Sensitivity of *Escherichia coli*, *Klebsiella sp*, *Pseudomonas sp* and *Staphylococcus aureus* to Aqueous and Alcoholic Extracts of Four Medicinal Plants

By

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Sensitivity of *Escherichia coli*, *Klebsiella sp*, *Pseudomonas sp* and *Staphylococcus aureus* to Aqueous and Alcoholic Extracts of Four Medicinal Plants

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DEDICATION

This thesis is dedicated to my parents, God bless my father and prolong the life of my mother.

Also I dedicated this work to my brothers and sisters who have supported this study especially Hawa and Abdalla.

I also dedicated this thesis to all my family members who has supported me all the way since the beginning of my studies.
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**ABSTRACT**

There is an increasing demand for the biologically active substances from plants origin.

The synthetic chemical pharmaceuticals showed various side effects on the functions of different parts of the human body, both internally and externally and as a result of increasing resistance to antibiotic drugs. This study investigates the antibacterial activity of aqueous and alcohol extracts of *Acacia senegal* (L.), *Guiera senegalensis*, *Cymbopogon proximus* and *Solenostemma argel* leaves against *E. coli*, *Klebsiella*, *Pseudomonas* and *Staphylococcus aureus*. This study was conducted at the Center of Plant Pathology, University of Gezira, during the year, 2015. The paper disc diffusion (Inhibition zone) method was used for studying the effects of the plant extracts on the different bacterial growth. The results showed that, in single phase Arabic gum, Mahreb, Gebbiesh aqueous extract tested against *E. coli*, *Klebsiella*, *Staphylococcus* and *Pseudomonas* with the high concentrations of (50%) had the highest inhibitory effects were (12.3, 10.7, 13 and 12.3 mm); (5.7, 8.3, 6 and 8.3 mm); (7, 6.7 and 6 mm), respectively. However, in the combination phase (Arab gum+Harjal); (Mahreb+Harjal); (Gebbeish+Harjal) with the highest concentration (50%) had an inhibitory effects of (7, 9, 6.7 and 10.7 mm); (6.7, 9, 8.7 and 8.3 mm); (8.3, 10, 11 and 7.7 mm), respectively. On the other hand, (Arab gum+Harjal); (Mahreb+Harjal); (Gebbeish+Harjal) alcoholic extract tested against *E. coli*, *Klebsiella*, *Staphylococcus eureus* and *Pseudomonas* in the high concentration (50%) indicated an inhibitory effects of (6, 7.3, 7 and 6 mm); (6, 6.3, 6 and 6 mm); (14.3, 16, 15 and 6.3 mm), respectively. The results showed that almost all plant aqueous and ethanolic extracts had significant inhibitory effects on the bacterial growth. Moreover, it was observed that the ethanolic extracts had more significant results than the aqueous extracts. The addition of *Hargel* showed a significant inhibition. The study recommended that, more research should be done to investigate the role of *Hargel* in the biological activity of the different herb extracts.
حساسية الإشريشية القولونية و كليبسيلا و سيدوموناس والمكورات العنقودية الذهبية

عبد الرزاق حاج حسين عبد الرحمن

ملخص الدراسة

هناك طلب متزايد على المواد الفعالة بيولوجيا من أصل نباتي. و ذلك لأن الأدوية الكيميائية الإصطناعية أظهرت آثارا جانبية مختلفة على أجزاء مختلفة من جسم الإنسان الداخلية وخارجيا و كنتيجة لزيادة المقاومة للمضادات الحيوية. أجريت هذه الدراسة لعرفة النشاط المضاد لأنواع البكتيريا من المستخلصات المانية والكحولية من أوراق نباتات الصمغ العربي، الغبيش، المحريب والحرجل ضد الإشريشية القولونية، كليبسيلا، سيدوموناس، والمكورات العنقودية الذهبية. أجريت هذه الدراسة في مركز أمراض النبات، جامعة الجزيرة خلال العام 2015. تم استخدام طريقة انتشار القرص الورقي (منطقة التثبيط) لدراسة تأثير المستخلصات النباتية على نمو البكتيريا المختلفة. أظهرت النتائج أن التركيزات العالمية من (50%) في الحالة الفردية للصمغ العربي، والمحريب، والغبيش للمستخلص الماني تم اختبارها ضد الإشريشية القولونية، كليبسيلا، المكورات العنقودية الذهبية والسيدوموناس، كان لها أعلى تأثيرات مثبطة (10.7، 12.3 و 13.0 مم) كليا، (5.7، 8.3 و 6.6 مم) ، (7، 6.7 و 6.6 مم) على التوالي. ومع ذلك ، وفي حالة الجمع (الصمغ العربي + الحرجل) ، (محريب + الحرجل) ، (غبيش + الحرجل) مع أعلى تركيز (50%) كان لها تأثيرات عالية في البكتيريا (9، 9.4، 9.7 و 10.7 مم) ، (8.3، 10، 11 و 7.7 مم) على التوالي. ومن ناحية أخرى ، تم اختبار المستخلص المائي للصمغ العربي، والمحريب، والغبيش ضد البكتيريا الإشريشية القولونية، كليبسيلا، المكورات العنقودية الذهبية والسيدوموناس، حيث أعطي التركيز العالي (50%) أثر مثبطة (6، 7.3 و 6 مم) ، (6.3، 6 و 6 مم) ، (14.3، 15 و 6.3 مم) على التوالي. وأظهرت النتائج أن جميع المستخلصات المائية والكحولية للنباتات تأتي كمارة على نمو البكتيريا تقريبا. علاوة على ذلك ، لوحظ أن المستخلصات الإيثانولية كانت لها نتائج أكثر اجابة من المستخلصات المائية. و كان لإضافة الحرجل تثبيط كبير. وأوصت الدراسة بأنه ينبغي إجراء المزيد من البحوث لدراسة دور الحرجل في تنشيط الفعال المضاد للبكتيريا لمستخلصات الأعشاب المختلفة.

حسين عبد الرحمن

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هناك طلب متزايد على المواد الفعالة بيولوجيا من أصل نباتي. و ذلك لأن الأدوية الكيميائية الإصطناعية أظهرت آثارا جانبية مختلفة على أجزاء مختلفة من جسم الإنسان الداخلية وخارجيا و كنتيجة لزيادة المقاومة للمضادات الحيوية. أجريت هذه الدراسة لعرفة النشاط المضاد لأنواع البكتيريا من المستخلصات المانية والكحولية من أوراق نباتات الصمغ العربي، الغبيش، المحريب والحرجل ضد الإشريشية القولونية، كليبسيلا، سيدوموناس، والمكورات العنقودية الذهبية. أجريت هذه الدراسة في مركز أمراض النبات، جامعة الجزيرة خلال العام 2015. تم استخدام طريقة انتشار القرص الورقي (منطقة التثبيط) لدراسة تأثير المستخلصات النباتية على نمو البكتيريا المختلفة. أظهرت النتائج أن التركيزات العالمية من (50%) في الحالة الفردية للصمغ العربي، والمحريب، والغبيش للمستخلص الماني تم اختبارها ضد الإشريشية القولونية، كليبسيلا، المكورات العنقودية الذهبية والسيدوموناس، كان لها أعلى تأثيرات مثبطة (10.7، 12.3 و 13.0 مم) كليا، (5.7، 8.3 و 6.6 مم) ، (7، 6.7 و 6.6 مم) على التوالي. ومع ذلك ، وفي حالة الجمع (الصمغ العربي + الحرجل) ، (محريب + الحرجل) ، (غبيش + الحرجل) مع أعلى تركيز (50%) كان لها تأثيرات عالية في البكتيريا (9، 9.4، 9.7 و 10.7 مم) ، (8.3، 10، 11 و 7.7 مم) على التوالي. ومن ناحية أخرى ، تم اختبار المستخلص المائي للصمغ العربي، والمحريب، والغبيش ضد البكتيريا الإشريشية القولونية، كليبسيلا، المكورات العنقودية الذهبية والسيدوموناس، حيث أعطي التركيز العالي (50%) أثر مثبطة (6، 7.3 و 6 مم) ، (6.3، 6 و 6 مم) ، (14.3، 15 و 6.3 مم) على التوالي. وأظهرت النتائج أن جميع المستخلصات المائية والكحولية للنباتات تأتي كمارة على نمو البكتيريا تقريبا. علاوة على ذلك ، لوحظ أن المستخلصات الإيثانولية كانت لها نتائج أكثر اجابة من المستخلصات المائية. و كان لإضافة الحرجل تثبيط كبير. وأوصت الدراسة بأنه ينبغي إجراء المزيد من البحوث لدراسة دور الحرجل في تنشيط الفعال المضاد للبكتيريا لمستخلصات الأعشاب المختلفة.
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### List of Abbreviations

<table>
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<th>Abbreviation</th>
<th>Full Form</th>
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<tr>
<td>E.coli</td>
<td>Escherichia coli</td>
</tr>
<tr>
<td>CLED</td>
<td>cystine-lactose-electrolyte deficient</td>
</tr>
<tr>
<td>S</td>
<td>Staphylococcus</td>
</tr>
<tr>
<td>Cm</td>
<td>Centimeter</td>
</tr>
<tr>
<td>MRSA</td>
<td>Methicillin-resistant Staphylococcus aureus</td>
</tr>
<tr>
<td>P</td>
<td>Pseudomonas</td>
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<tr>
<td>°C</td>
<td>Celsius Degree</td>
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<tr>
<td>µl</td>
<td>Micro liter</td>
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<tr>
<td>HIV</td>
<td>Human immunodeficiency virus</td>
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<tr>
<td>AIDS</td>
<td>acquired immune deficiency syndrome</td>
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<td>K</td>
<td>Klebsiella</td>
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<tr>
<td>CPS</td>
<td>capsular polysaccharide</td>
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<tr>
<td>LPS</td>
<td>Lipopolysaccharide</td>
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<tr>
<td>HMV</td>
<td>Hypermucoviscosity</td>
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<tr>
<td>WHO</td>
<td>World health organization</td>
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<tr>
<td>mm</td>
<td>Millimeter</td>
</tr>
<tr>
<td>GA</td>
<td>Gum Arabic</td>
</tr>
<tr>
<td>BC</td>
<td>Before christ</td>
</tr>
<tr>
<td>AGPs</td>
<td>arabinogalactan-protein</td>
</tr>
<tr>
<td>ml</td>
<td>Milliliter</td>
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<tr>
<td>G</td>
<td>Gram</td>
</tr>
<tr>
<td>UV</td>
<td>Ultraviolet light</td>
</tr>
<tr>
<td>BUN</td>
<td>blood urea nitrogen</td>
</tr>
<tr>
<td>CP</td>
<td>Cymbopogon proximus</td>
</tr>
<tr>
<td>TDS</td>
<td>total dissolved solids</td>
</tr>
<tr>
<td>pH</td>
<td>Power of hydrogen</td>
</tr>
<tr>
<td>NA</td>
<td>Nutrient Agar</td>
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CHAPTER ONE
INTRODUCTION

Herbal medicine sometimes referred to as herbalism or botanical medicine, which means the use of herbs for their therapeutic or medicinal value. A herb is a plant or a plant part valued for its medicinal, aromatic or savory qualities. Herb plants produce and contain a variety of chemical substances that act upon the body (Shelef, 1983). It has been estimated that approximately 80% of the world's inhabitants rely mainly on traditional medicines for their primary health care; where plant based systems still play a vital role in health care. In developed countries, plant drugs are also extremely important, currently at least 119 chemicals derived from plant species can be considered as important drugs in use (Mullholland, 2000).

Spices and herbs have been used for thousands of centuries by many cultures to enhance the flavor and aroma of food. Early cultures also recognized the value of using spices and herbs in preserving food and for their medicinal value (Shelef, 1983). Scientific experiments since the late 19th century have documented the antimicrobial properties of some spices, herbs, and their components (Zaika, 1988). Ten Sudanese plants were screened for their antibacterial activity, seven of them showed promising results (Mustafa et al., 1982). Crude extracts solution obtained from the plant Gordenia lutea, showed antibacterial activity against Bacillus subtilis, Staphylocous aureus, Escherichia coli and Pseudomonas aeruginoza (Ahmed et al., 1984). Badreledin (2006) reported that ginger oil showed antimicrobial activity against Staphylococcus aureus, while, ELboshra (2005) reported that Staphylococcus aureus was sensitive to clove oil. The fenugreek oil was also found to inhibit Salmonella typhimurium (Sulieman, 2009).

Despite of the growth of global market for herbal products, following complementary and/or alternative medicines, homeopathy, health foods and natural-pharmaceuticals therapy, yet the majority of these herbs were not assessed for quality, safety and efficacy. These antimicrobial substances are of natural origin, and it is thought that their influences on the environment are few and can be used as biological control agents. However, some medicinal herbs for some reasons have not found wider application and sometimes are referred as ‘forgotten plants’. Taking into account the increasing demand for natural ingredients that might be used as food additives, components of functional foods,
preventing plant diseases and nutraceuticals as well as for other applications. It is reasonable to revise the ‘forgotten plants’ by assessing their applicability and benefits using modern scientific analysis methods (Abdel Rahman et al., 2011). The synthetic chemical pharmaceuticals also showed various side effects on the functions of different parts of the human body, both internally and externally (Mohamed et al., 2015). Even though pharmacological industries have produced a number of new antibiotics in the last three decades, resistance to these drugs by microorganisms has increased. In general, bacteria have the genetic ability to transmit and acquire resistance to drugs, which are utilized as therapeutic agents (Gislene et al., 2000). as from previous studies like Mahgob,(2016) showed a significant role of Hargel in inhibiting different bacterial growth individually or in combination with other herbs.

1.2 Objectives

1.2.1 General objective

The study has been conducted to investigate the antibacterial activity of the extracts from Acacia senegal (Arabic gum), Guiera senegalensis (Gebbeish), Cymbopogon proximus (Maharaib) individually, and their combination with Solenostemma argel (Hargel).

1.2.2 Specific objectives

1-Extraction and preparation of crude plant extracts at different concentrations by different solvents on same pathogenic bacteria.
2-To evaluate their individual and combined effects.
2.1 Microbes

2.1.1 Escherichia coli

*Escherichia coli* is the most commonly encountered member of the family Enterobacteriaceae in the normal colonic flora and the most common cause of opportunistic infections (Sherris, 1984). All members of the family Enterobacteriaceae are facultative, all ferment glucose and reduce nitrates to nitrites and all are oxidase negative (Sherris, 1984).

2.1.1.1 Classification

- **Domain:** Bacteria
- **Class:** Gammaproteobacteria
- **Order:** Enterobacteriales
- **Family:** Enterobacteriaceae
- **Genus:** *Escherichia*
- **Species:** *coli* (Sherris, 1984).

2.1.1.2 Morphology and identification

*Escherichia coli* is gram-negative, non-sporing bacilli with most strains being motile and generally possessing both sex pili and adhesive fimbriae (Mahon and Manuselis, 1995). Because most strains rapidly ferment lactose, colonies grown on MacConkey media are smooth, glossy, and translucent and are rose-pink in colour. Some strains grown on blood agar result in colonies being surrounded by zones of haemolysis. Colonies are smooth, circular, 1 – 1,5 mm in diameter and yellow opaque if lactose fermenting (blue, if non-lactose fermenting) when grown on cystine-lactose-electrolyte deficient (CLED) medium (Mackie and McCartney, 1989).

2.1.1.3 Epidemiology

Strains of *Escherichia coli* predominate among the aerobic commensal bacteria present in the healthy gut (Mackie and McCartney, 1989).

2.1.1.4 Escherichia coli Infections

*Escherichia coli* was initially considered a non-harmful member of the colon flora, but is now associated with a wide range of diseases and infections including
meningeal, gastrointestinal, urinary tract, wound and bacteremia infections in all age groups (Mahon and Manuselis, 1995). Other infections caused by Escherichia coli include peritonitis, cholecystitis, septic wounds and bedsores. They may also infect the lower respiratory passages or cause bacteraemia and endotoxic shock especially in surgical or debilitated patients (Mackie and McCartney, 1989).

2.1.1.5 Antimicrobial Susceptibility
Within the community, *E. coli* strains are commonly susceptible to all agents active against the Enterobacteriaceae. However, because of the frequent occurrence of R plasmids, strains acquired in hospitals may be resistant to any combination of potentially effective antimicrobics and therapy must therefore be guided by susceptibility testing (Sherris, 1984).

2.1.2 *Staphylococcus aureus*
Members of the genus *Staphylococcus* (staphylococci) are Gram-positive cocci that tend to be arranged in grape-like clusters (Ryan and Ray, 2004).

2.1.2.1 Classification
- **Domain:** Bacteria
- **Phylum:** Firmicutes
- **Class:** Bacilli
- **Order:** Bacillales
- **Family:** Staphylococcaceae
- **Genus:** *Staphylococcus*
- **Species:** *aureus* (Ryan and Ray, 2004).

2.1.2.2 Morphology and identification
Staphylococci are spherical cells about 1 m in diameter arranged in irregular clusters. Single cocci, pairs, tetrads, and chains are also seen in liquid cultures. Young cocci stain strongly gram-positive; on aging, many cells become gram-negative. Staphylococci are non-motile and do not form spores (Brooks *et al.*, 2007). *Staphylococcus aureus* is a facultative anaerobe that grows at an optimum temperature of 37°C and an optimum pH of 7.5. *S.aureus* produces white colonies that tend to turn a buff-golden color with time, which is the basis of the species epithet *aureus* (golden). Most, but not all, strains show a rim of clear β-hemolysis surrounding the colony (Ryan and Ray, 2004).
On nutrient agar, following aerobic incubation for 24 hours at 37°C, colonies are 1 – 3mm in diameter, have a smooth glistening surface, an entire edge and an opaque pigmented appearance. In most strains, pigmentation is golden with orange, yellow and cream varieties. On MacConkey agar, colonies are small to medium in size and pink or pink-orange in colour (Mackie and McCartney, 1989).

2.1.2.3 Epidemiology
Staphylococci are highly successful colonizers of humans and animals. They reside mainly on the skin, particularly in moist areas such as the anterior nares (nose), axilla and groin. Between one-third and three-quarters of individuals carry these organisms at any one time. Staphylococcal infections occur worldwide, and newly emerging hyper virulent or multi resistant strains spread rapidly over wide geographical areas. The bacteria survive in the air, on objects or in dust for days, therefore they can contaminate environments (such as hospitals) and continue to be transmitted over long periods of time. Some individuals may shed the organism more heavily than others. Staphylococcal infections are acquired from either self (endogenous) or external (exogenous) sources (Irving et al., 2005).

2.1.2.4 Infections
S. aureus causes serious infections of the skin, soft tissues, bone, lung, heart, brain or blood (Irving et al., 2005), include pneumonia, bacteremia leading to secondary pneumonia and endocarditis, osteomyelitis secondary to bacteremia and septic arthritis, seen in children and in patients with a history of rheumatoid arthritis.

Diseases caused by Staphylococcal toxins include scalded skin syndrome and toxic shock syndrome (Sherris, 1984).

2.1.2.5 Antimicrobial Susceptibility
Resistance to penicillin G can be predicted by a positive test for β-lactamase; approximately 90% of S.aureus produce β-lactamase. Resistance to nafcillin (and oxacillin and methicillin) occurs in about 35% of S.aureus and approximately 75% of S epidermidis isolates (Brooks et al., 2007). Alternative antibiotics for resistant organisms (e.g. MRSA) include vancomycin, erythromycin and gentamicin. Some strains become resistant to multiple antibiotics (Irving et al., 2005).
2.1.3 *Pseudomonas aeruginosa*

*Pseudomonas aeruginosa* is a classic opportunist pathogen belonging to the genus *Pseudomonas* (Mackie and McCartney, 1989).

### 2.1.3.1 Classification

- **Domain:** Bacteria
- **Phylum:** Proteobacteria
- **Class:** Gammaproteobacteria
- **Order:** Pseudomonadales
- **Family:** Pseudomonadaceae
- **Genus:** *Pseudomonas*
- **Species:** *aeruginosa* (Mackie and McCartney, 1989).

### 2.1.3.2 Morphology and Identification

Is an obligate aerobe, motile, rod-shaped, measuring about 0.6 x 2 µm. It is gram-negative and occurs as single bacteria, in pairs, and occasionally in short chains. sometimes producing a sweet or grape-like or corn taco-like odor (Brooks *et al.*, 2007). Its production of blue, yellow, or rust-colored pigments differentiates it from most other Gram-negative bacteria. The blue pigment, pyocyanin, is produced only by *P. aeruginosa*. Fluorescin, a yellow pigment that fluoresces under ultraviolet light, is by *P. aeruginosa* and other free-living less pathogenic *Pseudomonas* species. Pyocyanin produced and fluorescin combined produce a bright green color that diffuses throughout the medium (Ryan and Ray, 2004).

*P. aeruginosa* grows well at 37–42 °C; its growth at 42 °C helps differentiate it from other *Pseudomonas* species. It does not ferment carbohydrates, but many strains oxidize glucose (Brooks *et al.*, 2007).

### 2.1.3.3 Epidemiology

*P. aeruginosa* normally inhabit soil, water, and vegetation and can be isolated from the skin, throat, and stool of healthy persons. They often colonize hospital food, sinks, nosocomial taps, mops, and respiratory equipment. Spread is from patient to patient via contact with fomites or by ingestion of contaminated food and water (Baron, 1996).
2.1.3.4 Infections

*Pseudomonas aeruginosa* causes infections in healthy individuals and those who are hospitalized or have a compromised immune system as a result of other diseases. A variety of human infections are commonly associated with this bacterium:

- Urinary tract infections
- Ventilator-associated pneumonia
- Surgical site infection
- Respiratory infections
- Ocular infections
- Ear infections (external otitis, malignant external otitis)
- Skin and soft tissue infections, including hot tub folliculitis, and osteomyelitis
- Burn sepsis

Individuals with compromising conditions, such as HIV/AIDS, cystic fibrosis, chemotherapy-related neutropenia, and diabetes have an increased risk of acquiring an infection and developing complications (*Trautmann et al.*, 2008).

2.1.3.5 Antimicrobial Susceptibility

*Pseudomonas aeruginosa* is frequently resistant to many commonly used antibiotics. Although many strains are susceptible to gentamicin, tobramycin, colistin, and amikacin, resistant forms have developed, making susceptibility testing essential (*Baron, 1996*).

2.1.4 Klebsiella pneumonia

*Klebsiella pneumoniae* is one of the most important pathogenic bacteria. It is gram negative, bacilli, nonmotile and causative agent of many diseases, such as pneumonia, urinary tract infections, bacteremia, burns and wounds infections and pyogenic liver abscesses (*Rahamathulla et al.*, 2016).

2.1.4.1 Classification

- **Domain**: Bacteria
- **Phylum**: Proteobacteria
- **Class**: Gammaproteobacteria
- **Order**: Enterobacteriales
- **Family**: Enterobacteriaceae
- **Genus**: *Klebsiella*
- **Species**: *pneumonia* (*Rahamathulla et al.*, 2016).
2.1.4.2 Description and significance

*Klebsiella pneumoniae* is a gram negative bacterium. It is facultative anaerobic. It is rod-shaped and measures 2 µm by 0.5 µm. In 1882, Friedlander C. Uber first discovered *Klebsiella* to be a pathogen that caused pneumonia (8). Many hospital cases around the world have been linked to *K. pneumoniae*. Therefore, more studies of the strains were important and performed. The bacterium was isolated and sequenced from a patient in 2004. *K. pneumoniae* is commonly found in the gastrointestinal tract and hands of hospital personnel (Podschu and Ullmann, 1998). The reason for its pathogenicity is the thick capsule layer surrounding the bacterium. It is 160 nm thick of fine fibers that protrudes out from the outer membrane at right angles (Lawlor et al., 2005; Amako et al., 1988). Another site on the human body that this bacteria can be found is the nasopharynx. Its habitat is not limited to humans but is ubiquitous to the ecological environment. This includes surface water, sewage, and soil (Brisse and Verhoef, 2001).

2.1.4.3 Ecology

*Klebsiella pneumoniae* is ubiquitous as it is found in mammals and ecological environment. It has pathogenic effects worldwide. There is evidence of community-acquired and hospital-acquired infections in countries such as Taiwan and South Africa.

Community-acquired *K. pneumoniae* has been found, in some places, to be associated with alcoholism. There are a large number of infections acquired when it affects different organs of the body. It can affect the liver, urinary tract, lungs, to name a few (Wen-Chien et al., 2002).

2.1.4.4 Pathology

*Klebsielle pneumoniae* is an important cause of human infections (Also see Description and significance). Infections or diseases are usually nosocomial or hospital-acquired.

In 1998, *K. pneumoniae* and *K. oxytoca* accounted for 8% of nosocomial bacterial infections in the United States and in Europe. Diseases include urinary tract infections, pneumonia, septicemias, and soft tissue infections (Podschu and Ullmann, 1998). The diseases caused by *K. pneumoniae* can result in death for patients who are immunodeficient. Differences in the diseases are determined by the different virulence factors. For example, mucoid phenotype varies as the strains for mucoid
vary (Victor et al., 2007). CPS and LPS O side chain are two of the most important virulence factors of K. pneumonia (Cortés et al., 2002). They serve to protect the bacterium from phagocytosis by the host. Treatment is done by antibiotics such as clinafloxacin (Sylvain et al., 1999). But, there are an increasing amount of antibiotic-resistance strains. Ciprofloxacin is an antibiotic that is becoming less effective (Brisse et al., 2000).

In California sea lions (Zalophus californianus) an isolate of the phenotypic characteristic hypermucoviscosity (HMV) of the bacteria Klebsiella pneumoniae has been found in a total of 25 cases. The HMV phenotype of K. pneumoniae was isolated from cases in which the sea lions had suppurative pneumonia and pleuritis; as well it was isolated from sea lions with abscesses. This is the first incidence of a pathogen that could be transmitted from marine animals to humans. Therefore, it is of great importance that marine mammals should be screened for pathogenic bacteria that could cause health problems in humans. Furthermore, K. pneumoniae HMV is starting to become more prevalent in marine coastal mammals as the primary pathogen. As a result, further studies of K. pneumoniae HMV are required to further improve and determine the extent of our understanding of this pathogen and its effects on epidemiology (Jang et al., 2010).

Klebsiella pneumoniae is a very common pathogen that is encountered by many health care providers. Other than being a hospital-acquired pathogen that causes several infections such as urinary tract, nosocomial pneumonia and intra abdominal infections, Pneumoniae has been identified as a community-acquired infection with fluctuating prevalence. Strong correlation has been established between the demographic and geographic distribution among world populations and the incidents of community-acquired infections caused by K. pneumoniae. K. pneumoniae has been considered a respiratory pathogen that causes Pneumoniae, the symptoms include: toxic presentation with sudden onset, high fever, and hemoptysis. Diagnosis through chest radiograph looks for abnormalities such as bulging interlobar fissure and cavitary abscesses. Over the years the contribution of K. pneumoniae to the total community-acquired cases of Pneumoniae has severely declined, while its contribution to other disease states increased. Community Acquired K. pneumoniae has been responsible for increased number of bacteremic liver abscess cases, especially in central and far Asia. Patients with Klebsiella liver abscess also showed higher rates of occurrence for the following complications: pulmonary emboli or
abscess, brain abscess, pyogenic meningitis, endophthalmitis, prostatic abscess, osteomyelitis, septic arthritis, or psoas abscess. It is important to note that the rates of infections caused by community-acquired *K. pneumoniae* vary among world populations. For example: the rate of meningitis caused by *K. pneumonia* increased more than two folds in some hospitals in central Asia, while the same infection caused by *K. pneumoniae* only accounted for less than 2% in a hospital in the United States (Wen-Chien *et al.*, 2002).

### 2.2 Botanical extract

#### 2.2.1 *Solenostemma argel* (Del.) Hayne

*Solenostemma argel* is a plant or plant part valued for its medicinal, aromatic or savory qualities. Herb plants produce and contain a variety of chemical substances (Shayoub, 2003). Herbs had been used all cultures throughout history. The primitive man observed and appreciated the great diversity of plants available to him. The plants provided food, clothing, shelter, and medicine (Shayoub, 2003). Certainly herbs used in some infections, cough, cold, stomach upset, indigestion, catarrh, constipation and so on (WHO, 2002; Mcntyres 2003). Herbal medicine has provided the world's population with safe, effective and low cost natural substances (medicine) for centuries (Shayoub, 2003).

The plant Hargel is a member of the family Asclepiadaceae that comprises numerous medicinal plants, like *Calotropis procera*, *Marsdenia obyssinica* and *Huernia mecrocarpa*, known for their cardiac activity. Hargel grows naturally in the northern parts of the Sudan and extends from Berber to Abu-Hamad, especially the Rubatab area. It is also widely distributed throughout North Africa (Egypt, Libya and Algeria and Saudi Arabia) (Ahmed, 2004). Hargel is used in the folk medicine as an effective fought remedy; infusion of leaves for gastrointestinal, cramps, as laxative (Filipescu *et al.*, 1985); stomachache; anticolic, antisyphilitic if used for prolonged period of 40 to 80 days (Boulos, 1983) and as anti inflammatory (Jobeen *et al.*, 1984). Hargel has antimicrobial effect to some bacteria and fungi. (Abd Elhad *et al.*, 1994a, b) and has antiviral activity to new castle disease virus. Hargel leaves are used in indigenous medicine for the treatment of some diseases such as the disease of liver and kidney. It is an effective remedy for bronchitis and is used to treat neuralgia. It is used as incense in the treatment of measles and sometimes crushed and used as remedy for healing wounds. The leaves are infused to treat gastro-intestinal cramps.
and stomach colic (Abd Elhady et al., 1994a). Sulieman et al. (2009) reported that Hargel aqueous extracts have antimicrobial activity against two fungi (Aspergillus niger and Penicillium italicum) and two Gram negative bacteria (Esherichia coli and Salmonella typhi).

2.2.1 Classification

Kingdom : Plantae
(unranked): Angiosperms
(unranked): Eudicots
(unranked): Asterids
Order : Gentianales
Family : Gentianales
Subfamily : Asclepiadoideae
Genus : Solenostemma
Species : argel (Shayoub, 2003).

Figure (1): Solenostemma argel (Salih, 2013)
Common English Name: arghel.

2.2.1.2 Morphological description

A perennial, 60 cm high, with several vigorous stems. The leaves are, oval, leathery and covered with fine hairs. The numerous flowers have white petals, and a strong smell. Their inflorescences are giving the plant attractive look. The fruits are thick, 5 cm. long and 1.5-2 cm wide, green with violet lines; they contain pubescent seeds. The plant has a long flowering period from March to June (El-kamali, 2001).

2.2.1.3 Regional

The genus Solenostemma is a member of the family Asclepiadaceae which is a perennial shrub widely distributed in the deserts of Arabia, Egypt, Libya, Chad, Sudan and Palestine, where it is known as “Argal” or “El Arghel” (Hegazi et al., 1994), the herb of the plant locally called “El Hargel” (El- Tahir et al., 1987).
2.2.1.4 Ecology
The plant grows in extremely dry conditions with a yearly rainfall of around 50-100 mm. It grows on the gravelly soils and on the stony and pebbly soils (El-kamali, 2001).

2.2.1.5 Part used
The leaves and stems which are collected in the spring and prepared as an infusion, a decoction or a powder. This can be taken by mouth or used externally Hegazi et al., (1994).

2.2.1.6 Constituent
Acylated phenolic glycosides, namely argelin and argelosid, choline, flavonoids, monoterpenes and pregane glucoside, sitosterol and a triterpenoid saponin (El-kamali, 2001).

2.2.1.7 Pharmacological action
Anti-inflammatory activity and antimicrobial activity. It is used for colds, diabetes, respiratory troubles, rheumatism, stomach pain, urinary infection. The bitter sap from the stem is used for cold (El-kamali, 2001).

2.2.1.8 Chemical composition
Leaf of Hargel is characterized by high carbohydrates (64.8%) and low crude fiber (6.5%). In addition the leaf contained 15% protein, 1.6% crude oil, 7.7% ash, and 4.4% moisture content. The results revealed that the leaf contained high potassium (0.54%), calcium (0.06%), magnesium (0.03%) and sodium (0.01%), but it is characterized by low copper (0.0001%), ferrous (0.002%), manganese (0.002%) and lead (0.001%). The protein fractionation of leaf is characterized by high albumins (16.7%), non-nitrogenous protein (15.3%), prolamine (11.7) and low globulins (8.7%), and glutelin (6.2%). Leaf contained phytic acid (3.2 g/100g) and tannin content (0.4%) as reported by Sabah El-Kheir and Murwa, (2010).

2.2.1.9 Folkloric use
used traditionally for colic, digestion disorders, diabetes, nephritis and measles (Elghazali et al., 1998). Also used as diuretic (Saad et al., 1988), and as spasmylytic, pain killer, colic’s accompanying dysmenorrhea (Eltahir et al., 1987). reported that the alcoholic extract of stems has got antifungal activity, antispasmodic effect in the leaves aqueous extract (uterine muscle relaxant) for the presence of flavonoids. The decoction of leaves and stems is used in Sudan, Egypt, Libya, Sudia Arabia and
Algeria for coughs, influenza, intestinal disorders and stomach aches. Leaves have diuretic properties, expectorant, antipyretic, digestant, for urinary infections, syphilis. Fine powdered leaves are used for treatment of infected wounds, fumigation of leaves for measles, in Libya and Chad the boiling leaves for neuralgia and sciatica (Elkamali, 1997).

2.2.2 Gum Arabic

Gum Arabic (GA) is edible, dried, gummy exudates from the stems and branches of Acacia senegal and Acacia seyal that is rich in non-viscous soluble fiber (Azeez, 2005; Badreldin et al., 2008; Abdul-Hadi et al., 2010). These trees are abundant in the central Sudan, central Africa and in West Africa. Gum Arabic is commonly used in the pharmaceutical and food industries as emulsifier and stabilizer as suspending agent for insoluble drugs (Abdul-Hadi et al., 2010; Lelon et al., 2010).

2.2.2.1 Classification:

Kingdom: Plantae – Plants  
Class: Magnoliopsida – Dicotyledons  
Subclass: Rosidae  
Order: Fabales  
Family: Fabaceae/Leguminosae  
Genus: Acacia  
Species: senegal (Azeez, 2005).

Figure (2): Senegalia (Acacia) senegal gum  
Franz Eugen Köhler, Köhler’s Medizinal-Pflanzen (1887)

2.2.2.2 History and Origins of Gum Arabic

Gum Arabic is certainly the most ancient and the most well known of all gum types. The term ‘gum Arabic’ was coined by European merchants who imported it from Arab ports such as Jeddah and Alexandria. Egyptians referred to it as ‘kami’ and
allegedly used it from the third dynasty onwards (around 2650 BC) to secure bandages around mummies (ITC, 2008).

This gum was supposedly also used to fix pigments into hieroglyphic paintings. According to a Sudanese researcher, the word ‘mana’ (manna) mentioned in the Koran (Surah Al baqarah) as the best food available to man is, in fact, a direct reference to gum Arabic. The word ‘mana’ seemingly also refers to gum Arabic in the Torah where it is described as an essential food and designated by Moses to the Israelis as God-given bread. There are close to 900 acacia species capable of producing gum. These are primarily located in tropical climates, with about 130 of them located specifically on the African continent (ITC, 2008).

Africa, therefore, quickly became the major site of the production of gum; this is the reason why it is also referred to as ‘senegal gum’. Gum is essentially the secretion of several acacia (leguminous) trees. Acacia gum species, of which there are up to seventeen, produce acacia gum of varying quality and quantity. Interestingly, close to 80% of gum Arabic is produced by the A. senegal (in Sudan). The remainder is produced either by the Acacia laeta or the A. seyal, with each species contributing 10% of the total supply of gum. The gum produced by the A. senegal is commonly referred to as “hard gum” and the gum from A. seyal, as “flaky gum” (Forestry Department, 1995). Gums obtained from other acacia species and occasionally from Albizia and Combretum, are also traded as “gum Arabic”. Current regulation surrounding gum Arabic does not distinguish between gum obtained from Acacia senegal and A. seyal (ITC, 2008).

2.2.2.3 For Sudanese Exports, the Distinction is Quite Clear

Gum from A. senegal is sold under the name “hashab gum” while gum from A. seyal is sold as “talhagum”. In Zimbabwe, the gum traded locally as gum Arabic comes from Acacia karoo. However, synthetic substitutes, namely “modified starches” such as xanthan and gellane, are rapidly replacing gum Arabic as dietary hydrocolloids. Gum Arabic is traditionally defined as a ‘substance, which exudes from A. Senegal or related species’. This definition encompasses a variety of species, which, from a taxonomy point of view, are not related. To date, though, only the gum from A. Senegal has been effectively demonstrated as an innocuous food additive (ITC, 2008).
2.2.2.4 Structure of Gum Arabic

Gum Arabic, a natural composite polysaccharide derived from exudates of *A. senegal* and *A. seyal* trees, is one of the most commonly used food hydrocolloids. *Gum Arabic* serves as a very efficient emulsifier and a long-term stabilizer in food and cosmetic products containing oil-water interfaces. Gum Arabic is recognized by many researchers that GA consists of mainly three fractions (Yael et al., 2006).

- The major fraction is a highly branched polysaccharide consisting of galactose backbone with linked branches of arabinose and rhamnose, which terminate in glucuronic acid found in nature as magnesium, potassium and calcium salt.
- A smaller fraction is a higher molecular weight arabinogalactan-protein complex (AGPs) in which arabinogalactan chains are covalently linked to a protein chain through serine and hydroxyl proline groups. The attached arabinogalactan in the complex contains glucuronic acid.
- The smallest fraction having the highest protein content is a glycoprotein which differs in its amino acids composition.

2.2.2.5 Physical Properties of Gum Arabic

The physical properties of gum Arabic, established as quality parameters include moisture, total ash, volatile matter and internal energy. Gum Arabic is a natural product complex mixture of hydrophilic carbohydrate and hydrophobic protein components emulsifier which adsorbs onto surface of oil droplets while hydrophilic carbohydrate component inhibits flocculation and coalescence of molecules through electrostatic and steric repulsions in food additives (Lelon et al., 2010). Moisture content facilitates the solubility of hydrophilic carbohydrates and hydrophobic proteins in gum Arabic. Total ash content is used to determine the critical levels of foreign matter, acid insoluble matter, salts of calcium, potassium and magnesium. The cationic compositions of ash content are used to determine the specific levels of heavy metals in Gum Arabic (Lelon et al., 2010).

2.2.2.6 Characteristics of Gum Arabic

Gum Arabic is a solid of a pale to orange brown colour which, when ruptured, secretes a vitreous substance. Gum Arabic of excellent quality is tear shaped, round, with an orange-brown colour. After it is crushed or shattered, the pieces are paler in colour and have a vitreous appearance. Contrary to other vegetable gums, gum Arabic
dissolves very well in water (up to 50%). The viscosity of A. Senegal is weak (16 ml/g on average).
The resulting solution is colourless, tasteless and does not interact easily with other chemical compounds (ITC, 2008). Chemically, gum Arabic is a slightly acidic complex compound, made up of glycoprotein and polysaccharides and their calcium, magnesium and potassium salts. The principal polysaccharide is Arabic acid, a polysaccharide linking a D-galactose with branches composed of L arabinose, L-rhamnose and D-glucuronic acids. Essentially, the proteins are classified as arabino galactanes, rich in hydroxproline (ITC, 2008).

2.2.2.7 Applications of Gum Arabic

Exudates gums are used in an overwhelming number of applications, mainly situated in the food area. However, there are also considerable non-food applications. Gum Arabic is being widely used for industrial purposes such as a stabilizer, a thickener, an emulsifier and an encapsulating in the food industry and to a lesser extent in textiles, ceramics, lithography, cosmetic and pharmaceutical industry. In the food industry, GA is primarily used in confectionery, bakery, dairy, beverage and as a micro encapsulating agent (Mariana et al., 2012). Gum Arabic readily dissolves in cold and hot water in concentrations up to 50%. Because of the compact, branched structure and therefore small hydrodynamic volume, gum Arabic solutions are characterized by a low viscosity, allowing the use of high gum concentrations in various applications. Solutions exhibit Newtonian behavior at concentrations up to 40% and become pseudo plastic at higher concentrations (Verbeken et al., 2003).

2.2.2.8 Food Applications

Gum Arabic is mainly used in the confectionery industry, where it is incorporated in a wide range of products. It has a long tradition of use in wine gums, where it produces a clarity that is higher than can be obtained with other hydrocolloids. Furthermore, it prevents sucrose crystallization, provides a controlled flavor release and slows down melting in the mouth, making the wine gum long lasting. It also provides the appropriate texture to the secandies, which are easily deformed in the mouth but do not adhere to the teeth (Arja et al., 2011). In lower-calorie candy, gum Arabic is used to compensate for the loss of texture, mouth feel and body, resulting from the replacement of sugars by artificial sweeteners. It is also used in chewing gum as a coating agent and as a pigment stabilizer. In aerated confectionery products,
such as marshmallows, nougats and meringues, gum Arabic acts as a whipping and stabilizing agent. It is also used in toffees and caramels as an emulsifier, to maintain a uniform distribution of the fat across the product. In jelly products, it is used to provide a fibrous, fruit-like texture (Tadesse et al., 2007).

2.2.2.9 Non-Food Applications

Gum Arabic was once extensively used in the pharmaceutical industry, but is now replaced by cellulosics and modified starches in many applications. It is still used as a suspending agent, emulsifier, adhesive and binder in tabletting and in demulcent syrups.

In cosmetics, gum Arabic functions as a stabilizer in lotions and protective creams, where it increases viscosity, imparts spreading properties and provides a protective coating and a smooth feel. It is used as an adhesive agent in blusher and as a foam stabilizer in liquid soaps (Arja et al., 2011).

Gum Arabic has several domestic uses namely in manufacturing ink, making adhesives, crafts making, in cosmetic products, in confectionary and in foodstuff. It is also utilized locally in special meals and as chewing gum. Focusing specifically on human consumption, gum Arabic is an excellent dietary product because it contains less than 1cal for every gram. The Hottentots in southern Africa can survive for several days on nothing but gums, while Moorish populations in northern Africa sustain a daily portion of 170g of gum (Arja et al., 2011). Ever since the pharaonic era, gum has been utilized in traditional medicine as a calming and softening agent. It is equally included in (traditional) medicine concoctions to address internal ailments such as cough, diarrhea, dysentery and hemorrhage and applied externally. It is also used in veterinary medicine, to treat skin diseases and inflammations for example. The Ebers manuscript (a medical document on papyrus written around 1550 BC) describes acacia gum or GA as a means of contraception when utilized with dates. Nevertheless, gum Arabic is most famous and prized by producing and exporting countries for its industrial uses, among others, in the nutrition, pharmaceutical and cosmetic branches (Verbeken et al., 2003).

2.2.3 Guiera senegalensis

*Guiera senegalensis* commonly known as Sabara in Hausa is a shrub of the savannah region of west and central Africa. *G.senegalensis* is widely being used in traditional medicine for the remedy of many ailments/diseases. Its leaves extract is
being used against dysentery, diarrhea, gastrointestinal pain and disorder, rheumatism and fever (Sule and Mohammed, 2006). In addition, partially purified anthocyanin fraction from leaf extract of *G. senegalensis* has been shown to possess antioxidant property against ccl₄– induced oxidative stress in rats (Sule and Mohammed, 2009). *G. senegalensis* and *Piliostigma reticulatum* commonly coexists with crops in the farmer’s field throughout the Sahel and their presence can potentially provide more organic inputs to cropped fields than any other source. Traditional management of these shrubs includes coppicing and burning of residues at the beginning of each cropping season, however, non-thermal management of these organic materials hold potential to add organic matter to soils and thus, be a source of nutrients such as nitrogen and phosphorus (Dossa *et al.*, 2009).

### 2.2.3.1 Scientific classification

**Kingdom:** Plantae  
**Phylum:** Magnoliophyta  
**Class:** Magnoliopsida  
**Order:** Myrtales  
**Family:** Combretaceae  
**Genus:** Guiera  
**Species:** senegalensis  
**Vernacular name:** Ghubeish (Arab.) (Elghazali, 1997).
2.2.3.2 Botanical description
Grey to mentose shrubs up to 2 m high, leaves opposite or sub opposite, elliptic-oblong, 6-12 x 0.5-2.8 cm, apex mucronate base slightly cordate to attenuate; margin entire (Elghazali et al., 1987).

2.2.3.2 Habitat
Lowland sandy plains, degraded savanna.

2.2.3.3 Distribution
Rashad, Wad ashana and central Sudan.

2.2.3.4 Chemical constituents
Flavonoids, saponins, alkaloids, mucilages and tannins (Elghazali, 1997).

2.2.3.5 Folkloric use
Leaves used as anti diabetic, antipyretic, anti-vomiting and anti leprosy (Elghazali et al., 1987). Also leaves in African traditional medicine for gastrointestinal disorders, coughs and topically for wound healing (Bosisio et al., 1997). Plant is used for Malaria in Mali and Sao-tome traditional medicine (Ancolio et al., 2002).

2.2.4 Cymbopogon proximus
The *Cymbopogon proximus* is a herbal plant. Common name is camel’s hay and is known locally as (Mahraib). It is a perennial herb, erect, tufted 9 cm long, culms slender, glabrous and 3 – 4 nodes. Leaf simple, alternate, linear 5-7 cm long, 1 cm wide, sheathed apex spiny entire, and inflorescence spikelets highly branched 5 cm log (Eltahir and Ereish, 2010). The plant is widely distributed in Africa (northwest tropical, northeast tropical and east tropical), temperate Asia (western Asia and Arabia) and tropical Asia (Indian and Indo-China). In addition, *Cymbopogon proximus* is found in northern and Central Sudan (Clayton et al., 2005).

2.2.4.1 Classification
Kingdom: Plantae.
Phylum: Magnoliophyta.
Class: Liliopsida.
Order: Poales.
Family: Poaceae.
Genus: Cymbopogon.
Species: Cymbopogon schoenanthus (L.) Spreng. (Global Biodiversity Information Facility, 2011).

2.2.4.2 Chemical composition:

Table (1): Proximate analysis of *Cymbopogon proximus*

<table>
<thead>
<tr>
<th>Moisture</th>
<th>Ash</th>
<th>Crude fiber</th>
<th>Crude protein</th>
<th>Crude lipid</th>
<th>Total carbohydrates</th>
</tr>
</thead>
<tbody>
<tr>
<td>11.5</td>
<td>6.5</td>
<td>32.0</td>
<td>11.0</td>
<td>8.5</td>
<td>42.0</td>
</tr>
</tbody>
</table>

(Faten and El-Khateeb, 2013).

Table (2): Preliminary phytochemical tests of aqueous extract of *Cymbopogon proximus*

<table>
<thead>
<tr>
<th>Terpenes</th>
<th>Tannins</th>
<th>Flavonoids</th>
<th>Saponins</th>
<th>Alkaloids</th>
<th>Carbohydrate or glycoside</th>
<th>Phenolic glycoside</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>+</td>
<td>+</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

(+++): High concentration; (++): moderate concentration; (+): low concentration (Faten and El-Khateeb, 2013).

Terpenes are naturally occurring substances produced by a wide variety of plants and animals. A broad range of the biological properties of terpenoids is described, including cancer chemopreventive effects, antimicrobial, antifungal, antiviral, anti hyperglycemic, anti-inflammatory, and antiparasitic activities. Terpenes are also presented as skin penetration enhancers and agents involved in the prevention and therapy of several inflammatory diseases. Moreover, a potential mechanism of their action against pathogens and their influence on skin permeability are discussed. The major conclusion is that larger-scale use of terpenoids in modern medicine should be taken into consideration (Paduch et al., 2007).

Tannins are polyphenolic compounds that are broadly categorized into two major groups: (1) hydrolyzable tannins, consisting of a central core of carbohydrate to which phenolic carboxylic acids are bound by ester linkage and (2) condensed tannins, or proanthocyanidins, consisting of oligomers of two or more flavan-3-ols, such as catechin, epicatechin, or the corresponding gallocatechin. Tannins have a very high affinity for proteins and form protein-tannin complexes. The ingestion of a plant containing condensed tannins decreases nutrient utilization, protein being affected to a great extent, and decreases feed intake. On the other hand, hydrolyzable tannins are
potentially toxic to animals. Consumption of feeds containing high levels of hydrolyzable tannins cause liver and kidney toxicity and lead to death of animals. Oak and yellow wood poisonings are attributed to hydrolysable tannins (Makkar et al., 2007).

Saponins are steroid or triterpenoid glycosides, common in a large number of plants and plant products that are important in human and animal nutrition. Several biological effects have been ascribed to saponins. Extensive research has been carried out into the membrane permeabilising, immune stimulant, hypocholesterolaemic and anti carcinogenic properties of saponins and they have also been found to significantly affect growth, feed intake and reproduction in animals. These structurally diverse compounds have also been observed to kill protozoans and molluscs, to be anti oxidants, to impair the digestion of protein and the uptake of vitamins and minerals in the gut, to cause hypoglycaemia, and to act as antifungal and anti viral agents. These compounds can thus affect animals in a host of different ways both positive and negative (Francis et al., 2002).

Flavonoids are plant pigments that are synthesized from phenylalanine, generally display marvelous colors known from flower petals, mostly emit brilliant fluorescence when they are excited by UV light, and are ubiquitous to green plant cells. The flavonoids are used by botanists for taxonomical classification. They regulate plant growth by inhibition of the exocytosis of the auxin indolyl acetic acid, as well as by induction of gene expression, and they influence other biological cells in numerous ways. Flavonoids kill many bacterial strains, inhibit important viral enzymes, such as reverse transcriptase and protease, and destroy some pathogenic protozoans. Yet, their toxicity to animal cells is low. Flavonoids are major functional components of many herbal and insect preparations for medical use, which have been used since ancient times. The daily intake of flavonoids with normal food, especially fruit and vegetables is 1–2 g. Modern authorized physicians are increasing their use of pure flavonoids to treat many important common diseases, due to their proven ability to inhibit specific enzymes, to simulate some hormones and neurotransmitters, and to scavenge free radicals (Havsteen, 2002).

Alkaloids many substances which interfere with the inflammatory response have been isolated from plants. Some alkaloids of vegetal origin which in the period of 1907 to 2000 were evaluated regarding a possible anti inflammatory activity.
The alkaloids were classified in sub groups in accordance with their chemical structures and the pharmacological data were obtained from different experimental models. Of the 171 evaluated alkaloids, 137 presented anti-inflammatory activity, and among those, the iso quinoline type was the most studied. The carrageen in-induced paw edema was the most used model for evaluating the anti-inflammatory activity (Filho et al., 2006).

A glycoside consists of two components, an aglycone (non-sugar) part and a sugar part. The aglycone portion may be of several different types of secondary metabolites, including coumarin, flavonoids or hydroxylanthracene. The sugar moiety is linked to the aglycone by a direct carbon to carbon bond (C-glycoside), or through oxygen to carbon bond (O-glycoside). Cyanide glycosides, release toxic hydrogen cyanide when cells are damaged and act as a defence mechanism. Glucosinolates contain nitrogen and sulfur and are pungent (Veitch et al., 2015).

### 2.2.4.3 Medical uses

According to phytochemical tests of *Cymbopogon proximus* the presence of saponins, flavonoids, glycosides and tannins may be a rationale for the use of the plant in medicine preparations. Flavonoids are known to protect against allergies, diabetes, inflammations, malaria, platelet aggregations and microbial infection (Okwu and Omodiromiro, 2005).

The indigenous *C. proximus*, grows as a wild aromatic plant in Sudan, and used locally as carminatives, simulative, antiseptics, and for treatment of rheumatism, cholera (Warrag et al., 2014).

*C. proximus* (*schoenanthus*) is used in Egypt as a renal antispasmodic agent (Elgamal and Wolff, 1987). *C. citrates* are used as traditional folk medicine in the treatment of nervous, gastrointestinal disturbances fevers and hypertension (Borrelli and Izzo, 2000).

The Cymbopogon proximus treated lower levels of serum calcium, serum blood urea nitrogen (BUN) and kidney calcium. The Cymbopogonproximus has a significant protective effect against ethylene glycol-induced nephrolithiasis in rats (Warrag et al., 2014).
2.2.4.4 Traditional uses

*Cymbopogon proximus* (Family Poaceae) is a traditional medicinal Sudanese plant commonly known as “Mahareb”, which is used in folkmedicine (*Eltahir and Ereish, 2010*).

It is extensively used as folk medicine to promote diuresis, to alleviate colic pain and as antipyretic plant against fever (*Khalid et al., 2012*). In the Egyptian folk medicine, it is famous as an effective diuretic and renal antispasmodic (*Eltahir and Abdel Kader, 2008; El-Askary et al., 2003; Selim, 2011*). A decoction of the entire dried herb has been used for centuries by certain tribes in South Egypt as a diuretic, colic pain killer, aid for removal of small stones from the urinary tract, and antipyretic. The plant has been found to possess antispasmodic, antioxidant (*Selim, 2011*). Antibacterial affects (*El-Kamali, 2010; Selim, 2011*). Hypotensive, antiemetic, anticonvulsant properties (*Eltahir and AbdelKader, 2008*). Hypoglycemic properties (*Sheweita et al., 2002*). Fungicidal properties (*Fawzi et al., 2009; El-Assiuty et al., 2006*). Ovicidal and larvicidal properties (*Bassole et al., 2003*).

It was also used to treat constipation, intestinal complaints, carminative, stomachic and as an appetizer (*EL-Kamali and EL-Amir, 2010*). Treatment of drinking water with herbal plants as traditional medicine is worldwide. In particular, the *Cymbopogon proximus* (CP) is widely used in Sudan in a purpose of folk medicine. The addition of *Cymbopogon proximus* (Mahraib) at a rate of 5g CP/1.5 litre water (3.3L-1) to groundwater removes NO3-in 48 h and at the same time increases total dissolved solids (TDS) and fluoride (F-) levels and consequently increases the risk of dental fluorosis incidence and other fluoride adverse effects (*Abdellah et al., 2012*).
CHAPTER THREE
MATERIAL AND METHODS

3.1 MATERIALS

3.1.1 bacteria

3.1.1.1 *Escherichia coli*
3.1.1.2 *Pseudomonas sp*
3.1.1.3 *Staphylococcus aureus*
3.1.1.5 *Klebsiella sp*

The four bacterial isolates were obtained from the Microbiological Laboratory of the Department of Pathology Medical lab, Faculty of Medicine, University of Gezira, Wad Medani, Sudan during the period from March, 2015 to May, 2015.

3.1.2 plants

3.1.2.1 *Solenostemma argel*
3.1.2.2 *Acacia Senegal*
3.1.2.3 *Guiera senegalensis*
3.1.2.4 *Cymbopogon proximus*

All Samples plants were obtained from Wad Medani local market and identified.

3.1.3 Equipment

13.3.1 Laboratory incubator Model: 6307 OGAWA SEIKLO.

13.3.2 An autoclave Model: LSB50L.

13.3.3 laminar flow cabinet.

13.3.4 Hot air oven OSK type 6248.

13.3.5 Other equipment including the following: Petri-dishes, sterile tube, flasks, filter papers, Wattman glass fiber, Aluminium foil, Refrigerator, Water bath, hole punch, forceps, permanent marker, disinfectants (Clorox, detergent (soap) and ethanol), gloves, masks, cotton, mortar, respiratory funnel, beakers and for weighting (Sensitive analytical balance).
3.2 METHODS

3.2.1 Preparation of Nutrient agar

This was a general-purpose cultured medium for bacteria. It was obtained in a dehydrated form. The constituent of the medium were beef extract, yeast extract, peptone, sodium chloride and agar. It was prepared according to the manufacturers instruction by suspending 28g in one liter distilled water. The medium was allowed to boil until it was completely dissolved. The pH of medium was adjusted to pH 7.4±0.2 and then the medium was sterilized in an autoclave at 121°C (115b\in²) for 15 min (Harrigan, 1998).

3.2.2 Preparation of Crude Extracts

All samples (Hargel, Mahreb, Arabic Gum, and Gebbeish) were taken from retailer’s stores. The leaves were freed from foreign materials like stones, sand and dust, before kept in the lab. The leaves were then washed with water, dried, and milled using laboratory mill into fine powder (3 g, 4g, 5g) each of the sample have been taken in a test tube with (7 ml, 6 ml, 5 ml) of 98% Ethanol and distilled water. Then each of the individual test tubes has been kept for 48 hour at room temperature. After 48 hour the extract have been filtered through filter paper and tested. Then different concentrations (30ml, 40ml, 50ml) were prepared in test tubes for experiments. we also used pure ethanol as control.

3.2.3 Preparation of test organisms

The nutrient agar were mixed well and poured on the sterile Petri-dishes. The agar media on Petri-dishes were allowed to set for few minutes. Nutrient agar plates were inoculated with respective bacteria (E. coli, S.aureus, Pseudomonas sp, and Klebsiella sp), and then incubated at 37°C for overnight. Each time, a fresh bacterial culture was prepared.

3.2.4 Antibacterial activity

Antibacterial activity was measured using paper disc diffusion method, (Saba et al., 2011)

The following steps were involved in paper disc diffusion (Inhibition zone) method. The agar were mixed well and poured on the sterile Petri-dishes. The agar media on Petri-dishes were allowed to set harden for few minutes. Agar plates were inoculated with respective bacteria. The small autoclaved discs of wattman filter paper were used.
The test organism was spread on the Petri-dishes by using sterilized glass spreader. During paper-disc diffusion method, the sterile discs were dipped in the different crude extracts of medicinal plants with the help of sterilized forceps and placed on the Petri plates. Distilled water was used as a control to check the comparison of antibacterial activity with different crude extracts of medicinal plants. The Petri-dishes were sealed with para film. Then, the Petri-dishes were left at room temperature for 30 minute, to allow the diffusion of the test sample and then incubated at 37° C for overnight. The diameter of the zones of inhibition were measured in cm.

3.3 Statistical analysis

The obtained data was statistically analyzed by computer software MSTATC according to analysis of variance (ANOVA); Duncan's Multiple Range Test was used for mean separation.
CHAPTER FOUR

RESULTS AND DISCUSSION

RESULTS

4.1 Study sample

The resuspension aqueous and ethanol extract of Arabian gum, Mahreb, Gebbeish and Harjal (used as combination) with other plants were investigated for their antimicrobial activity against *E.coli, Klebsiella sp, S.aureus* and *Pseudomonas sp*. The disc diffusion (Inhibition zone) method was used in testing various concentrations of this extract.

4.2 Effect of different concentration of aqueous extracts of Arabian gum, Mahreb Gebbeish and Harjal used as combination on the test microorganisms

The results (Table 1 and Figure1,2,3,4) shows that almost all plants aqueous extracts, have significant inhibitory effects on the tested bacterial growth. In single phase Arabian gum aqueous extract tested against *E.coli, Klebsiella sp, Staphylococcus* and *Pseudomonas sp* with the high concentrations of (50%) had the highest inhibitory effects (12.3,10.7,13 and 12.3 mm), respectively. Followed by the concentration (40%) which gave an inhibition zones of (10.3,10.3,11 and 11.7 mm), respectively and the concentration (30%) had the least inhibitory effects (8.7,10.7,10 and 10.3 mm), respectively. Mahreb aqueous followed Arabian gum where the highest concentrations of (50%) gave inhibitory effects of (5.7,8.3,6 and 8.3 mm), respectively. The other Mahreb concentration (40%) gave an inhibition zones of (8.7,8.7,6.3 and 8.3 mm), respectively and the concentration (30%) had an inhibitory effects of (7.7, 7.3, 6.7 and 7.3 mm), respectively. Gebbiesh aqueous extract had the lowest inhibitory effects, where the highest concentrations of (50%) gave inhibitory effects of (7.6,6.7 and 6 mm) respectively. The other gebbiesh concentration (40%) gave an inhibition zones of (6, 6.3,6.3 and 6.7 mm) respectively and the low concentration (30%) had an inhibitory effects of (6.3,6.7,7 and 6.3 mm) respectively.

It was noticed that, in single phases Arabian gum aqueous extract is the best one compared with the Mahreb and Gebbeish and gave an inhibition zones greater than others. On the other hand, in the combination phases (Arabian gum+Harjal) aqueous extracts tested against *E.coli, Klebsiella, Staphylococcus* and *Pseudomonas* were the
best one compared with (Mahreb+Harjal) and (Gebbeish+Harjal) aqueous extracts, where the high concentration (50%) used in this study had an inhibitory effects of (7, 9, 6.7 and 10.7 mm), respectively. However, this combined extract showed inhibition action of (9, 8.7, 10 and 9.7 mm) respectively in the concentration of (40%) used in this study. The other concentration of (30%) gave an inhibition zones of (6.3, 8.3, 8.3 and 11 mm), respectively. (Gebbeish+Harjal) aqueous extract followed (Arab gum+Harjal) aqueous extract, where the highest concentration (50%) had an inhibitory effects of (8.3, 10, 11 and 7.7 mm) respectively. However, the other concentration of (40%) showed inhibition action of (6.3, 7.7, 9.7 and 9.7 mm), respectively. The low concentration of (30%) gave an inhibition zones of (6.3, 8.3, 8.3 and 11 mm) respectively. (Mahreb+Harjal) aqueous extract was the least one compared with (Arab gum+Harjal) and (Gebbeish+Harjal) aqueous extracts, where the highest concentration (50%) had an inhibitory effects of (6.7, 9, 8.7 and 8.3 mm) respectively. The (40%) concentration showed inhibition action of (6.7, 8.7, and 8.7 mm) respectively. and the concentration (30%) gave an inhibition zones of (6.3, 8.3, 8.3 and 11 mm) respectively. Here, the results shows that, the Harjal combination phase with the other herbs was better than the single phase of the herbs, thus Harjal posses synergistic effect that activated the herbs significantly.

A NOVA analysis proved a significant difference ( p≤ 0.01 ) at the rows level (F calculated = 21.385, F-crit= 2.382823 ), and non-significant differences ( p > 0.05) at the columns level (F calculated = 0.699, F-crit= 2.01 ).
Table 1: Effect of different Concentration of aqueous extracts of Arabic gum, Mahreb and Gebbeish on the test microorganisms.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Test organisms</th>
<th>E.coli</th>
<th>Klebsiella</th>
<th>Staphylococcus</th>
<th>Pseudomonas</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Concentration%</td>
<td>30</td>
<td>40</td>
<td>50</td>
<td>30</td>
</tr>
<tr>
<td>Inhibition zones(mm)</td>
<td>arabic gum</td>
<td>8.7</td>
<td>10.3</td>
<td>12.3</td>
<td>10.7</td>
</tr>
<tr>
<td></td>
<td>mahreb</td>
<td>7.7</td>
<td>8.7</td>
<td>5.7</td>
<td>8.7</td>
</tr>
<tr>
<td></td>
<td>gebbeish</td>
<td>6.3</td>
<td>6</td>
<td>7</td>
<td>6.3</td>
</tr>
<tr>
<td></td>
<td>arabic gum + harjal</td>
<td>11</td>
<td>9</td>
<td>7</td>
<td>8.3</td>
</tr>
<tr>
<td></td>
<td>mahreb + harjal</td>
<td>6.3</td>
<td>6.7</td>
<td>6</td>
<td>8.3</td>
</tr>
<tr>
<td></td>
<td>gebbeish + harjal</td>
<td>9</td>
<td>6.3</td>
<td>8.3</td>
<td>8</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>8.1</td>
<td>7.8</td>
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<td>8.21</td>
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</table>

SUMMARY

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<tr>
<th>Source of Variation</th>
<th>Count</th>
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<th>Average</th>
<th>Variance</th>
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<td>131.3</td>
<td>10.94</td>
<td>1.433561</td>
</tr>
<tr>
<td>mahreb</td>
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<td>89.3</td>
<td>7.44</td>
<td>1.131742</td>
</tr>
<tr>
<td>gebbeish</td>
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Figure (1): Effect of different concentration of aqueous extracts of Arabic gum, Mahreb, Harjal and Gebbeish on the E. coli.

Figure (2): Effect of different concentration of aqueous extracts of Arabic gum, Mahreb, Harjal and Gebbeish on the Staphylococcus aureus.
Figure (3): Effect of different concentration of aqueous extracts of Arabic gum, Mahreb, Harjal and Gebbeish on the Klebsiella sp.

Figure (4): Effect of different concentration of aqueous extracts of Arabic gum, Mahreb, Harjal and Gebbeish on the Pseudomonas sp.
4.3 Effect of different Concentration of alcoholic extracts of Arabic gum, Mahreb, Gebbeish and Harjal on the test microorganisms.

The results (Table 2 and Figure 5,6,7,8) indicate that (Arab gum+Harjal), (Mahreb+Harjal) and (Gebbeish+Harjal) alcoholic extract tested against *E.coli*, *Klebsiella sp*, *Staphylococcus eureus* and *Pseudomonas* sp had the highest inhibitory effects compared with the aqueous extracts of (Arab gum, Mahreb, Gebbeish) singly and in the combination of (Arab gum+Harjal), (Mahreb+Harjal) and (Gebbeish+Harjal), specifically (Gebbeish+Harjal) alcoholic extract indicated the highest inhibitory effects on all the tested bacteria, where the highest concentration (50%) had an inhibitory effects of (14.3, 16, 15 and 6.3 mm) respectively. The other concentration of (40%) used in this study shows inhibition action of (15.3, 8.7, 15.3 and 6.3 mm) respectively. The low concentration of (30%) gave an inhibition zones of (14.3, 8.16 and 16.7 mm), respectively.

(Arab gum+Harjal) alcoholic extract in the highest concentration (50%) indicated an inhibitory effects of (6.7, 3.7 and 6 mm) respectively. The other concentration (40%) used in this study showed inhibition action of (9.3, 9.7, 7 and 6.7 mm) respectively. The low concentration (30%) gave an inhibition zones of (8.3, 7.6.7 and 6 mm) respectively. However, (Mahreb+Harjal) alcoholic extract in the highest concentration (50%) showed an inhibitory effects of (6.6, 3.6 and 6 mm) respectively. The other concentration (40%) used in this study indicated an inhibition action of (6.3, 6.6, 7 and 6.3 mm), respectively. The low concentration (30%) gave an inhibition zones of (6.8, 7.6.3 and 7 mm), respectively.

The results shows that, the alcoholic extracts of the herbs are better than aqueous extracts of the herbs singly and in combination with Harjal, and the inhibitory effect of the plant extracts in both solvents were much greater on *Pseudomonas* and *Staphylococcus eureus* compared with *Klebsiella* and *E.coli*.

It is worth to remember at this point that, the Ethanol which we used as control for comparison did not give us any result.

A NOVA analysis proved a significant difference (p≤0.01) at the rows level (Fcalculated= 21.33868, F-crit= 3.443357), and an non-significant differences (p > 0.05) at the columns level (Fcalculated = 0.891323, F-crit= 2.258518).
Table 2: Effect of different Concentration of alcoholic extracts of Arabic gum+Harjal , Mahreb+Harjal,Gebbeish+Harjal on the test microorganisms.

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<th>Klebsiella</th>
<th>staphylococcus</th>
<th>Pseudomonas</th>
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<td></td>
<td>30</td>
<td>40</td>
<td>50</td>
<td>30</td>
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<tr>
<td>Inhibition zones(mm)</td>
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<td>9.3</td>
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<tr>
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<td>15.3</td>
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SUMMARY

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ANOVA

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</table>
Figure (5): Effect of different concentration of alcoholic extracts of Arabic gum + Harjal, Mahreb+Harjal and Gebbeish + Harjal on the *E.coli*.

Figure (6): Effect of different concentration of alcoholic extracts of Arabic gum + Harjal, Mahreb+Harjal and Gebbeish + Harjal on the *Klebsiella sp.*
Figure (7): Effect of different concentration of alcoholic extracts of Arabic gum + Harjal, Mahreb+Harjal and Gebbeish + Harjal on the *Staphylococcus aureus*.

Figure (8): Effect of different concentration of alcoholic extracts of Arabic gum + Harjal, Mahreb+Harjal and Gebbeish + Harjal on the *Pseudomonas sp.*
**Discussion**

Medicinal plants are potential sources of novel antimicrobial compounds against pathogenic microorganisms, which are responsible for various human and animals’ infections. The use of medicinal plants still plays a vital role to develop the basic health needs in developing countries. Nearly 80% of the world population depends on the traditional medicine for primary health care, most of which involves the use of natural products (Sandhya et al., 2006). In Sudan many studies were carried out for testing the antimicrobial activity of some medicinal plants. Ahmed (2004) tested the extracts of 10 Sudanese medicinal plants against gram-positive and gram-negative bacteria as well as on Candida albicans. and was found a marked antibacterial effect against the gram-positive bacterium, *S. aureus*, followed by the *E. coli* and *Candida albicans*.

*E. coli, S. aureus, Klebsiella* and *Pseudomonas* are the most important pathogens in human nosocomial infection. *S. aureus* colonizes in the nasal cavity, nasopharynx, skin and mucous membranes of human and animals (Kluytmans et al., 1997; Wertheim et al., 2005), and causes a variety of significant economic disease in human and animals (Wisplinghoff et al., 2004).

Our present study was investigating the antibacterial activity of the most important and widely distributed plants in the Sudan, the *Acacia Senegal* (Gum Arabic), *Guiera senegalens* (Gebbeish), *Cymbopogon proximus* (Maharaib), and *Solenostemma argel* (Hargel) leaves in single phase and in combination of the other plants with Harjal. in aqueous and ethanolic phases. The paper disc diffusion (Inhibition zone) method was used for studying the effects of the plant extracts on *E. coli, Klebsiella, Pseudomonas,* and *Staphylococcus aureus*. The study showed antimicrobial activity of plant aqueous extract of Arab gum, Mahreb, Gebbeish in single phase and in combination phase with Harjal. and also in ethanolic extract of (Arab gum+Harjal), (Mahreb+Harjal), (Gebbeish+Harjal). The high concentration of the plant extracts (50%) and even at minimal concentration (30%) used in this study had an inhibitory effects, although the high concentration of (50%) gave higher inhibitory effects compared with the other concentrations (40% ) and (30%).

In earlier studies gum acacia extracts are reported to inhibit growth of 12 reference strains of *S. aureus, S. epidermidis, S. pneumoniae, P. aeruginosa, Proteus merabilis, Acinetobacter, Enterobacter, Klebsiella pneumoniae, Serratia spp., E. coli,*
Salmonella typhi, Candida albicans (Sravani P, et al., 2014). Williams et al., (2009) also reported that leave extract of G. senegalensis inhibited the growth of various microorganisms at different concentrations. Literature reports several recorded use for G. senegalensis in traditional medicine to treat various illnesses (Aniagus, et al., 2005). The observed antibacterial effect of the isolate is believe to be due to the presence of tannins, flavonoids and saponins which have been shown to possess anti-bacterial properties (Osadebe, 2004). (Gasal et al., 2016) who concluded The C.proximus essential oil was effective against Escherichia coli, Staphylococcus aureus and Klebsiella pneumoniae. (Alessandra et al., 2005) and (Kelemba and Kunicke, 2003) those reported, the antimicrobial activity of essential oil from C. proximus could in part, be associated with major constituents such as α-terpinene, linalool, α-pinene, γ-terpinene, α-elemol, β-elemol, guainol, cuparene and thymol. These components have been reported to have antimicrobial effects. There are many reports of antimicrobial activity of Pomegranate. Showing that pomegranate juice is inhibitory to Staphylococcus and Klebsiella pneumoniae. Similar results are recorded in present study which shows highest antibacterial activity after rind extract (Kirilenko et al., 1978). Some of the advantages that herbal preparations have over the synthetic ones are that they do not act directly on bacteria but create an adverse environment for them, thus threatening their survival and they have also been found to deter the development of resistant strains of microorganisms (Adeshina et al., 2007).

In this study, it was observed that the ethanolic extracts had a significantly higher antimicrobial activity than the aqueous extract. This difference is attributed to the solubility of the active component in different solvents. Karou et al. 2007). Our present study showed that, the plants aqueous and ethanolic extracts of ( Arab gum + Harjal ), ( Mahreb+Harjal ) and ( Gebbeish+Harjal ) marry were highly effective against E.coli, S.aureus, Klebsiella sp and Pseudomonas sp. In another study El-Shayeb and Mabrouk (1984) and Osman (1986) reported that the extracts of Sweet pepper and a Hibiscus sp caused a complete inhibition of aflatoxin production by A. flavus, although both were non-effective on fungal growth. However, there were no reports about the direct effect of Harjal extracts on fungi and bacteria in the literature. Lai (2004) said that many herbs and spices included Harjal can yield medicinal compounds. Spices and herbs have been used for thousands of years by man to enhance the flavour and aroma of
foods. Antibacterial activity of spices and other plants are well documented (Alicia, 1981). Vlietinek et al. (1995) screened about 100 medicinal plants, used by traditional healers to treat infections in Rwanda, for their antibacterial, antifungal, and antiviral properties. Their study showed that 45% were active against bacteria; *Staph aureus*, 2% against *E. coli*, 16% against *Pseudomonas aerogenosa*, and 7% against fungus; *Candida albicans*. However, about 27% of the plant species tested exhibited antiviral properties.
CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

5.1 CONCLUSION

1. From the results it could be concluded that, the plant extracts used in this study are effective against the four bacteria tested.

2. In this study, it was observed that the ethanolic extracts had a significantly higher antimicrobial activity than the aqueous extract. This difference is attributed to the solubility of the active component in the alcohol more than water.

3. The inhibitory effect of the plant extracts in both solvents were much greater on *Pseudomonas sp* and *Staphylococcus eureus* compared to *Klebsiella sp* and *E.coli*.

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

5.2 RECOMMENDATIONS

1. The extracts of these medicinal plants should be further analyzed to isolate the specific antibacterial principles in them.

2. More research should be done to investigate the role of Hargel in the biological activity of the different herb extracts.

3. Research on the effectiveness of other parts of the plant as the roots or flowers, etc should be done.

4. Toxicity studies of the effective plants should be done to determine the safety indices of the extracts. Clinical trials should be carried out to explore the potential of these plant extracts in the treatment of the infectious diseases, the pure extract kinetic at the molecular level should be also done in the future.
Reference


Baron, S (1996). Medical Microbiology, 4th edition. The University of Texas Medical Branch at Galveston, Texas, USA.


Global Biodiversity Information Facility (GBIF), 2011.


