the Standard bacteria and their activities were compared to the activity of plants extract.

Gas Chromatography analysis exhibited 33 Compounds of A.nilotica sub and 15 compounds for sub adansonii, this different in the component may due to the environmental difference, also these compounds were identified and quantified by the Retention time and quantitatively by the area under the curve.

مستخلص الاطروحه

هدف هذه الدراسه كان دراسة النشاط المضاد للبكتريا لتراكيز مختلفه للمستخلصات المائيه والميثانوليه لاحد النباتات الطبيه وهو نبات السنط باستخدام طريقة اختبار تخفيف الاجار.

اخذت مائة مسحه من الجروح في مستشفى سوبا التعليمي و مستشفى البان جديد التعليمي في الفتره من مارس الي اكتوبر 2015 م.

وجد في هذه الدراسه ان التهاب الجروح يكون اكثر تكررا في الفئه العمريه من 21–40 سنه و المكورات العنقوديه الذهبيه كانت اكثر تكررا.

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

اختبرت النشاطات المضاده للبكتريا لمستخلصات الميثانول والماء لنبات السنط ضد البكتريا القياسيه واحده موجبة الجرام(المكورات العنقوديه الذهبيه.واربعه سالبة الجرام(الكلبسيلا الرئويه والزائفه الزنجاريه والاشريكيه القولونيه والمتقلبه الاعتياديه).

كذلك اختبرت البكترياء المعزوله وهي (المكورات العنقوديه الذهبيه والمكورات العنقوديه الذهبيه المقاومه للميثيسلين والكلبسيلا الرئويه والزائفه الزنجاريه والاشريكيه القولونيه والمتقلبه الاعتيادي).

المستخلصات الميثانوليه لنبات السنط كانت اكثر نشاطا ضد واحد او اكثر من البكتريا السته (المكورات العنقوديه الذهبيه والكلبسيلا الرئويه والزائفه الزنجاريه والاشريكيه القولونيه والمتقلبه الاعتياديه).

اما المستخلص المائي لنبات السنط قضى على واحد او اكثر من البكترياء المختبره (المكورات العنقوديه الذهبيه والكلبسيلا الرئويه والزائفه الزنجاريه والاشريكيه القولونيه والمتقلبه الاعتياديه).

حدد النشاط المضاد للبكتريا لاربعه عقاقير مرجعيه وهي الجنتامايسين والتتراسايكلين والامبسيلين والايرثرومايسين ضد البكتريا القياسيه وقورن نشاطها مع نشاط المستخلص النباتي.

اظهر التحليل اللوني للغاز عن 33 مركبا لنبات السنط(اكاسيا نايلوتيكا) و15مركبا (اكاسيا ادانسوناي) ,هذا الاختلاف في عدد المركبات قد يكون لاختلاف البيئه.

وحددت هذه المركبات نوعيا باستخدام الوقت المحتفظ وكميا عبر المنطقه تحت المنحنى.

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CHAPTER ONE
INTRODUCTION

CHAPTER ONE

1. INTRODUCTION

1.1. Introduction

Herbs have medicinal properties due to presence of different active principle like alkaloids, volatile essential oils, glycosides, resins, oleoresins, steroids, tannins, terpenes and phenols. In the last few years there was an exponential growth in the fields of herbals medicine because of natural origin, easy availability, efficacy, safety and less side effects with efficient to cure age-related disorders like memory loss, osteoporosis, immune disorders, etc. (Malviya *et al*., 2011).

Some plants have been investigated significantly for the antimicrobial activity, and large number of plants products have been shown to inhibit the growth of pathogenic microorganisms (Suleiman, 2013).

Microbial infection is major public health problem in developed countries and Antibiotics used to treat this infection. Due to indiscriminate use of commercial antibiotics, the incidence of multiple antibiotic resistances in human pathogens is increased this has forced the scientists to search for new antimicrobial substance from various sources like medical plants. Medical plants constitute the main source of new pharmaceutical and health care products (Ivanona *et al*., 2005).

The World Health Organization (WHO) has listed more than 21,000 plants, which are used for many medical purposed around the world. They observed that about 74% of 119 plant- derived pharmaceutical medicines are used in modern medicine. It also estimates that 4 billion people (80 percent of the world population) presently use herbal medicine in health care (Malviya *et al*., 2011).

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 and consequently new drugs must be sought for, so herbal treatment is one possible way to treat diseases caused by multidrug 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. Rationale

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 detect antibacterial activity of *Acacia nilotica* sub spp *nilotica* and *Acaica nilotica* sub spp *adansonii* against bacteria isolated from wound.

1.3.2. Specific objectives

1. To isolate and identify the bacteria isolated from wound infections.

2. To study antibacterial activity of *Acacia nilotica* methanol and water extract against bacteria cause wound infection.

3. To compare between two species of *A.cacia nilotica* and the antibacterial activity of each one.

4. To determine the Minimum Inhibitory Concentration (MICs) of selected plants and to compare their activity with the commonly used antibacterial agent in Sudan.

5. To identify the major chemical components of the most effective extracts of the tested plants using gas chromatography technique.

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 (Hailu *et 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 19^{th} 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, Shiella 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. mirablis, Salmonella para typhi 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: Unani Tibbi: 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 (Malviya *et 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 (Malviya *et 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 Siddhuraju *et al*., 1996).

2.2.1.5. Ethopharmacological Studies

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

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

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

Anti bacterial 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:

Wadood *et 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: Agunua *et 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: Nath *et 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* leave 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.

Glactagogue 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 therapeutic used as: anti cancer, anti tumors, antiscorbutic, astringent, anti-oxidant, natriuretic, antispasmodial, diuretic, intestinal pain and diarrhea, nerve stimulant, cold, congestion, cough, dysentery, fever, hemorrhages, leucorrhea, ophthalmia and sclerosis .Seed have 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 is obtains 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 (Abd-elnabi *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 (Pranee *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. 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.

◙ **Dilution method:**

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).

2.2.5. 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 *et 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 (Grenet *et 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 (Pfaller *et al*, 2001; Muhammed and Muhammad, 2005).

2.2.6. Bacterial pathogens causing wound infection

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

A. Gram positive bacteria

- *Staphylococcus aureus.*
- *Streptococcus pyogens.*
- *Enterococcus faecalis.*
- *Bacillus anthracis.*
- *Bacillus cereus.*
- *Corynebacterium diphtheria.*
- *Colistridium perfringens type A.*

B. Gram negative bacteria

- *Escherichia coli.*

• *Proteus mirabilis.*

• *Klebsiella pneumonia.*

• *Pseudomonas aeruginosa.*

• *Aeromonas hydrophilia*.

2.2.7. General characteristics of tested bacteria

2.2.7.1. *Staphylococcus aureus*

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

2.2.7.2. *Klebsiella pneumoniae*

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 wound infections (Greenwood *et al*., 1998).

2.2.7.3. *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 (pyocyamin + pyoverdin). It grows over a wide temperature range (4-42ºC)

with optimum of (35-37ºC). 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 H_2S 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).

2.2.7.4. *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 35-37ºC. 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).

2.2.7.5. *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 METHODS

CHAPTER THREE

3. MATERIAL AND METHODS

3.1. Study design

This was descriptive prospective and cross sectional study.

3.2. Study area

Soba Teaching Hospital Khartoum State and Alban Gadeed Teaching Hospital, Khartoum Sudan.

3.3. Study population

All clinical suspected cases of wound infection arrived the unit of emergency during period of the study from March to September 2015.

3.4. Inclusion criteria

Patients admitted to hospital with wound infection.

3.5. Exclusion criteria

Patients under medical treatment or those already on antimicrobial treatment were excluded.

3.6. Sampling

None - Probability Sampling.

3.7. Sample size

A total of hundred wound swab samples (n=100) were collected.

3.8. Data collection

Data were collected from the patients using structural questionnaire containing all study variables **(Appendix) [1].**

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 37ºC 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 check for purity using Gram's staining procedure.

Obtained pure cultures were presented through inoculation into nutrient agar slope and stored at 4ºC after 24hrs incubation at 37ºC.

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% H_2O_2 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 with a 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 37ºC 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-phenylene diamine 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. Indole production test

Indole production was carried out as described by Barrow and Felham (1993). The tested organism was inoculated into pepton water and incubated at 37ºC 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 37ºC 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 37ºC for 24-48 hrs. The development of a pink color was indicative of production of NH_3 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* sub spp nilotica and adansonii were cleaned from dust and grass; 100 grams of each plant sample were separately crushed to a powder from using sterilized mortar and pestle.

3.13.2. Preparation of crude extract

Extraction was carried out according to method described by Sukhdev *et al*., (2008). 50 grams of each sample was grinded using mortar and pestle and extracted with methanol using soxhelt extractor apparatus. Extraction carried out for about eight hours till the solvent returned colorless at the last siphoning times. Solvent was evaporated under reduced pressure allowed air in petri dish till complete dryness and the yield percentages were calculated as followed:

Weight of extract obtained / weight of plant sample x 100

3.13.3. Preparation of the aqueous extract

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 - 20ºC extract was dried using freeze dryer -40ºC 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 37ºC for 24hrs. The bacterial growth was harvested and washed off with 100 ml sterile normal saline, to produce suspension containing about (10^8-10^9) colony forming units/ml. The inocula density was compared with Macfarland standard solution of $BaSO_4$ (0.1ml of 1% $BaCl_2$ + 9.9ml of 1% H_2SO_4). The suspension was stored in the refrigerator at 4ºC until used.

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 (10^8-10^9) colony forming unit/ml was thoroughly mixed with 100ml of Muller-Hinton agar (HiMedia, India) which was maintained at 45ºC

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 (10 mm in diameter) were cut using a sterile cork borer (No.4) and agar disc were removed. Alternate cups filled with 0.1ml samples of each of the extracts using automatic micro titer pipette, and allowed to diffuse at room temperature for 2hrs. The plates were then incubated in the upright position at 37ºC 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 (MICs) 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 50, 25, 12.5, 6.25 mg/ml. the bottom of each plate was marked off in to 4segments. The tested organisms were grown in broth over night to contain 10^8 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 37ºC 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.

3.15. Gas chromatography of *Acacia nilotica* (Methanol extract)

In gas chromatography, the mobile phase (or moving phase) is a carrier gas, usually an inert gas such as helium or an unreactive gas such as nitrogen. The stationary phase is microscopic layer of liquid or polymer on an inert solid support, inside a piece of glass or metal tubing called a column (a homage to the fractionating

column used in distillation). The instrument used to perform gas chromatography is called a gas chromatography (or aerograph, gas separator).

The gaseous compounds being analyzed interact with the walls of the column, at different time, known as the retention time of compound. The comparison of retention time is what gives GC its analytical usefulness (Suleiman, 2013).

3.15.1. Methylation steps:

Two grams from the samples were taken and transferred in a test tube, then 7 ml of alcoholic sodium hydroxide was prepared by {2 g (NaOH) → 100 ml (methanol)}, and 7 ml of alcoholic sulphuric acid was added which was prepared by {1 ml (H_2SO_4) → 100 ml (methanol)}. After that the sample was left at overnight in room temperature.

Then a suitable volume from normal hexen (n-hexen) was added, also a suitable volume of super saturated sodium chloride was added. Then it was shaken gently, after that two layers were appeared, the upper one is organic layer and the lower is aqueous layer. Finally a volume of the organic was taken and diluted by Di ethylene and injected in Gas Chromatography Mass Spectrometer (GC-MS) by using 1µl syringe.

3.16. Data analysis

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

CHAPTER FOUR

RESULTS

Chapter Four

4. Results

4.1. Frequency and percentage of sampling according to age group

Out of 100 specimens, 31 samples were taken from patients of with mean age $^{+}_{<}$20 age group, 42 from 21-40 age group, 15 from 41-60 age group, 9 from 61-80 age group and 3 from >80 age group as shown in table 1.The age grouped in to five grouped majority samples taken from age group 21-40 Year.

Table 1: Frequency and percentage of sampling according to age group

Age group	Frequency	Percentage
< 20	31	31%
21-40	42	42%
41-60	15	15%
16-80	9	9%
>80	3	3%
Total	100	100%

4.2. Frequency and percentage of sampling according to gender

Out of 100 specimens, 57(57%) were males whereas 43(43%) were females as shown in table 2 and figure 1.

Table 2: Frequency and percentage of sampling according to gender

Gender	Frequency	Percentage
Male	57	57%
Female	43	43%
Total	100	100%

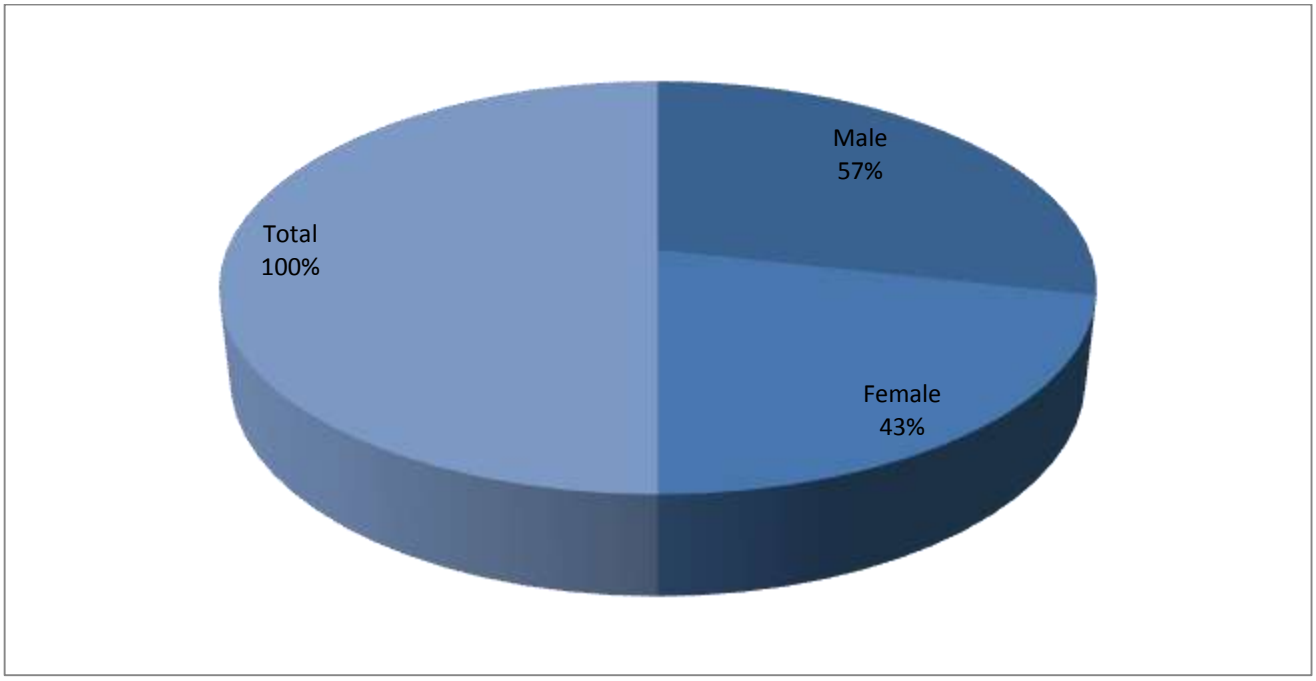

Fig 1: Frequency and percentage of sampling according to gender.

4.3. Bacteriological Result

4.3.1. Frequency and Percentage of bacterial growth.

Out of the 100 studied specimen, 94 (94%) showed bacteria growth while the remaining 6 (6%) were negative for bacterial growth as shown in (table 3 and Fig 2).

Table 3: Frequency and Percentage of bacterial growth.

Result of culture	Frequency	Percentage
Growth	94	94%
No growth	6	6%
Total	100	100%

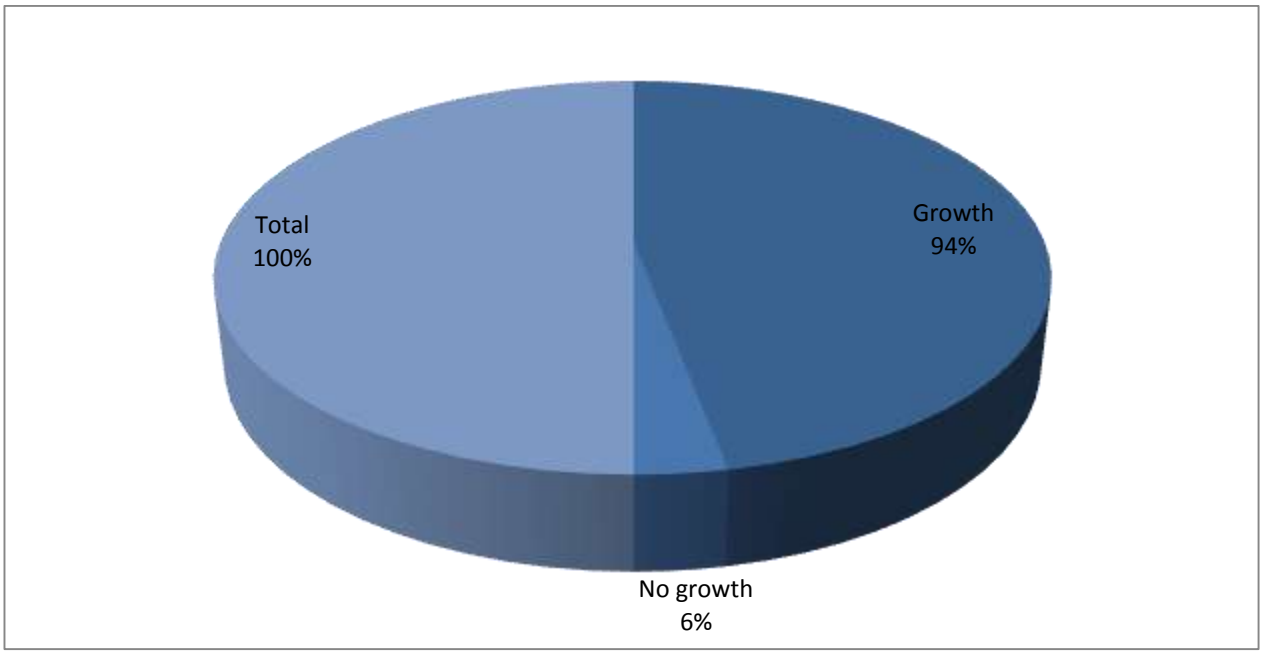

Fig 2: The frequency and percentage of bacterial growth.

4.3.2. Gram Stain for isolated bacteria species

Out of the 94 positive cultures for bacterial growth 40 were Gram positive (42.6%) and 54(57.4%) are shown in (table 4 and Fig 3).

Table 4. Gram stain for isolated bacteria

Gram reaction	Frequency	Percentage
Gram positive	**40**	**42.6%**
Gram negative	**54**	**57.4%**
Total	**94**	**100**

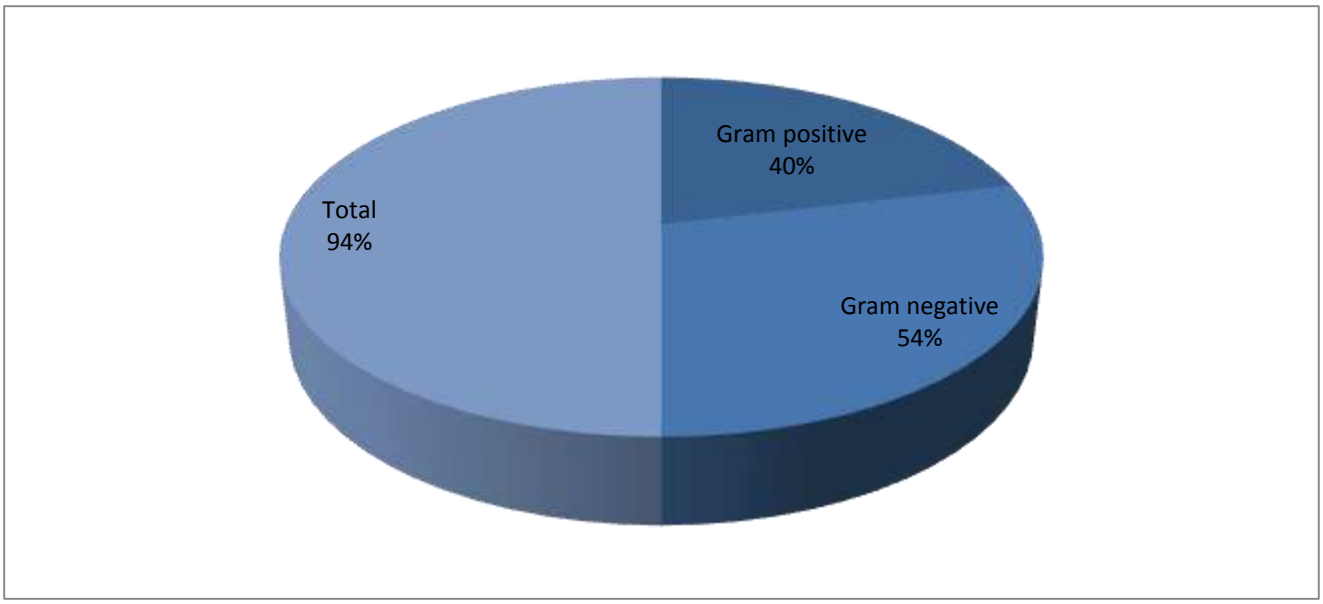

Fig 3: Gram Stain for isolated bacteria.

4.3.3. Characters and biochemical tested of *Staphylococcus aureus*.

The biochemical tests for both Gram positive and Gram negative stain were shown in Table5 and Table 6 respectively.

Table 5. Characters and biochemical tested of *S.aureus*.

Character	***S.aureus***
Macconkey's agar	Yellow colonies
Blood agar	White creamy colonies
Haemolysis	B-haemolysis
Catalase	+
DNAse	+
Co-agulase	+
Lactose fermentation	_
Mannitol	+

Note:

(+) positive

(-) negative

Table 6. Characteristics and biochemical properties of tested Gram negative bacteria

Character	***E.coli***	***P.aeurginosa***	***K.pneumonae***	***Proteus***
Macconkey's agar	pink	Yellow	Pink	Yellow
Blood agar	Large white, non haemolytic colonies	Large haemolytic colonies	Large mucoid colonies	Fishy odour and swarming
Shape	Rod	Rod	Rod	Rod
Oxidase test	-	+	-	-
Catalase test	+	+	+	+
Citrate test	-	+	+	-
Urease test	-	-	+	+
KIA Slope/butt	Y/Y	R/R	Y/Y	R/Y
Gas production	+	-	+	+
H2S production	-	-	-	+
Lactose fermentation	+	-	+	-
Indole test	+	-	-	+

Note:

(-) negative

(+) positive

Y (Yellow)

R (Red)

KIA (Kligler Iron Agar).

4.3.4. Frequency and percentage of isolated bacteria species

The isolate bacteria were identified phenotypically as follows: *Staphylococcus aureus* 40(42.5%), two of *S.aureus* was found resistant to Methicillin and considered as *MRSA*, while 3 Gram negative species were identified as follows: *E.coli* 18(19.1%), *K.pneumonia 12*(12.7%), *Pseudomonas aeurginosa* 22(23.4%), and *Proteus spp* 2(2.13%) as shown o (Table 7 and Fig 4).

Table 7. Frequency and percentage of isolated bacteria.

Isolate	Frequency	Percentage
S. aureus	40	42.5%
E. coli	18	19.1%
K.pneumonia	12	12.7%
p. aeurginosa	22	23.4%
proteus spp	2	2.13%
Total	94	100%

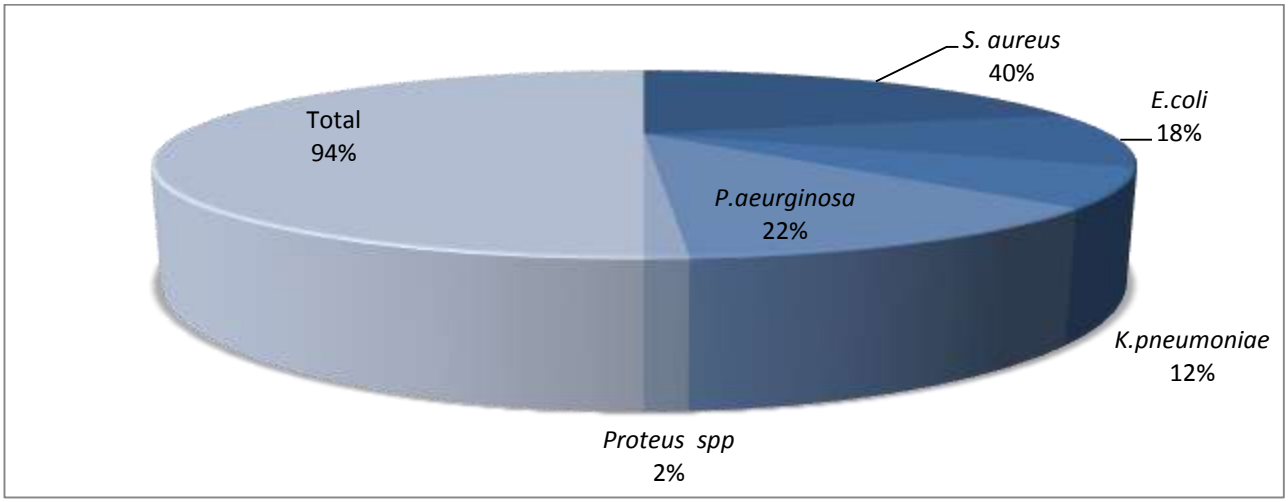

Fig 4: Frequency and percentage of isolated bacteria.

4.3.4. Frequency and percentage of isolated bacteria species according gender

Out of 94 isolated bacteria species, 51\94 isolated from male, and 43\94 isolated from females, as shown in table 8. There was insignificant association between the isolated bacteria and the gender in the studied group {P. value (0.656)}.

Table 8. Frequency and percentage of isolated bacteria species according gender

Gender	Growth					Total
	S.aureus	***E.coli***	***K.pneumonae***	***P.aeruginosa***	***Proteus***	
Male	21	9	9	11	1	51
Female	19	9	3	11	1	43
Total	40	40	12	21	2	94

p.value 0.656.

4.4. Distribution of wound infection according to history of disease

Distribution of wound infection according to history of disease is shown in table. There was insignificant association between history of disease and wound infection in the studied group, {P.value (0.872)}.

Table 9. Distribution of wound infection according to history of disease

History of disease	Growth					Total
	S. aur Eus	***E.coli***	***K. Pneumonia***	***P. Aeruginosa***	***Proteus spp***	
1 month	12	7	5	9	0	33
2 month	9	3	3	5	2	21
3 month	7	3	1	4	0	16
4 month	4	1	1	3	0	9
5 month	5	3	1	1	0	10
6 month	3	1	1	0	0	5
Total	40	18	12	22	2	94

P.value 0.872

Weight and yield of *A.nilotica* extracts are shown in table 10.

Table 10. Weight and % of different plant extracts obtained from methanol and water solvents.

Plant Sample Wt./g		**Methanol**		**Aqueous**	
		Weight of extract	**Yield %**	**Weight of extract**	**Yield %**
A.nilotica* subspp *adansonii	100g	13.798g	27.596%	8.175g	16.35%
A.nilotica* Subspp *nsilotica	100g	17.744g	35.488%	10.556g	21.112%

4.5. Antibacterial activity of *Acacia nilotca* sub species

4.5.1. Antibacterial activity of *Acacia nilotca* sub species against Standard bacteria

The methanolic of *A.nilotica* sub spp *nilotica* & *adansonii* exhibited inhibitory effects against all standard bacteria while aqueous extract showed medium activity while the aqueous one showed high activity to all standard bacteria with exception of *K.pneumonae* with medium activity. As shown in table 11.

Table 11. **Antibacterial activity of *Acacia nilotca* sub spp *nilotica* and *Acacia nilotica* sub spp *adansonii* against Standard bacteria**.

Microorganism	Mean zone of inhibition (in mm) according solvents used			
	A.nilotica nilotica		*A. nilotica adansonii*	
	Me OH	H_2O	Me OH	H_2O
S.aureus	22	18.5	25	22
E.coli	23	18	23	20.5
Proteus	22	24	23	21
P.aeruginosa	23	18	22	23
K.pneumoniae	17	17	19	17

Note:

Me OH (Methanol)

H_2O (Water).

Concentration used 10 mg/ml (100%) at 0.1 ml/cup.

Interpretation of the result

MDIZ mean diameter of growth inhibition zone in (mm) average of (2) replicates.

If MDIZ

- **>** 18 Sensitive**.**
- 14-18 Moderate**.**
- **<** 14 Resistant.

4.5.2. Antibacterial activity of *Acacia nilotca sub species* against isolated bacteria

The methanolic and aqueoua extracts of *A.niloica* sub spp showed antibacterial high activity against all isolated bacteria species, with exception that aqueous extract showed antibacterial medium activity against *K.pneumonae*. The mean zone of inhibition was used to measure the antibacterial activity of different extracts against the isolated bacteria are shown in table.12.

Table 12. Antibacterial activity of *Acacia nilotca* sub spp *nilotica* and *Acacia nilotica* sub spp *adansonii* against isolated bacteria.

Microorganism	Mean zone of inhibition (in mm) according solvents used			
	A.nilotica nilotica		*A. nilotica adansonii*	
	Me OH	H_2O	Me OH	H_2O
S.aureus	21.5	20	22	19
MRSA	24	21	22.5	20.5
E.coli	32	17	24	17
Proteus	31.5	17	32.5	19
P.aeruginosa	29	21	30	18
K.pneumoniae	24	18.5	25	17

MeOH (Methanol)

H_2O (Water).

Concentration used 10 mg/ml (100%) at 0.1 ml/cup.

Interpretation of the result

MDIZ mean diameter of growth inhibition zone in (mm) average of (2) replicates.

If MDIZ.

- > 18 sensitive.
- 14-18 moderate**.**
- < 14 Resistant.

4.6. The Minimum Inhibitory Concentration (MIC) of *A.nilotica*

The inhibition of bacterial growth was observed to increase with the increase in extract concentration. Detection of MIC depends on the activity of plant and solvent used in extraction to give high activity. In this study, MIC performed to methanol extract of *A.nilotica* pods.The minimum inhibitory concentration (MIC) of *A.nilotica* methanolic and aqueous extract to standard organisms revealed in table 13 and for isolated bacteria on table 14.

Table 13. The MIC of *A.nilotica* sub spp *nilotica* & *adansonii* to standard bacteria

Extract	Solvent	Conc	*S.aureus*	*E.coli*	*K.pneu*	*P.aer*	*Prot*
A.nilotica Sub *nilotica*	MeOH	50	21	22	15	21	20
		25	18	19	13	18	18
		12.5	15	14	11	15	15
		6.25	12	12	-	12	13
A.nilotica Sub *Nilotica*	Water	50	16	17	13	14	23
		25	14	15	13	12	20
		12.5	12	13	12	11	17
		6.25	-	-	-	-	15
A.nilotica Sub *Adansonii*	MeOH	50	23	22	17	20	21
		25	20	18	15	17	19
		12.5	16	15	13	13	15
		6.25	14	12	-	11	12
A.nilotica Sub *adansonii*	Water	50	21	19	15	22	20
		25	18	17	13	18	17
		12.5	14	15	11	15	16
		6.25	12	13	-	12	12

Note

MeOH (Methanol),

H_2O (Water)**.**

Concentration used 10 mg/ml (100%) at 0.1 ml/cup.

Interpretation of the result

MDIZ mean diameter of growth inhibition zone in (mm) average of (2) replicates.

If MDIZ

- > 18 sensitive.
- 14-18 moderate.
- < 14 resistant**.**

Tables 14. The Mic of *A.nilotica* sub spp *nilotica* & *adansonii* to isolated bacteria.

Extract	Solvent	Conc	*S.aureus*	*Mrsa*	*E.coli*	*K.pneu*	*P.aer*	*Prot*
A.nilotica* Sub *nilotica	**MeOH**	50	19	20	18	21	25	25
		25	17	18	15	17	19	21
		12.5	15	15	13	14	15	16
		6.25	11	11	11	11	11	11
A.nilotica* Sub *Nilotica	**Water**	50	18	19	15	15	19	15
		25	16	17	13	3	17	14
		12.5	13	14	2	12	14	12
		6.25	11	11	11	11	11	11
A.nilotica* Sub *Adansonii	**MeOH**	50	19	17	20	21	25	29
		25	17	15	18	17	20	23
		12.5	14	13	15	14	18	21
		6.25	11	11	11	11	11	11
A.nilotica* Sub *adansonii	**Water**	50	17	19	15	15	16	17
		25	15	17	13	13	14	15
		12.5	13	14	12	12	12	13
		6.25	11	11	11	11	11	11

Antibacterial activity of reference drugs against isolated bacteria.

As shown in table (15), Gentamicin was active against *Staphylococcus aureus, Escherichia coli, Klebsiella pneumonia, Pseudomonas aerginosa, MRSA* and *Proteus vulgaris.*

Erythromycin was active against *Staphylococcus aureus* while it had no activity to other isolates. *P. aerginosa sensitive* to ampicillin had activity against *Pseudomonas aerginosa*, where other no activity.

Tetracycline was active against *Staphylococcus aureus, MRSA* while it had no activity to *E.coli, Pseudomonas aerginosa, Proteus vulgaris and Klebsiella pneumoniae.*

Table 15. Antibacterial activity of reference drugs against isolated bacteria.

Organisms / Antibiotics	***S.aerus***	***MRSA***	***E.coli***	***K.pneu***	***Ps.are***	***Proteus***
Gentamicin	**S**	**S**	**S**	**S**	**S**	**S**
Tetracycline	**S**	**S**	**R**	**R**	**R**	**R**
Ampicillin	**R**	**R**	**R**	**R**	**S**	**R**
Erythromycin	**S**	**R**	**R**	**R**	**R**	**R**

Note:

(S) Sensitive.

(R) Resistant**.**

4.7. Gas Chromatography (GC) analysis

4.7.1. Gas chromatography analysis of Acacia nilotica sub spp nilotica.

In this study 33 components and chemical structure of *A.nilotica* sub *nilotica* and 15 components for sub *adansonii* pod were recognized and determined by using gas chromatography analysis as shown in table 12 and figure 4 for sub *nilotica* and on table 16 and figure 5 for sub *adansonii*.

Table 16: Gas chromatography analysis of *Acacia nilotica* sub spp *nilotica.*

Peak	R.Time	Area	Area %	Name
1	3.587	27566	0.03	Cyclohexane,(2-methylpropyi)-
2	4.154	31552	0.03	2-Oxopentanedioic acid
3	4.888	40811	0.04	Eucaiyptol
4	6.132	79132	0.08	Dimethyl dI-malate
5	6.370	73203	0.07	Methyl nicotinate
6	6.422	119096	0.12	4H-Pyran-4-one,2,3-dihydro-3,5dihydrox
7	7.275	2576513	2.53	Catechol
8	8.111	242861	0.24	1,2Benzenediol,3-methoxy
9	8.563	161026	0.16	1,2Benzenediol,4-methyl-
10	8.679	87837	0.09	2-Heptanol,acetate
11	8.871	278968	0.27	1,2-Benzenedicarboxylic acid
12	9.213	30704	0.03	Neric acid
13	9.294	50716	0.05	Phenol,2,6-dimethoxy-
14	9.658	40732589	39.97	1,2,3-Benzenetriol
15	10.070	2484377	2.44	Phenol,4,4-methylenebis[2,didimethyl-
16	11.006	2035247	2.00	.beta.-D-Glucopyranose,1,6-anhydro-
17	11.817	41258	0.04	Dodecanoic acid
18	12.483	875473	0.86	1,3,5-Benzenettriol
19	12.849	243777	0.24	Trans-4-Nonenedioic acid
20	13.423	10105996	9.92	4-O-Meethhllmannose
21	13.944	346520	0.34	Imidazole-4,5-dicarboxylic acid,1-methyl-
22	14.468	495612	0.49	Benzenemethanol,3,4,5-trimethoxy-
23	15.544	299078	0.29	(4-Methoxy-phenyl)-(10-methyl-10H-acrid
24	16.010	38776543	38.05	Benzoic acid,3,4,5-trihydroxy-methyl

				ester
25	16.284	287257	0.28	Hexaadecanioc acid,methyl ester
26	16.708	339029	0.33	Pentadecanoic acid
27	17.122	50501	0.05	Ethyl13-methyl-tetradecanoate
28	17.616	67757	0.07	Quiline,2-phenyl-
29	18.404	378899	0.37	9,12-Octadecadienoic acid(Z,Z)-methyl ester
30	18.467	244601	0.22	9-Octadecenoic acid, methyl ester,(E)-
31	8.759	90274	0.09	Methyl stearate
32	20.025	42272	0.04	(E)-.alpha.,.alpha.-Dicyanostillbene
33	20.202	198348	0.19	4-n-Dodecylresorcinol
	Total	10915393	100.00	

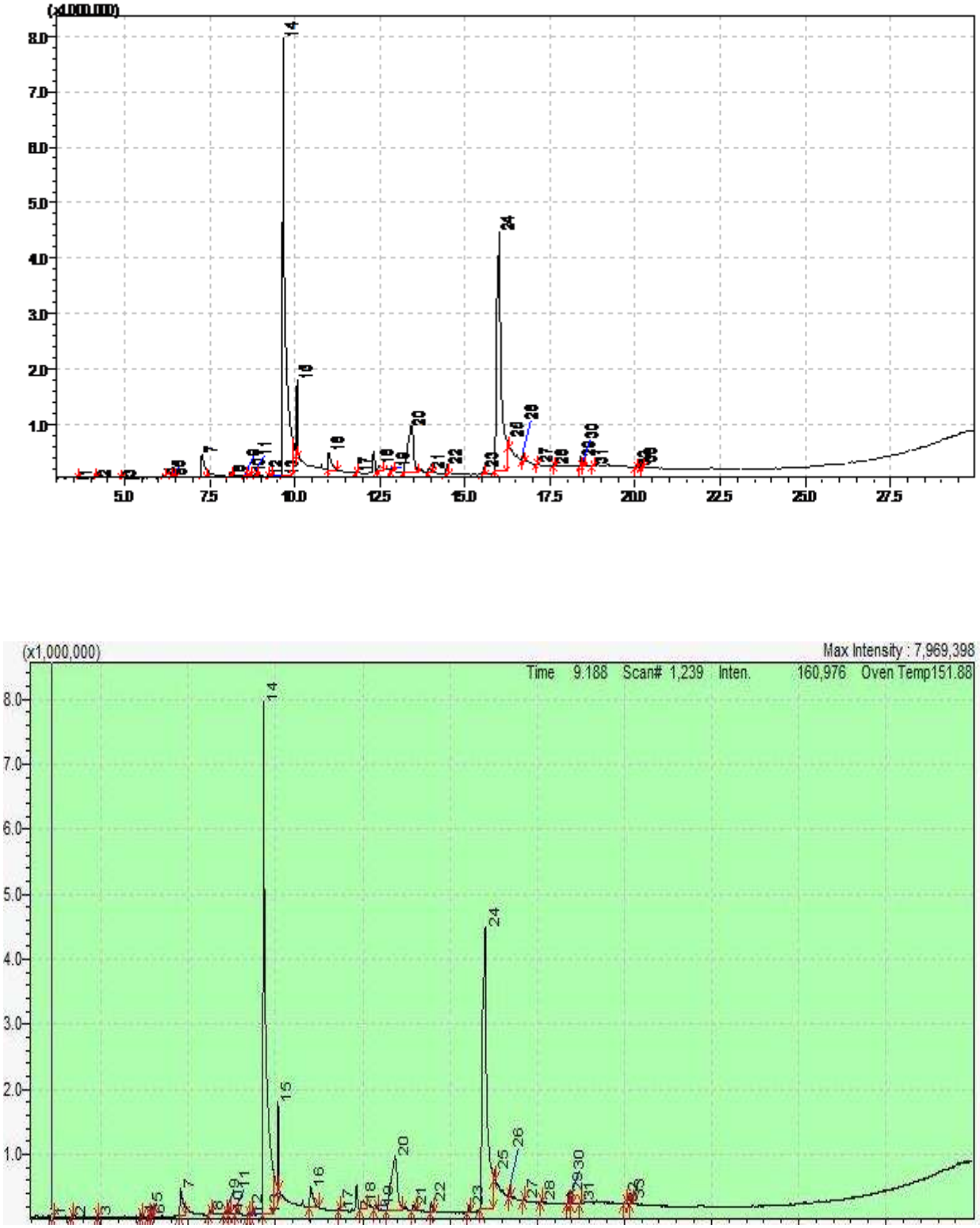

Fig 5: The phytochemical components found in *Acacia nilotica* sub *nilotica.*

4.7.2. Gas chromatography analysis of *Acacia nilotica* sub spp *adansonii*.

Table 17: Gas chromatography analysis of *Acacia nilotica* sub spp *adansonii*.

Peak	R.Time	Area	Area%	Name
1	3.600	19731	0.05	Cyclohexane, (2-methylpropyl)-
2	4.167	33971	0.09	2-Oxopentanedioic acid
3	7.304	1023322	2.62	Catechol
4	9.703	118867416	30.35	1,2,3-Benzenetriol
5	12.524	394284	1.01	1,3,5-Benzenetriol
6	13.348	10436539	26.69	4-O-Methylmannose
7	14.491	70487	0.18	Benzenemethanol,3,4,5-trimethoxy-
8	15.758	188128	0.48	Tonalid
9	15.945	13598999	34.77	Benzoic acid,3,4,5-trihydroxy- ,methyl ester
10	16.289	201322	0.51	Hexadecanoic acid, methyl ester
11	18.409	93815	0.24	9,12-Octadecadienoic acid(Z,Z)-, methyl ester
12	18.471	94658	0.24	9-Octadecenoic acid, nmethyl ester, (E)-
13	18764	43053	0.11	Methyl stearate
14	20.204	49280	0.13	6-Methyl-6-(5-methylfuran-2-yl)heptan-2-
15	29.023	992294	2.54	9,19-Cycloanost-23-ene-3,25-diol,3-aceta
	Total	39107299	100.00	

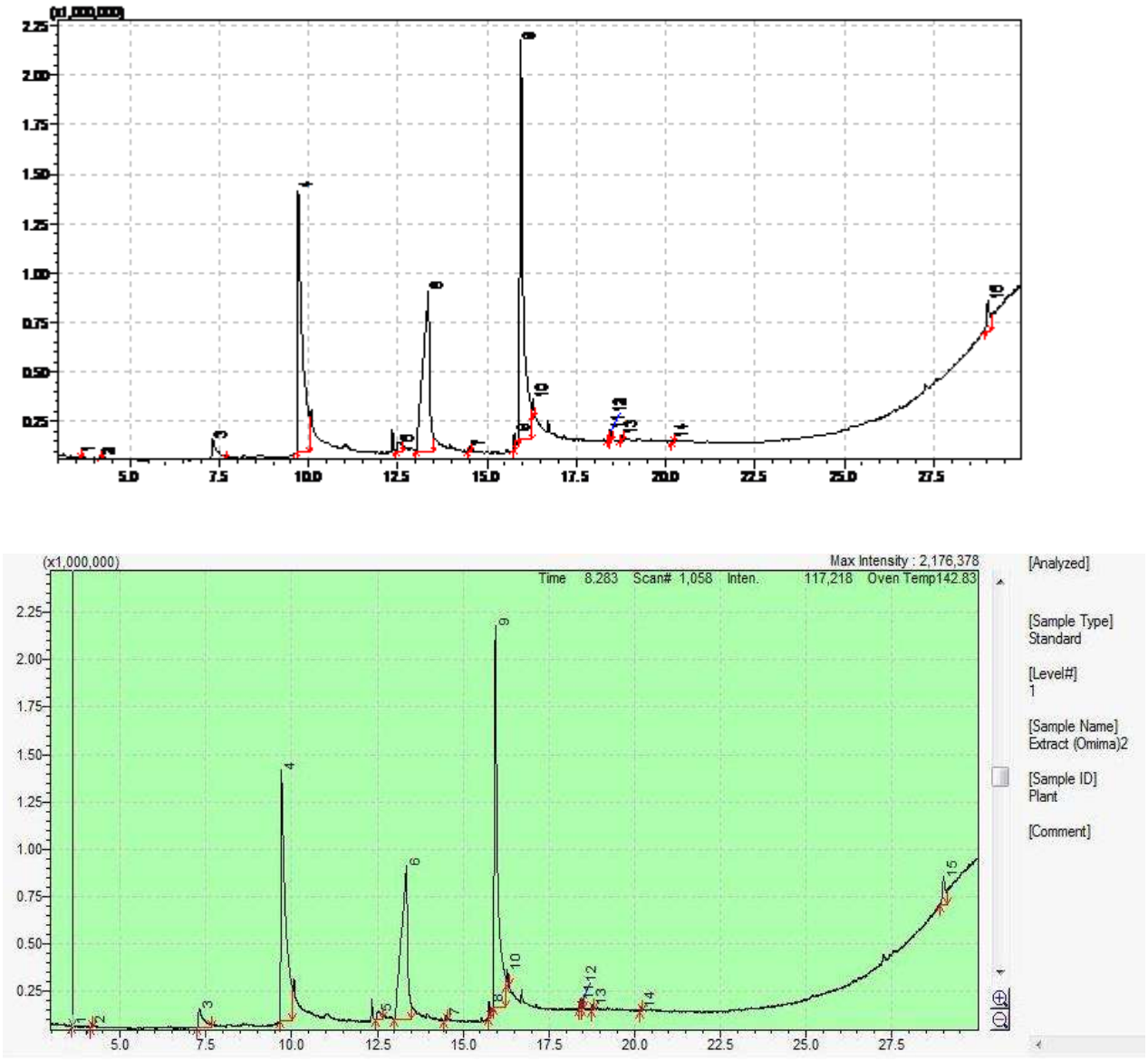

Fig 5: The phytochemical components found in *A. nilotica* sub *adansonii.*

CHAPTER FIVE

DISCUSSION

Chapter Five
5. Discussions

5.1. Discussions

The current study was carried out to screen the antibacterial activity of *Acacia nilotica* sub spp *nilotica* and *Adansonii*, Methanolic and aqueous extract was used against wound infection bacterial isolates *E.coli*, *S.aureus*, *MRSA*, *P. aerginosa, K. pneumonia and Proteus spp*. The results showed high activity of the methanolic& aqueous extract of *A.nilotica adansonii* had same activity to methanolic and aqueous extract of *A.nilotica* sub spp *nilotica* that mean the two sub spp had same activity.

A.nilotica is most active against isolated bacteria. This results agree to the reported by El-kamli and El- karim (2009).

The Means Diameter of growth Inhibition Zone (MDIZ) of microorganism isolates increases with the increase in drug concentration. This result is in agreement with report of Suleiman, (2013).

Methanolic extract of *A.nilotica* sub spp had higher activity against isolated bacteria. It was in agreement with the report of Saini 2008.Abass and Elhag 2015 and Satish 2008.

The aqueous extract of *A.nilotica* sub spp. exhibited high antibacterial activity against *S.auerus* and *MRSA* and moderate antibacterial activity against *E.coli*. This was consistent with reported by Saini 2008 and Ahmed *et al* 2007.

The methanolic extract of *A.nilotica* sub spp exhibited high antibacterial activity against *Proteus spp, P. aerginosa, k. pneumonia, MRSA, E.coli, S.aureus* respectively. It was agreed with report of Abass and Elhag 2015 and Ahmed *et al* 2007.

Gentamicin was found effective against all tested bacteria.

Tetracycline showed subjective results against *S.aureus* (ATCC 25923), and *Proteus vulgaris* (ATCC 6380) was found to be Ampicillin resistance. This result agrees to report by El-kamli and El- karim 2009.

The results of gas chromatography exhibited 33 Compounds of *A. nilotica sub nilotica and* 15for *A.nilotica* sub *adansonii*, and also these compounds were identified qualitatively by Retention time, and quantitatively by area under the curve.

These results were agreed to the reported by (Ruttoh, 2009). Who reported tannins, terpenoids, cardiac glycosides and alkaloid have been found to have the antibacterial properties.

The results of preliminary phytochemical analysis of methanol extract of *A.nilotica* in the present study revealed the presence of alkaloid, flavonoid, phenol, tannin and other like CHO, fatty acid, sugars and antioxidant. The alkaloid [24] and tannin [7] were mentioned by Seigler, (2003) Ruttoh, (2009), Malviya *et al*, (2011), Basno (2009) and, Deshpande (2013). The flavonoid [14] was mentioned by Seigler, (2003) Siddiqui *et al* (2009) and Deshpande (2013). While trepenoid [3] were mentione Seigler, (2003) d by Ruttoh, (2009), Malviya *et al*, (2011), and the phenol were mentioned by Malviya *et al*, (2011) also the Fatty acid [30] mentioned by Seigler, (2003) as showed on table 16 and Figure 4.

5.2. Conclusion

The current study showed that *A.nilotica* is rich in phytochemicals, this plants showed potent antibacterial activity.

A.nilotica had high activity against some bacteria, which justify their traditional use as antiseptic for treatment of skin infection.

The methanolic extract showed high antibacterial activity while the aqueous extract showed medium to high activity.

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

5.3. Recommendation

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 (MICS) for active ingredient of each bacteria including in this study.

3. Determination of the toxicity of the active ingredients.

4. More research is required for verify these results.

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REFERNCES

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Appendixes

Appendix 1

Sudan University of Science and Technology
College of Graduate Studies

In vitro Antibacterial activity of *Acacia nilotica* methanolic and aqueous extract against wound pathogens.
Questionaire

Name……..

Date…….

Sex……...

Age……..

History of Disease……..

……
……
……
……
……
……

Appendix 2

Fig 7: Pods of *A.nilotica* sub *nilotica.*

Fig 8: *A. nilotica* flowers.

Fig 9: *Babool* Gum

Fig 10: Pods of A. *nilotica* sub spp *nilotica*.

Fig 11: pods of *A.nilotica* sub spp *adansonii*

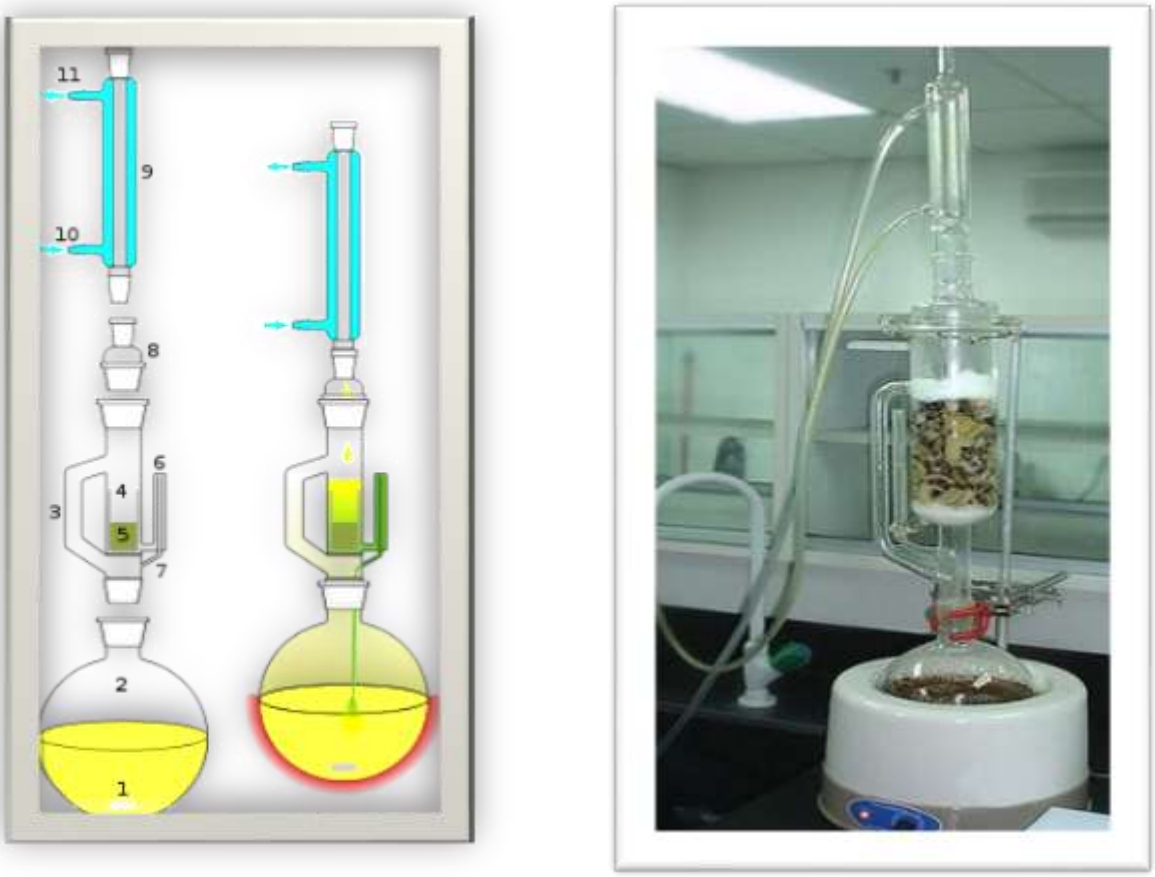
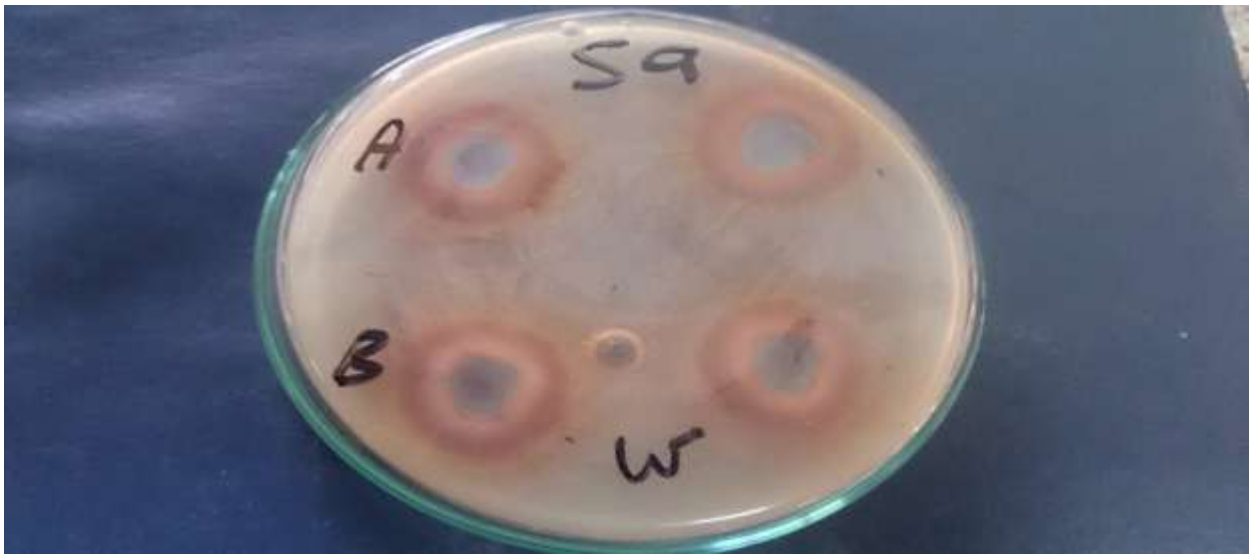


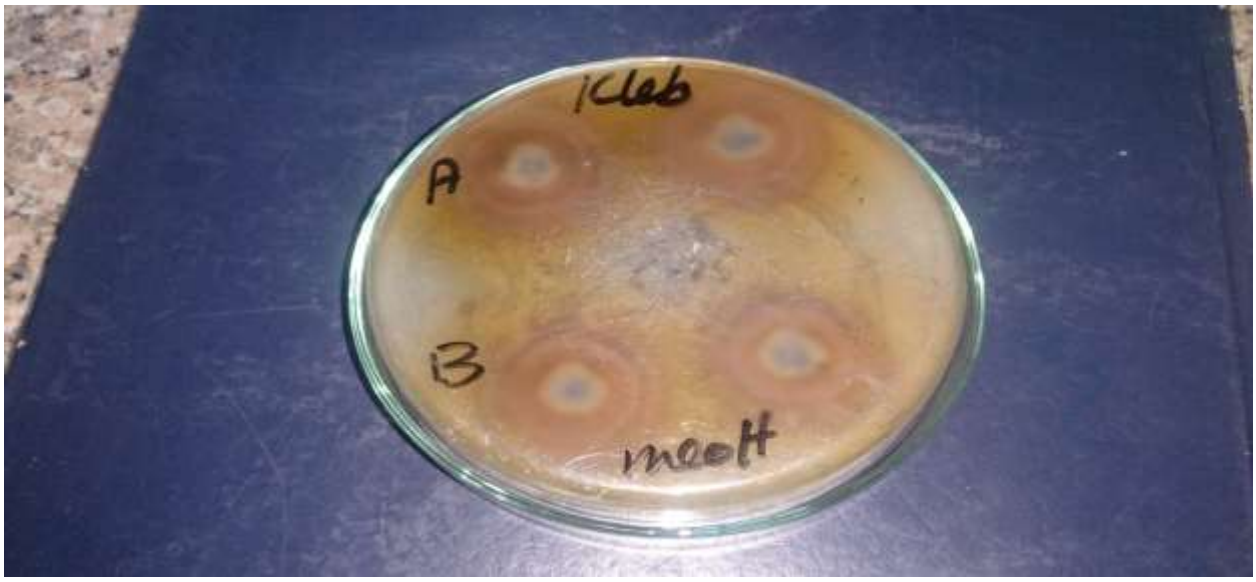
Fig 12: The Soxhelt extractor apparatus.



Activity of aqueous extract of: A. *Anilotica* sub *nilotica* B. *A.nilotica* sub *adansonii* against *S.aureus*.

Activity of aqueous extract of: *A. Anilotica* sub *nilotica* B. *A.nilotica* sub *adansonii* against *MRSA*.

Activity of methanolic extract of: *A. Anilotica* sub *nilotica* B. *A.nilotica* sub *adansonii* against *S.aureus.*



Activity of methanoic extract of: *A. Anilotica* sub *nilotica* B. *A.nilotica* sub *adansonii* against *K.pneumonae.*

Activity of methanolic extract of: *A. Anilotica* sub *nilotica* B. *A.nilotica* sub *adansonii* against *Proteus* spp.

Activity of different concentrations of methanol extract of *A. Anilotica* sub *adansonii* against isolated *P.aeruginosa*.

Activity of different concentrations of methanol extract of *A. Anilotica* sub adansonii against *Proteus vulgaris* ATCC 6380).

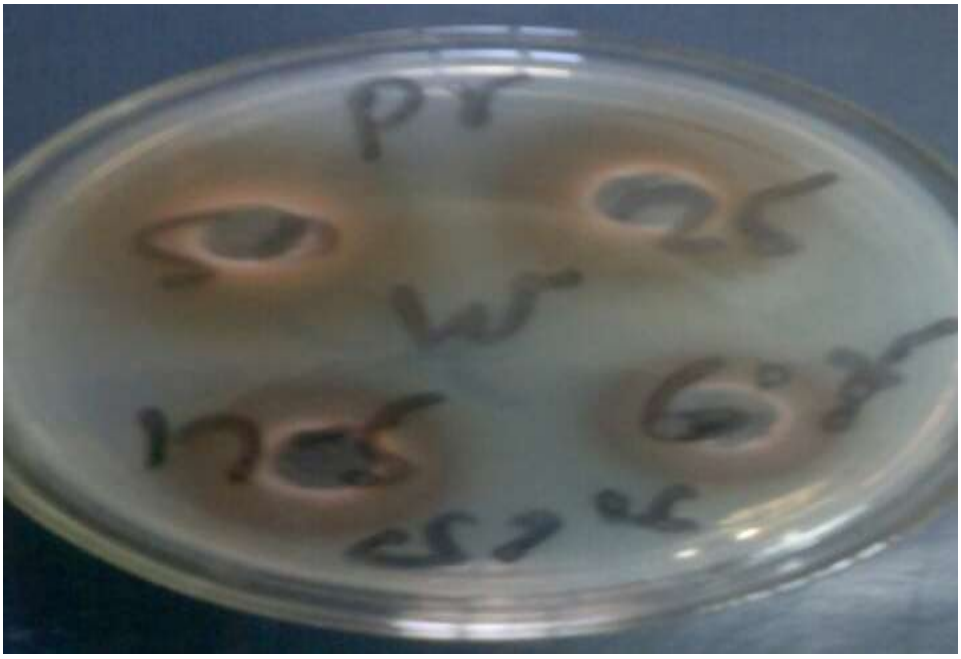
Activity of different concentrations of methanol extract of *A. Anilotica* sub *adansonii* against *K. pneumonae* ATCC53657.

Activity of different concentrations of methanol extract of: *A. Anilotica* sub *nilotica against isolated S.aureus.*

Activity of different concentrations of methanol extract of: *A. Anilotica* sub *nilotica against isolated MRSA.*

Activity of different concentrations of methanol extract of: *A. Anilotica* sub *nilotica against isolated Klebsiella pneumonae.*

Activity of different concentrations of methanol extract of *A. Anilotica* sub *nilotica against isolated E.coli.*



Activity of different concentrations of methanol extract of:

A. Anilotica* sub *nilotica* against isolated *Proteus.

Activity of different concentrations of methanol extract of:

A. Anilotica* sub *adansonii against isolated K.pneumonae.

Activity of different concentrations of methanol extract of:

A. Anilotica* sub *adansonii against isolated S.aureus.

Activity of different concentrations of aqueous extract of:

A. Anilotica* sub *adansonii against E. coli ATCC 25922 .

Activity of different concentrations of aqueous extract of:

A. Anilotica* sub *adansonii against isolated K.pneumonae.

Activity of different concentrations of aqueous extract of:

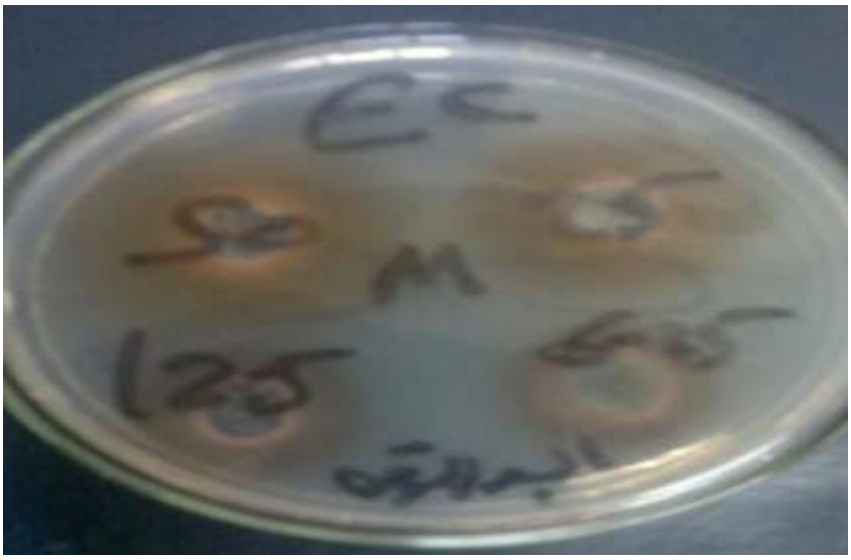
A. Anilotica* sub *adansonii against isolated MRSA.

Activity of different concentrations of aqueous extract of:

A. *Anilotica* sub *adansonii against Staphylococcus aureus ATCC25923.*

Activity of different concentrations of aqueous extract of:

A. Anilotica* sub *adansonii against isolated Proteus.



Activity of different concentrations of methanol extract of:

***A. Anilotica* sub *adansonii* against isolated *E.coli*.**

Activity of different concentrations of methanol extract of:

***A. Anilotica* sub *adansonii* against isolated *MRSA*.**