University of Khartoum
Faculty of Medical Laboratory Sciences
Department of Microbiology

Study on:

Antimicrobial activity of \textit{Honey and Acacia nilotica extracts against bacteria isolated from wound swabs}

A thesis submitted to Faculty of Medical Laboratory Sciences in partial fulfilment for the requirement of Bsc (Honors) degree in Medical Laboratory Sciences (Microbiology)

By:

Fatima Moutasim Dafallah
Hind El-Jaily mohammed
Hind Tariq El-Waseela

Supervisor:

Mr. Loai Osman Ibrahim

2012
بسم الله الرحمن الرحيم

قال تعالى:
(و الأرض محدناها والدين فيها وربنا فيها من كل شيء موزون) (الحجر الآية "19")

وقال تعالى:
(و اوحي ربك إلى النحل أن اتخذني من الجبال ديارا ومن الشجر مما يعرشون ثم عملي من خلق الثمرات فاسلقي سبل ربك حلالا يخرج من بطونها شرابا مختلطة في الوانه فيه شفاء للناس إن في ذلك لآية لقوم يتفكرون) (النحل الآية "69","68")
Dedication

To our teacher prophet Mohammed
Who is sent for all population as a mercy
To our mothers
Who have given us endless Love
To our fathers
Who have given us encouragement and strength
To our teachers
Who have given us advices
To our colleagues
Who have supported us
And to every one
have Participated in this project
Acknowledgement

Firstly we are deeply grateful for Allah favours.

We are greatly indebted to our supervisor Mr. Luai Osman Ibrahim (Msc) for his help and supervision in this work.

It is pleasure to thank Prof. Naser Eldin Bilal (PhD), Mr. Elamin Mohammed Ibrahim, Mr. Musa Abdalla (Msc), Dr. Ahmed Abdelhaleem and all Microbiology staff.

Special thanks to the staff members of Microbiology Laboratory in both Soba University Hospital and Military Hospital for their help in collecting the samples. And also we will not forget the role of Medicinal and Aromatic plants research plants institute National research Centre.

Finally we wish to thank the Faculty of Medical Laboratory Sciences students association, our friends and our seniors for their support and encouragement over the prolonged period in which we conduct the study.
Abstract

This study tested the invitro antibacterial activity of *Honey* and *Acacia nilotica* extract against bacteria isolated from wound swabs. Standards and clinical isolates tested were (*Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus mirabilis* and *Klebsiella Pneumoniae*). The clinical isolates were isolated from wound swabs, and were identified using biochemical tests. The invitro antimicrobial Susceptibility testing was performed using cup plate diffusion method.

The results showed that *Acacia nilotica* extracts and *Honey* exhibited a high antibacterial activity against all types of tested organisms both clinical isolates and standards.

The activity of *Honey* and *Acacia nilotica* extracts was controlled with some reference antibiotics include Azithromycin and Ciprofloxacin. The Minimum Bactericidal Concentrations (MBCs) were determined.
1-Introduction and literature review:

1-1-Preface:

Many researches to date have addressed honey and Acacia nilotica antibacterial properties and their effects not only on wound healing but also on many other infections. The following Laboratory studies and clinical trials have shown that honey and Acacia nilotica are effective broad-spectrum antibacterial agents.

Since 1992 a study showed the antibacterial activity of honey on wound-infecting species of bacteria. The results proved the high efficiency of honey to inhibit all bacteria tested (Willix, et al., 1992).

While in 1999 a study on the antibacterial activity of honey against Staphylococcus aureus isolated from wounds showed that the minimum inhibitory concentrations of honey tested were all between 2%-3% for mauka honey (from New Zeeland), and between 3%-4% for the pasture honey (Cooper, et al.,1999).

Also in 1999 another study was done on the antimicrobial activity of honey on 20 strains of Pseudomonas isolated from infected wounds. The minimum inhibitory concentrations were 5.5%-8.7% (v/v) for mauka honey and 5.8%-9.0% for pasture honey (Cooper and molan, 1999).

In 2001 the antimicrobial activity of honey on bacteria isolated from wounds was also studied showing that 100% of organisms isolated failed to grow at concentration 30% of honey in MH medium (Subrahmanyam, et al., 2001).

In 2004 both invitro and invivo investigations of the antimicrobial activity of honey on the pathogenic bacterial infection of surgical wounds and conjunctiva was made. The results of the invitro investigation proved that 100% of isolated bacteria was inhibited by tested honey, while the local application of tested honey on surgical wounds and infected conjunctiva of experimental mice reduced the redness swelling, time for complete resolution of lesions, bus discharge and time for complete eradication of bacterial infection (Noori and Alwali, et al., 2004).
Also In 2007 an invitro study of the effectiveness of honey dressing for healing pressure ulcers showed that after 5 weeks of treatment by honey, patients were completely healed (Yupucu Gunes, et al., 2007).

While in 2010 another study on the antibacterial properties of honey and its effect in wound management was made; resulting in that both Gram-positive and Gram-negative bacteria isolated were completely inhibited by the honey tested (Nur Azida, et al., 2010).

On the other hand in 2000 a study under the title of the inhibitory activity of honey against food borne pathogens as influenced by the presence of hydrogen peroxide and the level of antioxidant power, this study proved the inhibitory action of honey against Escherichia coli O157:H7, Salmonella typhimurium, Shigella sonnie, Listeria monocytogenes, Staphylococcus aureus and Bacillus cereus (Peter, et al., 2000).

Also in 2004 the bactericidal activity of honey against pathogenic bacteria was studied; showing that 93% of bacteria were inhibited by tested honey (Patricia, et al., 2004).

A study in 2005 on the antibacterial activity of honey against coagulase negative Staphylococci showed that honey were inhibitory at dilutions down to 3.6% for pasture honey and 3.4% for mauka honey (French, et al., 2005).

The activity of honey on Helicobacter pylori was studied in 2006 proving that all honey tested had inhibitory action against Helicobacter pylori (Basil, et al., 2006).

In 2007 a study was made by, John J. and randy W. Worobo on the antimicrobial activity of different floral sources of honey against bacterial isolation, the results showed that 92.5% of bacterial isolates was inhibited by honey (Hyungjaer, et al., 2007).

While in 2009 a study under the title of the effectiveness of honey on Staphylococcus aureus and Pseudomonas auroginosa biofilms showed that 100% of the isolates were effectively inhibited by honey (Alandejani, et al., 2009).
In study done in 2009 on the antibacterial activity of the stem park extract of *Acacia nilotica*. The antimicrobial activity of the extract was assayed against *Streptococcus viridians*, *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis* and *Shigella sonnei* using the agar diffusion method the most susceptible to plant extract while *Candida albicans* was the most resistant the minimum inhibitory concentration 35-50g/mL, while the minimum bacteriocidal concentration 35-60g/mL (Banso, 2009).

In study done in 2008 the results proved the broad spectrum inhibition activity of *acacia nilotica* against clinical isolates of *Citrobacter species*, *Klebsiella species*, *Escherichia coli*, *Proteus mirabilis*, *Pseudomonas aeroginosa*, *Staphylococcus aureus*, *Streptococcus faecalis*, *Salmonella typhi*, *Salmonella typhimurium*, *Salmonella para typhi A*, *Salmonella para typhi B*, *Shigella flexneri*, *Shigella sonnei*, *Shigella boydii* (Mahesh, et al., 2008)

In study done in 2010 on *Candida albicans* cured extract of *Acacia nilotica* 30ML showed zone with dimeter 22 mm on four different *Candida*, (Mithun, et al., 2011).

In study done in 2011 cured extract of methanol- ethyle-water by sox helt shown antimicrobial activity on *Staphylococcus aureus* and *Escherichia coli* methanol extraction of *Acacia nilotica* showed maximum zone on both 29mm while either 13 in *Escherichia coli* 15mm in *Staphylococcus aureus* while water extraction 20mm, (Amol, et al, 2011).

In study done in 2010 by using methanolic extraction of *Acacia nilotica* and chloroform and ethyle acetate and water showed antimicrobial activity in clinical isolates. By disc diffusion method the methanolic extraction inhibited *Klebsiella pneumoniae*, *Shigella dysenteriae* and *Staphylococcus aureus* water extraction inhibited the isolates with big zone (Yabaya and Salis, 2010).
1-2-Traditional medicine

1-2-1-1-1-Definition:

The World Health Organization (WHO) definition of Traditional Medicine is the health practices, approaches, knowledge and believes incorporating plant, animal and animal based medicines, spiritual therapies, manual technique and exercises, applied singularly or in combination to treat, diagnose and prevent illnesses or maintain well being. (WHO, 2008)

1-2-1-1-2-Olderly:

Traditional medicine is the oldest form of medicine known to Human Kind. It has been developed through observations and by trial and error. The pharmacological treatment of disease began long ago with the use of herbs. People did not know why something worked, they just knew what worked. When something worked, it was written down and considered medical knowledge that was passed on from generation to generation. (Fahd and Toufic, 1996).

1-2-1-1-3-Presently:

The World Health Organization (WHO) estimates that 4 billion people – 80% of the World population — presently use traditional Medicine for primary health care. (WHO, 2008)

Traditional medicine remains the foundation of modern Pharmacology. Animal and plant derived substances have been used as the basis for a large proportion of mainstream medicines; salicylic acid, Aspirin precursor, originally derived from meadow sweet plant. Quinine derived from cinchona bark. The opium poppy yields morphine. (WHO, 2008).

1-3- Honey

1-3-1-2-1-Bees:

Bees are flying insects closely related to wasps and ants, and are known for their role in pollination and for producing honey and beeswax. Bees are a monophyletic lineage within the superfamily Apoidea, presently classified by the unranked taxon name Anthophila. There are nearly 20,000 known species of bees in seven to nine recognized families. Although many are undescribed and the actual number is probably higher. (Danforth B.N, et al,2006).
1-3-3-1-1-Scientific classification: (Michael, et al, 2009).

<table>
<thead>
<tr>
<th>Scientific classification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kingdom: Animalia</td>
</tr>
<tr>
<td>Phylum: Arthropoda</td>
</tr>
<tr>
<td>Class: Insecta</td>
</tr>
<tr>
<td>Order: Hymenoptera</td>
</tr>
<tr>
<td>Suborder: Apocrita</td>
</tr>
<tr>
<td>Superfamily: Apoidea</td>
</tr>
<tr>
<td>Series: Anthophila</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Families</th>
</tr>
</thead>
<tbody>
<tr>
<td>Andrenidae</td>
</tr>
<tr>
<td>Apidae</td>
</tr>
<tr>
<td>Colletidae</td>
</tr>
<tr>
<td>Dasypodaidae</td>
</tr>
<tr>
<td>Halictidae</td>
</tr>
<tr>
<td>Megachilidae</td>
</tr>
<tr>
<td>Meganomiidae</td>
</tr>
<tr>
<td>Melittidae</td>
</tr>
<tr>
<td>Stenotritidae</td>
</tr>
</tbody>
</table>

1-3-3-Honey Bees:

Honey bees (or honeybees) are a subset of bees in the genus *Apis*, primarily distinguished by the production and storage of honey and the construction of perennial, colonial nests out of wax. Honey bees are the only extant members of the tribe Apini, all in the genus *Apis*. 
Currently, there are only seven recognised species of honey bee with a total of 44 subspecies. Though historically, anywhere from six to eleven species has been recognised. Honey bees represent only a small fraction of the approximately 20,000 known species of bees. Some other types of related bees produce and store honey, but only members of the genus *Apis* are true honey bees. (Michael, 1999).

**1-3-3-1-Origin and distribution:**

*Honey* bees as a group appear to have their centre of origin in South and South East Asia (including the Philippines). The first *Apis* bees appear in the fossil record at the Eocene–Oligocene boundary, in European deposits. The origin of these prehistoric *honey* bees does not necessarily indicate that Europe is where the genus originated, only that it occurred there at that time. There are few known fossil deposits in South Asia, the suspected region of honey bee origin, and fewer still have been thoroughly studied. (Deborah R. Smith, *et al.*, 2000).

No *Apis* species existed in New World in human time before introduction of *Apis melifera* by Europeans. There is only one fossil species documented from the New World, *Apis nearctica*, known from a single 14-million-year old specimen from Nevada. (Michael, *et al.*, 2009).
1-3-3-2-Scientific classification: (Michael, et al, 2009)

<table>
<thead>
<tr>
<th>Scientific classification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kingdom: Animalia</td>
</tr>
<tr>
<td>Phylum: Arthropoda</td>
</tr>
<tr>
<td>Class: Insecta</td>
</tr>
<tr>
<td>Order: Hymenoptera</td>
</tr>
<tr>
<td>Family: Apidae</td>
</tr>
<tr>
<td>Subfamily: Apinae</td>
</tr>
<tr>
<td>Tribe: Apini, Latreille, 1802</td>
</tr>
<tr>
<td>Genus: Apis, Linnaeus, 1758</td>
</tr>
</tbody>
</table>

**Species**

- Subgenus Micrapis:
  - *Apis andreniformis*
  - *Apis florea*
- Subgenus Megapis:
  - *Apis dorsata*
- Subgenus Apis:
  - *Apis cerana*
  - *Apis koschevnikovi*
  - *Apis mellifera*
  - *Apis nigrocincta*
1-3-3-3-Honey production

Firstly, the foraging bees collect nectar from flowers using tube-like structure called *proboscis*, in the bees' stomach the nectar metabolized by certain enzymes such as amylase and glucose oxidase. The metabolized nectar then dropped into the beeswax comb and finally converted into thickened *honey* after being evaporated by the bees' wings. (Subramanian, *et al.*, 2007).

1-3-4- Chemical composition: (Subramanian, *et al.*, 2007).

<table>
<thead>
<tr>
<th>NUTRIENT:</th>
<th>Amount in 100g of <em>honey</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>17.1 g</td>
</tr>
<tr>
<td>Carbohydrates (total)</td>
<td>82.4 g</td>
</tr>
<tr>
<td>....Fructose</td>
<td>38.5 g</td>
</tr>
<tr>
<td>....Glucose</td>
<td>31.0 g</td>
</tr>
<tr>
<td>....Maltose</td>
<td>7.20 g</td>
</tr>
<tr>
<td>....Sucrose</td>
<td>1.50 g</td>
</tr>
<tr>
<td>Proteins, amino acids, vitamins and minerals</td>
<td>0.50 g</td>
</tr>
<tr>
<td>Energy</td>
<td>304 Kcal</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Vitamins</th>
<th>Amount in 100g of <em>honey</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>....Thiamin</td>
<td>&lt; 0.006 mg</td>
</tr>
<tr>
<td>....Riboflavin</td>
<td>&lt; 0.06 mg</td>
</tr>
<tr>
<td>....Niacin</td>
<td>&lt; 0.36 mg</td>
</tr>
<tr>
<td>....Pantothenic acid</td>
<td>&lt; 0.11 mg</td>
</tr>
<tr>
<td>....Pyridoxine (B6)</td>
<td>&lt; 0.32 mg</td>
</tr>
<tr>
<td>....Ascorbic Acid (C)</td>
<td>2.2 - 2.4 mg</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Minerals</th>
<th>Amount in 100g of <em>honey</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>....Calcium</td>
<td>4.4 - 9.20 mg</td>
</tr>
<tr>
<td>....Copper</td>
<td>0.003 - 0.10 mg</td>
</tr>
<tr>
<td>....Iron</td>
<td>0.06 - 1.5 mg</td>
</tr>
<tr>
<td>....Magnesium</td>
<td>1.2 - 3.50 mg</td>
</tr>
<tr>
<td>....Manganese</td>
<td>0.02 - 0.4 mg</td>
</tr>
<tr>
<td>....Phosphorus</td>
<td>1.9 - 6.30 mg</td>
</tr>
<tr>
<td>....Potassium</td>
<td>13.2 - 16.8 mg</td>
</tr>
<tr>
<td>....Sodium</td>
<td>0.0 - 7.60 mg</td>
</tr>
<tr>
<td>....Zinc</td>
<td>0.03 - 0.4 mg</td>
</tr>
</tbody>
</table>
1-4-Acacia nilotica:

1-4-1-Scientific classification: (Backlund and K. Brener, 1998).

Kingdom: Plantae
Division: Magnoliophyta
Class: Magnoliopsi
Order: Fabales
Family: Fabaceae
Genus: acacia
Species: A. nilotica

1-4-2-Botanical Description:

Its gum Arabic tree 5–20 m high with a dense spheric crown stems and branches usually dark to black coloured, fissured bark, grey-pinkish slash, exuding a reddish low quality gum. The tree has thin, straight, light, grey spines in axillary pairs, usually in 3 to 12 pairs, 5 to 7.5 cm (3 in) long in young trees, mature trees commonly without thorns. The leaves are bipinnate, with 3-6 pairs of pinnulae and 10-30 pairs of leaflets each, tomentose, rachis with a gland at the bottom of the last pair of pinnulae. Flowers in globulous heads 1.2-1.5 cm in diameter of a bright golden-yellow colour, set up either axillary or whorly on peduncles 2–3 cm long located at the end of the branches. Pods are strongly constricted, hairy, white-grey, thick and softly tomentose. Its seeds number approximately 8000/kg. (Backlund and K. Brener, 1998).

1-4-3-Distribution:-

Acacia nilotica, Scented Thorn Acacia, is native from Egypt, across the Maghreb and Sahel, south to Mozambique and Natal, and east through Arabian Peninsula to Pakistan, India and Burma. It has become widely naturalised outside its native range including Zanzibar, and Australia. Acacia nilotica is restricted to riverine habitats and seasonally flooded areas within its native range however in its introduced range it is spread by livestock and grows outside riparian areas. (Backlund and K. Brener, 1998).
1-4-4-Traditional uses:-

1-4-4-1-Forage and fodder

In part of its range small stock consume the pods and leaves, but elsewhere it is also very popular with cattle. Pods are used as a supplement to poultry rations in India. Dried pods are particularly sought out by animals on rangelands. In India branches are commonly lopped for fodder. Pods are best fed dry as a supplement, not as a green fodder. (Clemens, et al, 1977)

1-4-4-2-Hedges

A. nilotica makes a good protective hedge because of its thorns. (Clemens, et al, 1977)

1-4-4-3-Medicine

A. nilotica may also be used for medicinal purposes, as a demulcent or for conditions such as gonorrhoea, leucorrhoea, diarrhea, dysentery or diabetes. It is styptic and astringent. In Siddha medicine, the gum is used to consolidate otherwise watery semen. (Clemens, et al, 1977)

1-4-4-4-Bark

To Hartwell, African Zulu take bark for cough. It acts as an astringent and it is used to treat diarrhea, dysentery, and leprosy. (Clemens, et al, 1977)

1-4-4-5-Bark and root

Maasai are intoxicated by the bark and root decoction, said to impart courage, even aphrodisia, and the root is said to cure impotence. (Clemens, et al, 1977)

1-4-4-6-Bark or gum

In West Africa, the bark or gum is used to treat indurations of liver and spleen, condylomas, and excess flesh. Sap or bark, leaves, and young pods are strongly astringent due to tannin, and are chewed in Senegal as an antiscorbutic. (Clemens, et al, 1977)

1-4-4-7-Leaves

The bruised leaves are poulticed and used to treat ulcers. (Clemens, et al, 1977)
1-4-4-8-Resin

In Lebanon, the resin is mixed with orange-flower infusion for typhoid convalescence. (Clemens, et al, 1977)

1-4-4-9-Root

The Chipi use the root for tuberculosis. In Tonga, the root is used to treat tuberculosis. (Clemens, et al, 1977)

1-4-4-10-Seed pods

Egyptian Nubians believe that diabetics may eat unlimited carbohydrates as long as they also consume powdered pods. (Clemens, et al, 1977)

1-4-4-11-Wood

In Italian Africa, the wood is used to treat smallpox. In Ethiopia, certain parts of the tree are used as a lactagogue. (Clemens, et al, 1977)

1-4-4-12-Lumber

The tree's wood is "very durable if water-seasoned" and its uses include tool handles and lumber for boats. The wood has a density of about 1170 kg/m. (Clemens, et al, 1977)

1-4-4-13-Twigs

In most parts of Indian sub-continent, thin twigs are chewed and used as a toothbrush. (Clemens, et al, 1977)

1-4-5- Constituents:-

The stem bark extract of the plant possessed the active compound( terpenoids, tannins, alkaloids, saponins glycosides phenol resin oleosin steroids terpnes phalobatanin gallic acid protocatecuicacid pytrocathol catechin epi-gallocatechin-7-gallate epi galloclatechin-5 7-digallate. (Clemens, et al, 1977)
1-5-wound infection:-

Wound is a breach in the skin and the exposure of subcutaneous tissue following loss of skin integrity which provides a moist, warm, and nutritive environment that is conducive for microbial colonization and proliferation. Wound infections have been regarded as the most common nosocomial infections and are associated with increased morbidity and mortality. (George, 2005)

Infection in a wound delays healing, causes wound breakdown, prolonged hospital stay, increased trauma care and treatment costs. (George, 2005)

Bacteriological studies have also shown that wound infections is universal and that the types of bacteria vary with geographical locations, bacteria resident on the skin, clothing at the site of wound, time between wound and examination. (George, 2005)

The control of wound infections has become more challenging due to widespread bacterial resistance to antibiotics and due to an increasing incidence of infections caused by methicillin-resistant Staphylococcus aureus (MRSA) and polymicrobial flora. (George, 2005)

1-5-1-Etiological agent:- (George, 2005)

- *Escherichia coli.*
- *Klebsiella spp*
- *Proteus spp.*
- *Pseudomonas aeruginosa.*
- *Staphylococcus aureus.*

**Organisms:**

1-5-1-1- *Staphylococcus aureus*:-

**Description:**

It is Gram +ve cocci, non-motile, non-capsulated. (Monica Cheesbrough, 1991).
Normal habitat: -

*Staphylococci* are distributed in environment. This form part of normal microbial flora of the skin, upper respiratory tract and intestinal tract. (Monica Cheesbrough, 1991).

**Antigenic structure:**

1) protein.
2) capsule (some strain).
3) peptidoglycan (Monica Cheesbrough, 1991).

**Production of enzyme and toxin:**

*Staphylococci* can produce many enzymes and toxins:

**Enzymes:**

1) bound and free coagulase, nucleases.
2) hyaluronidase.
3) proteinase.
4) fibrinolysin,

**Toxins:**

1) enterotoxin (A, E).
2) Toxic shock syndrome.
3) Haemolysin,
4) leucocidin. (Monica Cheesbrough, 1991).

**Pathogenicity:**

*Staphylococci aureus* is the most important Staphylococcal pathogen, it causes:

- Pyogenic infection: folliculitis, impetigo, osteomyelitis.
- Disseminated infection: septicaemia.
1-5-1-2- *Escherichia coli*: -

**Description:**
It is one of the most important members of *Enterobacteriaceae*. Gram -ve rods, non-spore, some strain are capsulated, lactose fermenting ,most strains are gas producer (Monica Cheesbrough, 1991).

**Normal habitat:**
*Escherichia coli* organism form part of normal microbial flora of human and animal. Also found in environment, water, vegetation and soil. (Monica Cheesbrough, 1991).

**Pathogenicity:**
From their normal site in the human body they are able to cause frequent opportunistic infection:
- Urinary tract infections mainly cystitis, pyelitis and pyelonephritis.
- Bacteraemia and meningitis especially of the new born.
- Wound infection.
- Diarrheal disease especially in infant but also in adult. (Monica Cheesbrough, 1991).

1-5-1-3- *Pseudomonas aeruginosa*:

**Description:**
Gram –ve, rod, aerobic, non fermentative, motile with polar flagella, monotrichosis (Monica Cheesbrough, 1991).

**Normal habitat:**
*Pseudomonas aeruginosa* frequently present on hospital environment especially in moist places (Monica Cheesbrough, 1991).

**Pathogenicity:**
It is pathogenic only when introduced into area devoid of normal defences. Also it is classic opportunistic pathogen with innate resistance to antibiotics and disinfectants. (Monica Cheesbrough, 1991).
- Skin infection, especially at burn sites, wounds.
- Urinary tract infections, following catheterization.
-Respiratory infection especially in patients with cystic fibrosis or condition that cause immunosupression.
-External ear infection (otitis externa).
-Eye infection
-Septicaemia (Monica Cheesbrough, 1991).

**1-5-1-4-Proteus:-**

**Description:-**
Gram -ve rod actively motile non-capsulated pleomorphic (Monica Cheesbrough, 1991).

**Normal habitat:-**
Found in intestines of humans and animals and in soil sewage and water (Monica Cheesbrough, 1991)

**Pathogenicity:**
This species mostly cause urinary tract infection – abdominal and wound infection and septicaemia (Monica Cheesbrough, 1991)

**1-5-1-5-klebsiella:-**

**Description:-**
Non motile , Gram negative ,rod, capsulated . (Monica Cheesbrough, 1991)

**Normal habitat:-**
Found in intestines of humans and animals and in soil sewage and water also can be found as commensal in the mouth and upper respiratory tract. (Monica Cheesbrough, 1991)

**Pathogenicity:-**
This species mostly cause :
-urinary tract infection
-abdominal and
-wound infection
-septicaemia and chest infection. (Monica Cheesbrough, 1991)
1-6-Antimicrobial activity:

This includes:
A-antibiotics
B-chemical antimicrobials (chemotherapeutic agents)

A-Antibiotics:

Are antimicrobial substances produced by living microorganisms. They include the culture extracts and filtrates of fungi such as *penicillium* and *cephalosporin*, and bacteria such as *sterptomyces* and *bacillus* speices. Many antibiotics in common use are synthetic derivative of microbial antibiotics. Important antibacterial antibiotics include the penicillins, cephalosporins, aminoglycosides, tetracyclines, polymyxins, rifampicin, fucidin, chloramphenicol, erythromycin, clindamycin and vancomycin. Antifungal antibiotic include amphotericin B and griseofulvin. (Monica Cheesbrough, 1991)

B-Chemical antimicrobials (Chemotherapeutic agents):

Are synthetically produced antimicrobial compounds. They include the sulphonamides, trimethoprim, cotrimoxazole, metronidazole, p.aminosalicyclic acid, isoniazid, ethambutol and thiosemicarbazone. Antifungal agents include nystatin, flucytosine and imidazole agents. A few antiviral drugs have seen developed in recent years but most of them have serious toxic effects. (Monica Cheesbrough, 1991)

1-6-1-Classification:

Antimicrobial can be classified according to;

1) Target site:

Not all antimicrobials, at the concentration required, to be effective are completely non toxic to human cells. Most of them, however, show sufficient selective toxicity to be of value in the treatment of microbial diseases. The various ways of antimicrobials that act on bacteria are as follows: (Monica Cheesbrough, 1991)
1-Inhibiting cell wall formation leading to cell lysis, for example the penicillins, cephalosporins and vancomycin

2-Damaging the bacterial cell membrane, leading to loss of cell contents and so cell death, for example the polymyxins and amphotericin B

3-Inhibiting protein production and therefore arresting bacterial growth, for example the aminoglycosides, tetracyclines, erythromycin, chloramphenicol and clindamycin.

4-Inhibiting the production of nucleic acids and therefore preventing bacteria from reproduction, for example nalidixic acid prevents DNA synthesis and rifampicin inhibits RNA synthesis.

2) Effect on antimicrobial growth (bactericidal and bacteriostatic activity):

In some clinical situation it is essential to use bactericidal drug rather than bacteriostatic one. A bactericidal drug kills bacteria, for example the penicillins, cephalosporins, polymyxin and aminoglycosides. Whereas a bacteriostatic drugs inhibit their growth by prevent the active multiplication of but does not kill them, for example chloramphenicol, tetracyclines and erythromycin. (Monica Cheesbrough, 1991)

3) Chemical structure:

1- **B-lactum antibiotics:** example B-lactmase enzyme of bacteria.
2- **Aminoglycosides:** example gentamycin and toegomycin.
3- **Quinolones:** example ciprofloxacin and nalidixic acid.
4- **Immidazoles:** example metronidazole.
5- **Tetracyclines:** example dihydrotetracycline and deoxytetracycline
6- **Macrolides:** example erythromycin and clindamycin.
7- **Miscellaneous:** example rifampicin and chloramphenicol. (Monica Cheesbrough, 1991)

4) Spectrum or range of activity:

1- **Broad spectrum antibiotics:** are active against several types of microorganisms, for example tetracyclines are active against many Gram negative rods, *chlamydiae*, *mycoplasmas* and *rickettsiae*. 
2-Narrow spectrum antibiotics: are active against one or very few types of organisms, example vancomycin is primarily used against certain Gram positive cocci, namely *staphylococci* and *enterococci*. (Monica Cheesbrough, 1991)

1-6-2-Antimicrobial resistance:

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

1-Bacteria produce enzymes that inactivate the drug, example B-lactmases can inactivate penicillins and cephalosporins by cleaving the B-lactum ring of the drug.
2-Bacteria synthesize modified targets against which the drug has no effect, e.g: a mutant protein in the 30s ribosomal subunit can result in resistance to streptomycin and a methylated 23s rRNA can result in resistance to erythromycin. (Monica Cheesbrough, 1991)
3-Bacteria decrease their permeability so that effective intracellular concentration of the drug is not achieved, e.g: change in porins can reduce the amount of penicillin entering the bacterium.
4-Bacteria actively export drugs using a multidrug resistance pump (MDR pump), the MDR pump imports protons and exports a variety of foreign molecules including certain antibiotics, such as quinolones. (Monica Cheesbrough, 1991)

1-6-3-Antimicrobial susceptibility tests:

Susceptibility tests measure antimicrobial activity against bacteria under laboratory condition (in vitro activity), not in side the patient’s body (in vivo activity). It cannot be assured, therefore, that an antimicrobial which kills or prevents an organism from growing in vitro will be a successful treatment. (Monica Cheesbrough, 1991)

1-6-4-Susceptibility test techniques:

Laboratory antimicrobial susceptibility testing can be performed using:

1) A disc diffusion method. 2) A dilution technique. (Monica Cheesbrough, 1991)

1-6-4-1-Diffusion sensitivity tests (disc diffusion technique):

These method are suitable for organisms that grow rapidly over night at 35-37C, are technically simple, cheap and reliable. (Monica Cheesbrough, 1991)
Disc diffusion method; a disc of blotting paper is impregnated with a known volume and appropriate concentration of an antimicrobial, and this is placed on a plate of Muller Hinton agar inoculated with the test organism. In the stokes comparative disc diffusion technique, a control organism is inoculated on the same plate, in other disc techniques including the Kirby Bauer method, the control strain inoculated on a separate plate. The antimicrobial diffuses from the disc into the medium, following overnight incubation the culture is examined for areas of no growth around the disc (zone of inhibition). Bacterial strains susceptible to the antimicrobial are inhibited at a distance from the disc whereas resistant strains grow up to the edge of the disc. In the stokes technique the zone of inhibition produced by the test organism is compared directly with that of control strain. In Kirby Bauer technique the zone is measured and compared against a previously prepared scale, the scale correlates zone size with the minimum inhibition concentration (MIC). The MIC is the lowest or minimum concentration of antimicrobial that is required to prevent in vitro bacterial multiplication under specified condition.

1-6-4-2-Dilution susceptibility tests:

Are performed when the patient does not respond to treatment thought to adequate, replaces while being treated, or when there is immunosupression. (Monica Cheesbrough, 1991)

Dilution techniques measure the minimum inhibitory concentration (MIC), they can also be used to measure the minimum bactericidal concentration (MBC) which is the lowest concentration of antimicrobial required to kill bacteria. (Monica Cheesbrough, 1991)

A dilution test is carried out by adding dilutions of an antimicrobial to a broth or agar medium, a standardized inoculums of the test organism is then added, after overnight incubation the MIC is reported as the lowest concentration of antimicrobial required to prevent visible growth, by comparing the MIC value with known concentration of the drug obtainable in serum or other body fluids, the likely clinical response can then be assessed. If required the MBC can be determined by sub culturing the last tube showing visible growth and all the tubes in which there is no growth, the MBC is the lowest concentration of antimicrobial required to produce a sterile culture. (Monica Cheesbrough, 1991)
1-6-5-Factor affecting antimicrobial activity:

1) Component of medium: the medium ingredients support good overnight growth of test and control organisms, no substances affect in the antimicrobial agent or the organism, slow growth can result in the inhibition zones being abnormally large. Suitable media include Muller Hinton agar available from many manufacturers of culter media, Oxoid iso-sensitest agar, Gibco sensitivity testing agar, and Wilkins Chalgren agar is recommended for testing anaerobic bacteria. The medium must be prepared exactly according to the manufacturer’s instructions and poured to a depth of 4 mm (25 ml medium) in a flat bottomed 9 cm Petri dishes on a level surface. When set the plates may be stored for up to a week at 4°C. Their agar surface should be dried with their lids before use. (Monica Cheesbrough, 1991)

2) PH of the medium: should be maintained at 7.2 to 7.4 (slightly alkaline). A more acid PH decreases the activity of aminoglycosides and macrolides, a more alkaline PH favours the action of tetracyclines, novobiocin and fusidic acid, but interfere seriously with the activity of nitrofurantoin. (Monica Cheesbrough, 1991)

3) Size of inoculums: the ideal inoculums after overnight incubation gives even semi confluent growth. This can be obtained by comparison with an opacity standard (Mc. Farland) which is composed of 0.5 ml of barium chloride and 99.5 ml of the sulphuric acid (Barium sulphate). Failure to standardize the inoculums is a common source of error. Too heavy inoculums reduces the size of the inhibition zones produced by many antibiotics. Too light inoculums not only makes zone diameters difficult to read accurately, but may fail to reveal resistance mediated by the bacterial production of drug destroying enzymes. (Monica Cheesbrough, 1991)

4) Incubation: plates should be incubated in air(aerobically) at 37°C for minimum of 16 hours and maximum of 24 hours, some antimicrobial demanding organism, e.g: Neisseria species require incubation in 10% Co2. (Monica Cheesbrough, 1991)
5) Concentration of antimicrobial agent: high concentration of antimicrobial agent give large inhibition zone and vice versa. (Monica Cheesbrough, 1991)

6) Antibiotic disc: commercially prepared discs 6 mm in diameter should be used e.g. Oxoid, Unipath. Manufacturers produce discs with accurate antibiotics content coded in sealed container with a desiccate, bulk stock being kept at 20°C if possible, otherwise at less than 8°C. Working stock, also kept in sealed container with a dessiccate, should be stored at less than 8°C. (Monica Cheesbrough, 1991)
2-Rationale and Objectives

2-1-Rationale:

Recently, modern societies face serious problems with using of the synthetic chemotherapeutic agents in order to their multiple disadvantages such as; harmful side effect, high cost and development of multi-resistant strains due to the recurrent usage. So the Traditional medication -specially in the MIDDLE and Far East societies- started to play an important role as a safer and cheaper alternative solution.

In Sudanese culture and as a part of the traditional medication, *Honey* and *Acacia nilotica* are used for the treatment of many infections as Respiratory tract and Urino-genital tract infections. Therefore it is of interest to test the antibacterial activity of their extracts on bacterial causative agents of Wound infection as well.
2-2-objectives:

2-2-1-general objective:
To measure the antibacterial activity of Honey and Acacia nilotica pods on the certain bacterial causative agents of Wound infection.

2-2-2-specific objectives:
To measure the anti-bacterial activity of honey on the bacterial causative agents of wound infection.

• To measure the anti-bacterial activity of Acacia nilotica on the bacterial causative agents of wound infection.

To determine the extract that has the highest anti-bacterial activity.

• To determine what of the two sterilization methods used (Filtration and autoclaving) yields the most effective extract.

• To compare between the degree of sensitivity of pigmented and non-pigmented Pseudomonas species to each extract.

• To determine the Minimum Bactericidal Concentration (MBC) of each extract.
3-Materials and Methods

3-1-Materials:

3-1-1-Equipments:
1-Autoclave
2-Incubator
3-Hot air oven
4-Refrigerator
5-Sensitive balance
6-Light microscope with oil immersion lenses
7-Wire loops with handles
8-Straight loops with handles
9-cork borer (0.5 cm in diameter)
10-Bunsen burner
11-Rack
12-syrings

3-1-2-Glassware:
1-Petri dishes
2-Flasks with different size
3-Measuring cylinder
4-Beakers
5-Sterile containers (bijou bottles)
6-Test tubes

3-1-3-Disposable materials:
1-Disposable syringes
2-Wooden applicators
3- swabs

**3-1-4-Chemical and reagents:**
1- Methanol

**3-1-5-Culture media:**
There are various types of synthetic bacteriological media which are essential for isolation, inoculation and identification of organisms from wound swabs used in our study.

The media are:
1- Nutrient agar
2- Media for biochemical reactions
3- Muller Hinton agar media (see appendix)

**3-1-6-Test organisms:**

**3-1-6-1-Standard bacterial strains (controls):**
The following strains of the bacteria were tested for their susceptibility to: reference antibiotics, honey and cured extracts of *Acacia nilotica*

1- Escherichia coli ATCC: 25922.
2- Staphylococcus aureus ATCC:29213.
3- Pseudomonas aeruginosa ATCC:27853.
ATCC American Type Culture Collection.

**3-1-6-2-Clinical bacterial isolates:**
3-1-6-2-1-Sampling:
Hundred samples of wound swabs from various types of wound were received by Soba teaching hospital (Khartoum) and Alselah altebby hospital (Omdurman) in the period between December 2011 and May 2012.

3-1-6-2-2-Culture:
All wound swabs were inoculated on MacConkey agar and Blood agar, incubated for (16-18hrs) at CO2 enriched atmosphere.

3-1-6-2-3-Identification:
The isolated organisms were fully identified by Gram stain, then followed by performing biochemical tests.

3-1-6-2-3-1-Gram stain:
1-Add three drops of sterile normal saline in a clean dry slide using sterilized wire loop.
2-From pure culture of the tested organism touch one colony by sterilized wire loop, mix it with normal saline and spread it evenly on an area of about 15-20 mm.
3-Fix the dried smear by heating using the flame.
4-Cover the fixed smear with crystal violet stain for 30 minutes.
5-rapidly wash off the stain with clean water.
6-Cover the smear with lugol’s ioidine for 30-60 minutes, then wash.
7-Decolourize rapidly (few seconds) with acetone alcohol, then wash.
8-Cover the smear with neutral red for 2 minutes, then wash.
9-Let the smear dry, add drop of immersion oil and examine the smear microscopically using X100.

Results:
Gram +ve bacteria: Dark purple.

3-1-6-2-3-2-Biochemical reaction:
I-Catalase:

**Required:** hydrogen peroxide (3% H₂O₂) and wooden stick

**Principle:** Catalase acts as a catalyst in the breakdown of hydrogen peroxide to oxygen and water. An organism is tested by bringing it into contact with hydrogen peroxide. Bubbles of oxygen are released if the organism is a catalase producer.

**Method:** Pour 2-3ml of the hydrogen peroxide solution into tube test, using sterile wooden stick, remove a good growth of the test organism and immerse it in the hydrogen peroxide solution and look for immediate bubbling.

**Results:** The positive test indicated by production of active bubbling. (Monica Cheesbrough, 1991)

II-Coagulase test:

**Required:** undiluted human plasma, slide and physiological saline.

**Principle:** Coagulase lead to plasma clotting by converting fibrinogen to fibrin. Two types of coagulase are produced by most strains of *Staphylococcus aureus*:

a) free coagulase detected by tube method

b) bound coagulase (clumping factor) which converts fibrinogen directly to fibrin without requiring a coagulase reacting factors. It can be detected by the clumping of bacterial cells in the rapid slide test.

**Method:** Place a drop of physiological saline in each end of a slide, emulsify a colony of the test organism in each of the drops to make two thick suspensions and add a drop of plasma to one of the suspension and mix gently by rotating. Look for clumping of the organism within 10 seconds.

**Result:** positive reaction was indicated by clumping within 10 seconds. (Monica Cheesbrough, 1991)

III-Deoxyribonuclease (DNase) test:

**Required:** DNAse agar plate, 1ml of hydrochloric acid (1%HCL)

**Principle:** Deoxyribonuclease hydrolyze deoxyribonucleic acid (DNA).
- **Method**: The test organism is cultured on a medium which contain DNA. After overnight incubation, the colonies are tested for DNAse production by flooding the plate with a weak hydrochloric acid solution. The acid precipitates unhydrolyzed DNA. DNAse producing colonies are therefore surrounded by clear areas indicating DNA hydrolysis.

**Result**: Positive reaction was indicated by clearing around colonies. (Monica Cheesbrough , 1991)

### IV-Oxidase test:

**Required**: filter paper impregnated with oxidase reagent (tetra methyl para phenylene diamine dihydrochloride), clean slide and wooden stick.

**Principle**: if the organism is oxidase producer, the para phenylene diamine in the reagent will be oxidized to a deep purple colour.

**Method**: a piece of filter paper is placed on a clean glass slide and three to four drops of freshly prepared oxidase reagent were added using sterile Pasteur pipette, wooden stick was used to pick a colony of the test organism and placed on the filter paper.

**Result**: the positive reaction is indicated by the production of blue-purple colour within 10 seconds. (Monica Cheesbrough , 1991)

### V-Indole test:

**Required**: sterile peptone water in small test tube, Kovac’s reagent, wire loop and Pasteur pipette.

**Principle**: organism having tryptophase enzyme can breakdown amino acid tryptophan to produce indole as an end product and is detected by adding Kovac’s reagent.

**Procedure**: the tested colony is inoculated in sterile peptone water using sterile wire loop and then incubated at 37°C aerobically overnight. Few drops of Kovac’s reagent were added to the medium using Pasteur pipette.
**Result:** positive indole is indicated by immediate production of red ring on the surface of the medium. Negative reaction is indicated by a yellow production. (Monica Cheesbrough, 1991)

**VI-Citrate utilization test:**
**Required:** Simmon’s citrate agar slope medium and straight loop.

Principle: an organism when cultured in medium containing citrate as only source of carbon, it will utilizes citrate and give ammonia which change the PH and is detected by change in the colour of indicator in the medium.

**Procedure:** a small part of the tested colony is picked off using sterile straight loop and inoculated on the surface of the slope of the medium in a zigzag manner, and then incubated at 37°C aerobically overnight.

**Result:** positive reaction is indicated by the change in the colour of the indicator in the medium (bromothymol blue) into blue colour. Negative reaction was indicated by no change in the colour. (Monica Cheesbrough, 1991)

**VII-Urease test:**
**Required:** Christensen’s urea agar medium and straight loop.

Principle: if the organism is urease producer, it will breakdown urea to ammonia and carbon dioxide which is indicated by the change in the colour of indicator.

**Procedure:** the tested colony is inoculated on the surface of the slope medium by sterile straight loop in zigzagging manner and then incubated overnight at 37°C aerobically.

**Result:** positive reaction is indicated by the colour change in the indicator (phenol red) to pink colour. Negative reaction is indicated by no change in the colour. (Monica Cheesbrough, 1991)

**VIII-Motility testing:**
**Required:** semisolid media and straight loop.

Principle: if an organism is motile, it will spread easily within the semisolid.

**Method:** the tested colony is taken by a sterile straight loop, and inoculated by stabbing the media, then incubated aerobically at 37°C overnight.
**Result:** the motility was shown by spreading turbidity from the stab-line, or turbidity throughout the medium. (Monica Cheesbrough, 1991)

**IX-KIA (kligler iron agar):**

**Required:** KIA medium in a slope position and a straight loop.

Principle: KIA reactions are based on the fermentation of lactose and glucose and the production of hydrogen sulphide (H$_2$S)

The fermentation is indicated by colour change of the media to yellow colour due to the phenol red indicator in the media.

H$_2$S is produced when sulphur-containing amino acid are decomposed. It is detected by production of black colour due to ferric citrate indicator in the medium.

**Method:** a small part of the tested colony was picked off using a straight loop and inoculated in KIA medium, first stabbing the butt, then streaking the slope in zigzag pattern, and then incubate at 37°C aerobically overnight.

**Result:** a yellow butt red-pink slope indicates the fermentation of the glucose only. The slope is pink-red due to a reversion of the acid reaction under aerobic condition.

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

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

Blackening along the stab line or throughout the medium indicates H$_2$S production.
Cracks and bubbles in the medium indicates gas production from glucose fermentation.

---

Five bacteria (*Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Klebsiella pneumoniae*) each organism we work with it replicated five time with different clinical isolate. (Monica Cheesbrough, 1991)
3-2-Method:

3-2-1-Plant material: -

Plant were collected from the market.

3-2-1-1-Preparation of cured extracts: -

1- Methanolic extract of the stem bark of the plant: -

Methanolic extract of the stem bark of the plant was extracted according to the method described by Okogun (2000) with slight modifications. A 700 g sample of the stem bark of the plant was air-dried, ground into powder using an electric blender. The blended material was transferred into a beaker and 700 ml of 95% ethanol was added at ambient temperature (28 ± 2°C). The mixture was extracted by agitation by hand for 3 hours.

Extraction was allowed to proceed for removed by evaporation at room temperature (28 ±2°C) to obtain the extract.

2- Aqueous extract of the stem bark of the plant: -

A 700 g sample of the stem bark of the plant was air-dried, ground into powder using an electric blender. The blended material was transferred into a beaker and 1400 ml of distilled water was added at ambient temperature (28 ± 2°C). The mixture was extracted by agitation by hand for 3 hours.

Extraction was allowed to proceed for 48 h. The mixture was decanted and the solvent was removed by evaporation at room temperature (28 ± 2°C) to obtain the extract.

3-2-1-2-Preparation of serial dilution of bark extracts and honey: -

1- Preparation of serial dilution of bark extract both methanolic and aqueous extracts:

The cured extract considered as 100% (stock), then serial dilutions performed as follow:

100% ———— 10 ml of the stock extract.

80% ———— 8 ml of the stock dissolved in 2 ml of distilled water.
60%——— 6ml of the stock dissolved in 4ml of distilled water.
40%——— 4ml of the stock dissolved in 6ml of distilled water.
20% ———— 2ml of the stock dissolved in 8ml of distilled water.
5% ———— 0.5ml of the stock dissolved in 9.5ml of distilled water.

2-- Preparation of serial dilutions of honey:

The stock honey sample considered as 100% (stock), then serial dilutions performed as follows:

100%——— 10g of the stock honey.
80%——— 8g of the stock honey dissolved in 10ml of distilled water.
60% ———— 6g of the stock honey dissolved in 10ml of distilled water.
40% ———— 4g of the stock honey dissolved in 10ml of distilled water.
20% ———— 2g of the stock honey dissolved in 10ml of distilled water.
5% ———— 5g of the stock honey dissolved in 10ml of distilled water.

The above dilutions were used to determine minimum bacteriocidal concentration.

3-2-1-3 Sterilization of honey and Acaia nilotica cured extracts:

Bottles of serial dilutions of honey and aqueous extract of Acacia nilotica were sterilized by autoclave at 121°C for 20 minutes, (Monica Cheesbrough , 1991)

While methanolic extract of Acacia were sterilized by both; autoclaving and filtration using 0.04um filter paper. . (Monica Cheesbrough , 1991).

3-2-2 Preparation of serial dilutions of reference antibiotics:

3-2-2-1 Azithromycin:

Commercially available syrup form of Azithromycin was used as stock (100%) for the following serial dilutions:

40%——— 4ml of the stock dissolved in 6ml of distilled water.
20% ———— 2ml of the stock dissolved in 8ml of distilled water.
10% ———— 1ml of the stock dissolved in 9ml of distilled water.
5% ———— 0.5ml of the stock dissolved in 9.5ml of distilled water.
3-2-2- Ciprofloxacin:

Commercially available tablets (250 mg) were dissolved in distilled water, and the prepared solution was used as stock (100%) for the following serial dilutions:

- 40% ———————— 4ml of the stock dissolved in 6ml of distilled water.
- 20% ———————— 2ml of the stock dissolved in 8ml of distilled water.
- 10% ———————— 1ml of the stock dissolved in 9ml of distilled water.
- 5% ———————— 0.5ml of the stock dissolved in 9.5ml of distilled water.

3-2-3- Preparation of standard bacterial suspension:

Ten ml normal saline were placed in test tubes and sterilized in autoclave at 121°C for 15 minutes, a loop full of purified bacteria were inoculated in sterile normal saline and compare with McFarland standard. (Monica Cheesbrough, 1991)

3-2-4- Modified diffusion technique:

The diffusion method was adopted with some minor modification to assess the antimicrobial activity of the prepared extracts and honey dilution. Two ml of bacterial suspension (standards and clinical isolates) were taken with disposable syringe and added to twenty ml of molten Muller Hinton media and mixed, then allow the media to set and solidify for few minutes, make wells using sterile cork borer of 5 mm diameter. Alternated cups were filled with 0.1 ml of sterilized reference antibiotics, Acacia nilotica extracts and honey dilutions using sterile disposable syringes.

Allowed to diffuse at room temperature for 30 min then the plate incubated in incubator in upright position at 37°C for 18 hours, (Monica Cheesbrough, 1991).

The diameters of the resultant growth inhibition zones were measured – averaged and the mean values were tabulated, (see results).

The reference antibiotics were tested against standard bacteria, while Acacia nilotica and Honey were tested against standard bacteria and clinical isolates.
4-Results

From the hundred wound swabs specimens tested only twenty five isolates were fully identified and selected to conduct our study. There are:

*Staphylococcus aureus* (five isolates).

*Escherichia coli* (five isolates).

*Pseudomonas aeruginosa* (five isolates).

*Proteus mirabilis* (five isolates).

*Klebsiella pneumonia* (five isolates).

In this work two reference antibiotics (Azithromycin and Ciprofloxacin) were used as controls or reference antibiotics. The Minimum Bactericidal concentration of these antibiotics (serial dilutions) against standard bacteria tested (Gram +ve and Gram –ve) were calculated and compared with that of tested *Honey* and *Acacia nilotica*, to determine which one has the most effective antibacterial activity.

The Azithromycin was only effective against *Staphylococcus aureus* (gram positive) with the minimum bactericidal concentration 20%, while *Staphylococcus aureus* and *Pseudomonas aeruginosa* were resistant. The second antibiotic (i.e. Ciprofloxacin) exhibited antibacterial activity only against gram negative standard organisms, by showing (MBC) of 5% with *E.coli*, and 10% with *P.aeruginosa* (Table 1).

In the other hand The inhibition zone diameter of *honey* against standard bacteria showed that *Staphylococcus aureus* was the most susceptible organism, followed by *Escherichia coli* and lastly *Pseudomonas aeruginosa* which was the most resistant standard organism, therefore the minimum bactericidal concentration (MBC) of *honey* against standard bacteria was 5% for *Staphylococcus aureus*, 20% For *Staphylococcus aureus* and 40% for *Pseudomonas aeruginosa* (Table 3).
The testing of antimicrobial activity of *Acacia nilotica* methanolic extract (sterilized by filtration) against standard bacteria showed that all organisms were resistant to the 5% concentration of the extract. The minimum bactericidal concentration (MBC) was 20% for all standard bacteria tested (Table 4).

Also the testing of *Acacia nilotica* methanolic extraction (sterilized by autoclave) against standard bacteria showed that no zones of inhibition for these organisms at 5% (Table 5).

The minimum bactericidal concentration (MBC) of *Acacia nilotica* methanolic extraction (sterilized by autoclave) was 20% for all standard organisms (Table 5). Concluding that both *Acacia nilotica* methanolic extract sterilized by filtration and that sterilized by autoclaving exhibited the same activity against standard organisms.

The most sensitive standard organism was *Staphylococcus aureus*, followed by *Escherichia coli* and *Pseudomonas aeruginosa* when the testing of *Acacia nilotica* aqueous extract activity was performed against the standard organisms. Accordingly the minimum bactericidal concentration (MBC) of *Staphylococcus aureus* was 20%, while it was 40% for both *Escherichia coli* and *Pseudomonas aeruginosa* (Table 6).

**Table (7)** shows the mean inhibition zone diameter in (mm) of honey against clinical isolates tested and the number of isolates were inhibited by each concentration. The 100% and 80% concentrations of honey were successfully inhibited all the number of isolates for each organism, while the 60% concentration inhibited the five isolates of only *Staphylococcus aureus*, *Escherichia coli* ([Figure 1](#)) and *Klebsiella pneumoniae* ([Figure 2](#)), four and three isolates of the *Proteus mirabilis* and *Pseudomonas aeruginosa* isolates respectively. Both 40% and 20% were effectively inhibited all *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella pneumoniae* isolates, but only four *Proteus mirabilis* isolates and one isolate of *pseudomonas aeruginosa* were inhibited by 40% concentration, while the 20% concentration inhibited only one *Proteus mirabilis* isolate and failed in inhibition of any *Pseudomonas aeruginosa* isolate. Finally the 5% concentration inhibited three *Staphlococcus aureus* isolates, only one isolate of both *Escherichia coli* and *Klebsiella pneumoniae* and it entirely failed in inhibition of both *pseudomonas aeruginosa* and *proteus mirabilis* isolates. The mean of the minimum bactericidal concentration (MBC) of *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella pneumoniae* was 5%, while it was 40% for *Pseudomonas aeruginosa* and 20% for *Proteus mirabilis*.
The mean inhibition zone of *Acacia nilotica* methanolic extraction (Sterilized by filtration) against clinical isolates tested is shown in (Table 8). Actually the isolates of all organisms were inhibited by all concentrations of the extract except of 5% concentration which inhibited only one isolate of *Escherichia coli*.

The mean of the minimum bactericidal concentration (MBC) of *Acacia nilotica* methanolic extraction (sterilized by filtration) against clinical isolates tested was 20% for all organisms except of *Escherichia coli* which was 5%. (Table 8).

The testing of *Acacia nilotica* methanolic extraction (Sterilized by autoclave) showed that all isolates were inhibited by all concentrations except of 5% concentration which inhibited only three *Staphylococcus aureus* isolates, four *Escherichia coli* isolates, one *Pseudomonas aeruginosa* isolate, two *Klebsiella pneumoniae* isolates and failed in inhibition of any *Proteus mirabilis* isolate. (Table 9).

The mean of the minimum bactericidal concentration (MBC) of *Acacia nilotica* methanolic extraction (sterilized by autoclave) against clinical isolates tested, which it was 5% for all organisms except *Proteus mirabilis*, was 20%. (Figure 3-4-5).

On the other hand the testing of *Acacia nilotica* aqueous extraction against clinical isolates tested showed that all isolates of *Staphylococcus aureus* were inhibited by all concentrations of this extract (Figure 6), except of 5% concentration which is failed. All *Escherichia coli* isolates were inhibited by the 100% concentration, while four isolates were inhibited by 80%, three by 60%, one by 40% and none of the isolates were inhibited by the 20% and 5% concentrations. *Pseudomonas aeruginosa* isolates were entirely inhibited by all concentrations except of 40% which inhibited three isolates, 20% that inhibited two isolates while 5% failed in inhibition of any isolate. *Proteus mirabilis* isolates were inhibited by all concentrations except the 20% which inhibited one isolate and 5% that failed in inhibition of any isolate. *Klebsiella pneumoniae* isolates were inhibited by two concentrations of the extract, firstly 100% that inhibited all isolates and secondly 80% that inhibited three isolates, whereas the rest of concentrations failed in inhibition of any isolate. (Table 19). Therefore the mean of the minimum bactericidal concentration (MBC) was 20% for *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Proteus mirabilis*. While it was 40% for *Escherichia coli* and 80% for *Klebsiella pneumoniae*. (Table 10)
Table (1): the inhibition zone diameter in (mm) and the minimum bactericidal concentration of Azthromycin (reference antibiotic) against standard bacteria tested:

<table>
<thead>
<tr>
<th>Conc. % (v/v)</th>
<th>40%</th>
<th>20%</th>
<th>10%</th>
<th>5%</th>
<th>MBC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Organisms</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>24</td>
<td>16</td>
<td>_</td>
<td>_</td>
<td>20%</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>_</td>
<td>_</td>
<td>_</td>
<td>_</td>
<td>_</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>_</td>
<td>_</td>
<td>_</td>
<td>_</td>
<td>_</td>
</tr>
</tbody>
</table>
Table (2): the inhibition zone diameter in (mm) and the minimum bactericidal concentration (MBC) of ciprofloxacin (reference antibiotic) against standard bacteria tested:

<table>
<thead>
<tr>
<th>Organisms</th>
<th>Conc. %(v/v)</th>
<th>40%</th>
<th>20%</th>
<th>10%</th>
<th>5%</th>
<th>MBC</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>26</td>
<td>26</td>
<td>20</td>
<td>15</td>
<td>5%</td>
<td></td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>20</td>
<td>14</td>
<td>14</td>
<td>–</td>
<td>10%</td>
<td></td>
</tr>
</tbody>
</table>
Table (3): the inhibition zone diameter in (mm) and the minimum bactericidal concentration (MBC) of *honey* against standard bacteria tested:

<table>
<thead>
<tr>
<th>Conc. %(w/v)</th>
<th>100%</th>
<th>80%</th>
<th>60%</th>
<th>40%</th>
<th>20%</th>
<th>5%</th>
<th>MBC</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Organisms</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>37</td>
<td>31</td>
<td>29</td>
<td>28</td>
<td>25</td>
<td>6</td>
<td>5%</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>34</td>
<td>27</td>
<td>26</td>
<td>20</td>
<td>18</td>
<td>_</td>
<td>20%</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>25</td>
<td>23</td>
<td>19</td>
<td>14</td>
<td>_</td>
<td>_</td>
<td>40%</td>
</tr>
</tbody>
</table>
Table (4): the inhibition zone diameter in (mm) and the minimum bactericidal concentration (MBC) of *Acacia nilotica* methanolic extraction (sterilized by filtration) against standard bacteria tested:

<table>
<thead>
<tr>
<th>Conc. % (v/v)</th>
<th>100%</th>
<th>80%</th>
<th>60%</th>
<th>40%</th>
<th>20%</th>
<th>5%</th>
<th>MBC</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Organisms</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>20</td>
<td>19</td>
<td>17</td>
<td>16</td>
<td>16</td>
<td>_</td>
<td>20%</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>24</td>
<td>22</td>
<td>20</td>
<td>19</td>
<td>19</td>
<td>_</td>
<td>20%</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>19</td>
<td>13</td>
<td>12</td>
<td>11</td>
<td>10</td>
<td>_</td>
<td>20%</td>
</tr>
</tbody>
</table>
Table (5): the inhibition zone diameter in (mm) and the minimum bactericidal concentration (MBC) of *Acacia nilotica* methanolic extraction (sterilized by autoclaving) against standard bacteria tested:

<table>
<thead>
<tr>
<th>Conc. % (v/v)</th>
<th>100%</th>
<th>80%</th>
<th>60%</th>
<th>40%</th>
<th>20%</th>
<th>5%</th>
<th>MBC</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Organisms</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>27</td>
<td>26</td>
<td>24</td>
<td>23</td>
<td>22</td>
<td>_</td>
<td>20%</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>28</td>
<td>26</td>
<td>24</td>
<td>23</td>
<td>22</td>
<td>_</td>
<td>20%</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>24</td>
<td>18</td>
<td>17</td>
<td>16</td>
<td>15</td>
<td>_</td>
<td>20%</td>
</tr>
</tbody>
</table>
Table (6): the inhibition zone diameter in (mm) and the minimum bactericidal concentration (MBC) of *Acacia nilotica* aqueous extraction against standard bacteria tested:

<table>
<thead>
<tr>
<th>Conc. %(v/v)</th>
<th>100%</th>
<th>80%</th>
<th>60%</th>
<th>40%</th>
<th>20%</th>
<th>5%</th>
<th>MBC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphylococcus aureus</td>
<td>11</td>
<td>9</td>
<td>8</td>
<td>7</td>
<td>6</td>
<td>_</td>
<td>20%</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>12</td>
<td>10</td>
<td>9</td>
<td>7</td>
<td>_</td>
<td>_</td>
<td>40%</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>11</td>
<td>9</td>
<td>8</td>
<td>6</td>
<td>_</td>
<td>_</td>
<td>40%</td>
</tr>
</tbody>
</table>
Table (7): the mean inhibition zone diameter in (mm) and the minimum bactericidal concentration (MBC) of honey against clinical isolates tested:

<table>
<thead>
<tr>
<th>Organisms</th>
<th>Total</th>
<th>Conc. % (W/V)</th>
<th>MBC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>100%</td>
<td>80%</td>
</tr>
<tr>
<td>N</td>
<td>M (mm)</td>
<td>N</td>
<td>M (mm)</td>
</tr>
<tr>
<td>-----</td>
<td>--------</td>
<td>-----</td>
<td>--------</td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td>5</td>
<td>5</td>
<td>31</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>5</td>
<td>5</td>
<td>32</td>
</tr>
<tr>
<td><em>P. aeruginosa</em></td>
<td>5</td>
<td>5</td>
<td>25</td>
</tr>
<tr>
<td><em>P. mirabilis</em></td>
<td>5</td>
<td>5</td>
<td>30</td>
</tr>
<tr>
<td><em>K. pneumoniae</em></td>
<td>5</td>
<td>5</td>
<td>35</td>
</tr>
</tbody>
</table>

*N = number of inhibited isolates / *M(mm) = the mean inhibition zone diameter in millimeter*
Table (8): the inhibition zone diameter in (mm) and the minimum bactericidal concentration (MBC) of *Acacia nilotica* methanolic extraction (sterilized by filtration) against clinical isolates tested:

<table>
<thead>
<tr>
<th>Organisms</th>
<th>Total</th>
<th>Conc. % (V/V)</th>
<th>MBC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>100% 80% 60% 40% 20% 5% MBC</td>
<td></td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>M (mm) N</td>
<td>M (mm) N</td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td>5</td>
<td>23 5 22 5 19 5 18 5 16 0 _ 20%</td>
<td></td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>5</td>
<td>29 5 26 5 25 5 24 5 20 1 6 5%</td>
<td></td>
</tr>
<tr>
<td><em>P. aeruginosa</em></td>
<td>5</td>
<td>24 3 22 3 16 5 15 5 13 0 _ 20%</td>
<td></td>
</tr>
<tr>
<td><em>P. mirabilis</em></td>
<td>5</td>
<td>18 5 17 4 15 5 14 5 13 0 _ 20%</td>
<td></td>
</tr>
<tr>
<td><em>K. pneumoniae</em></td>
<td>5</td>
<td>24 5 22 5 21 5 20 5 18 0 _ 20%</td>
<td></td>
</tr>
</tbody>
</table>

*N = number of inhibited isolates / *M(mm) = the mean inhibition zone diameter in millimeter
Table (9): the inhibition zone diameter in (mm) and the minimum bactericidal concentration (MBC) of *Acacia nilotica* methanolic extraction (sterilized by autoclaving) against clinical isolates tested:

<table>
<thead>
<tr>
<th>Organisms</th>
<th>Total</th>
<th>Conc. % (V/V)</th>
<th>100%</th>
<th>80%</th>
<th>60%</th>
<th>40%</th>
<th>20%</th>
<th>5%</th>
<th>MBC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>N</td>
<td>M</td>
<td>N</td>
<td>M</td>
<td>N</td>
<td>M</td>
<td>N</td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td>5</td>
<td></td>
<td>5</td>
<td>29</td>
<td>5</td>
<td>27</td>
<td>5</td>
<td>24</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>5</td>
<td>21</td>
<td>5</td>
<td>19</td>
<td>5</td>
<td>18</td>
<td>5</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>5</td>
<td></td>
<td>5</td>
<td>34</td>
<td>5</td>
<td>31</td>
<td>5</td>
<td>30</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>5</td>
<td>25</td>
<td>5</td>
<td>24</td>
<td>5</td>
<td>23</td>
<td>5</td>
</tr>
<tr>
<td><em>P. aeruginosa</em></td>
<td>5</td>
<td></td>
<td>5</td>
<td>29</td>
<td>3</td>
<td>27</td>
<td>3</td>
<td>22</td>
<td>5</td>
</tr>
<tr>
<td><em>P. mirabilis</em></td>
<td>5</td>
<td></td>
<td>5</td>
<td>24</td>
<td>5</td>
<td>20</td>
<td>5</td>
<td>18</td>
<td>5</td>
</tr>
<tr>
<td><em>K. pneumoniae</em></td>
<td>5</td>
<td></td>
<td>5</td>
<td>28</td>
<td>5</td>
<td>26</td>
<td>5</td>
<td>25</td>
<td>5</td>
</tr>
</tbody>
</table>

*N = number of inhibited isolates / *M(mm) = the mean inhibition zone diameter in millimeter
Table (10): the inhibition zone diameter in (mm) and the minimum bactericidal concentration (MBC) of *Acacia nilotica* aqueous extraction against clinical isolates tested:

<table>
<thead>
<tr>
<th>Organisms</th>
<th>Total</th>
<th>Conc. % (V/V)</th>
<th>MBC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>100%</td>
<td>80%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>N</td>
<td>M (mm)</td>
</tr>
<tr>
<td>S.aureus</td>
<td>5</td>
<td>5</td>
<td>13</td>
</tr>
<tr>
<td>E. coli</td>
<td>5</td>
<td>5</td>
<td>16</td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>5</td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td>P. mirabilis</td>
<td>5</td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td>K.pneumoniae</td>
<td>5</td>
<td>5</td>
<td>7</td>
</tr>
</tbody>
</table>

*N = number of inhibited isolates / *M(mm) = the mean inhibition zone diameter in millimeter
Figure (1): the inhibition zones of honey against *Escherichia coli*.
Figure (2): the inhibition zones of honey against *Klebsiella Pneumoniae*. 
Figure (3): the inhibition zones of *Acacia nilotica* methanolic extract against *Pseudomonas aeruginosa*.
Figure (4): the inhibition zones of *Acacia nilotica* methanolic extract against *proteus mirabilis*. 
Figure (5): the inhibition zones of *Acacia nilotica* methanolic extract against *Staphylococcus aureus*. 
Figure (6): the inhibition zones of *Acacia nilotica* aqueous extract against *Staphylococcus aureus*. 
5-Discussion

Plants essential oil and extracts have been used for thousands of years, in food preservation, pharmaceuticals, alternative medicine and natural therapies. Therefore, it is necessary to investigate those plants scientifically which have been used in traditional medicine to improve the quality of health care.

Essential oils are potentials sources of novel antimicrobial compounds especially against bacterial pathogens. In vitro studies in this work showed that the essential oils inhibited bacterial growth but varying degrees of effect.

The finding of our study as regards the antimicrobial activity of honey are in agreement with several studies cited below. In 2010 Nur Azida proved that both Gram-positive and Gram-negative bacteria isolated were completely inhibited by the honey tested. Also in 2004, Patricia reported that the bactericidal activity of honey against pathogenic bacteria was studied; showing that 93% of bacteria were inhibited by tested honey. Hyungjae, et al in 2007 reported that 92.5% of bacterial isolates of different floral sources was inhibited by honey. In 2009 Alandejani T., conducted a study and proved that 100% of the isolates of Staphylococcus aureus and Pseudomonas auropgiosa were effectively inhibited by honey. However, our study in Acacia nilotica was in disagreement with a study done in 2010 reported that methanolic extraction inhibit Klebsiella pnemoniae, S.dysenteriae and S.aureus while water extraction inhibit the isolates with bigger zone.

In our study, honey and Acacia nilotica extracts exhibited activity against the selected bacterial strain. Methanol extracts of Acacia nilotica produced significant diameter of zone inhibition due to its major components. This is because methanol is an organic compound and liberate active component required for antimicrobial activity. Smaller zones of inhibition were seen with water extract. Water may not liberate active component required for antimicrobial.

Comparing standard organisms with reference antibiotics tested resulted in that Azithromycin and all Acacia nilotica extracts had equal effectivity against S.aureus, while honey was more effective than Azithromycin against S.aureus.

Against Gram negative standard bacteria tested, Ciprofloxacin was more effective than honey and Acacia nilotica extracts.

Comparing clinical isolates with standard organisms; honey, aqueous and methanolic (sterilized by filtration) extracts of Acacia nilotica were equally effective against S.aureus, while methanolic extract of Acacia nilotica sterilized by autoclave more effective against clinical isolates than standard S.aureus.

Regarding to Gram negative, show equal effectivity against P.aeruginosa and P.mirabilis, while more effective against clinical isolates than E.coli and K.pneumoniae standards.

Acacia nilotica sterilized by filtration exhibited equal activity against P.aeruginosa, P.mirabilis and K.pneumoniae, but showed more activity against clinical isolates than E.coli controls.
Methanolic extract of *Acacia nilotica* sterilized by autoclave was more effective against clinical isolates of *E.coli*, *P.aeruginosa* and *K.pneumoniae* than controls, while show equal activity against *P.mirabilis* isolates and controls.

Aqueous extract of *Acacia nilotica* has equal activity against clinical isolates and standards of *E.coli*, but exhibit more activity against *P.aeruginosa* and *P.mirabilis* clinical isolates than control, otherwise was more effective in *K.pneumoniae* control than isolates.

In sterilization of methanol extract, autoclaving gave the same MBC of extract which sterilized by filtration. Concluding that heating liberates the active gradients of *Acacia nilotica* and therefore empowers and does not effect on the antimicrobial activity of the extract.

Pigmented strains of *Pseudomonas* were more resistant to *honey* and *Acacia nilotica* extracts, unlike the unpigmented strains which gave wider zones of inhibition. This proves that pigmentation increase the virulence of the organisms and therefore increase the resistance to antibacterial agents.

Modified diffusion technique used in antimicrobial sensitivity testing selected to conduct our research, because some trials were done to perform the antimicrobial testing using disc diffusion technique, using filter paper discs impregnated with our sterilized extracts proved , that discs were source of contamination, may be at the level of impregnation.
6-Conclusion and recommendations

6-1- conclusion:

It was concluded that both; honey and Acacia nilotica; posses antimicrobial activities, but with varying degrees of effectiveness. Honey was the most potential antibacterial agent followed by, methanol extracts of Acacia nilotica, then Acacia nilotica aqueous extract.

We believe that this investigation together with previous studies provided support to the antimicrobial properties of honey and Acacia nilotica.

Traditional medicinal practice could provide a source for new drugs and therefore efforts should be directed to evaluate traditional medicinal practice based on scientific methodologies available. Resort new source of antimicrobial agents to treat antibiotic resistant microbes in order to avoid the high cost and the side effects of medications. These results justify the use of some plants as folk medicine.
6-2-Recommendations:

- Further advanced non-cost extraction techniques to determine the active components responsible for the antimicrobial activity. (for example using chromatography that is powerful way to extract the active gradients of any natural herbal material)
- Confirmatory invivo investigations to evaluate the antimicrobial activity of both honey and Acacia nilotica pods which need collaboration between many health and medical sectors
- Determination of the Minimum Inhibitory Concentration (MIC) using tube dilution method.


Backlund A. And K. Brener, To be or not to be – Principles of class and monotypic plant families, *Taxon*, 1998; 47: 391-400

Banso A., Phytochemical and antimicrobial investigation of bark extracts of *Acacia nilotica*, *Journal of Medicinal Plant Research*, 2009; 3(2): 82-86

Basil C. Nzeak and Faiza Almaamani. The activity of honey against *Helicobacter pylori*. *Sultan qaboos University medical journal* 2006; 6(2): 71-76


WHO (World health organization) Fact sheet no. 134, Traditional medicine, 2008

Fahd, Toufic, Botany and agriculture, pp: 815, Morelon and Rashid, 1996; 513-520


Hyungjae Lee, John J. Churey and Randy W. Warobo. The antimicrobial activity of honey from different floral sources on bacterial isolates. *International Journal of Food Microbiology* 2007; 126(1-2): 249-244.


Mithun N. M., Shashidhara S., Vivek Kumar R., Review on *Acacia nilotica* phytochemical and pharmacological profile, *Pharmacology online*, 2011; 1:345-357


Subrahmanyam M., ArchanHemady and Pawar S.G. The antibacterial activity of *honey* on bacteria isolated from wounds. *Anals of burns fire disaster* 2001;54(1)


Reagents:

**Turbidity Standard Equivalent to Mc. Farland 0.5 (Barium sulphate):**

**Content:**

- Concentrated sulphuric acid: 1ml
- Dihydrate barium chloride (BaCl2.H2o): 0.5g
- Distilled water: 150ml

**Procedure:**

Prepare 1% v/v solution of sulphuric acid by adding 1ml of concentrated sulphuric acid to 99ml of distilled water, and mix. Prepare 1% w/v solution of barium chloride by dissolving 0.5g of dehydrates barium chloride in 50ml of distilled water. Add 0.6ml of barium chloride to 99.4ml of sulphuric acid solution and mix well.

**Oxidase reagent:**

**Content:**

- Tetramethyl-P-phenylene diamine dihydrochloride: 0.1g
- Distilled water: 10ml

**Procedure:**

Dissolve the chemical in the distilled water. This reagent should be prepared immediately before use, because is unstable.
Kovac’s reagent:

Content: to prepare 20ml;
- 4-dimethylaminobenzaldehyde 1g
- Isoamylalcohol (3-methyl-1-butanol) 15ml
- Concentrated hydrochloric acid 5ml

Procedure:
- Weight the dimethyaminobenzaldehyde, dissolve in the isoamylalcohol. Add concentrated hydrochloric acid and mix well. Transfer to a clean brown bottle and stored at 2-8°C.

Preparation of media:

Nutrient agar: (oxoid code:CM 3)

Typical formula g/l

Contents:
- Lab-lemco powder 1.0
- Yeast extract 2.0
- Peptone 5.0
- Sodium chloride 5.0
- Agar 15.0
- PH 7.4 0.2

Direction:
- Suspend 208g of powder in 1 liter of distilled water, bring to dissolve completely. Sterilize by autoclaving at 121°C for 15 minutes. Cool to 50°C and pour into sterile Petri dishes. Dry the surface of the gel before inoculation.
**DNAse Agar (Oxoid code 321):**

Typical formula  

<table>
<thead>
<tr>
<th>Component</th>
<th>g/l</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Contents</strong></td>
<td></td>
</tr>
<tr>
<td>Tryptose</td>
<td>20</td>
</tr>
<tr>
<td>Deoxyribonucleic acid</td>
<td>2</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>5</td>
</tr>
<tr>
<td>Agar</td>
<td>12</td>
</tr>
<tr>
<td>PH</td>
<td>7.2</td>
</tr>
</tbody>
</table>

**Direction:**

Suspend 3.9g in 1 liter of distilled water. Bring to boil to dissolve completely. Sterilize by autoclaving at 121C for 15 minutes. Cool to 50C, and pour into sterile Petri dishes. Dry the surface of the medium before inoculation.

---

**Koser’s citrate medium (Oxoid code 65 CM):**

Typical formula  

<table>
<thead>
<tr>
<th>Component</th>
<th>g/l</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Contents</strong></td>
<td></td>
</tr>
<tr>
<td>Sodium ammonium phosphate</td>
<td>1.5</td>
</tr>
<tr>
<td>Potassium dihydrogen phosphate</td>
<td>1.0</td>
</tr>
<tr>
<td>Magnesium sulphate</td>
<td>0.2</td>
</tr>
<tr>
<td>Bromothymol blue</td>
<td>0.016</td>
</tr>
</tbody>
</table>

**Direction:**

Suspend 0.52 in 1 liter of distilled water. Bring to boil to dissolve completely. Sterilize by autoclaving at 121C for 15 minutes. Cool to 50C, and pour into sterile Petri dishes. Dry the surface of the medium before inoculation.
Kligler iron agar (Oxoid code CM33):

Typical formula \( g/l \)

**Contents:**

- Lab-lemco powder \( 3.0 \)
- Yeast extract \( 3.0 \)
- Peptone \( 20 \)
- Sodium chloride \( 5.0 \)
- Lactose \( 10 \)
- Dextrose (glucose) \( 1.0 \)
- Ferric citrate \( 0.3 \)
- Sodium thiosulphate \( 0.3 \)
- Phenol red \( 0.05 \)
- Agar \( 12.0 \)

PH 7.4 0.2

**Direction:**
Suspend 5.5g in 1 liter of distilled water. Bring to boil to dissolve completely. Sterilize by autoclaving at 121°C for 15 minutes. Cool to 50°C, and pour into sterile Petri dishes. Dry the surface of the medium before inoculation.

Muller Hinton agar (Oxoid code CM 337):

Typical formula \( g/l \)

**Contents:**

- Beef, dehydrated infusion form
- Gasien hydrolysate \( 17.5 \)
- Starch \( 1.5 \)
- Agar \( 17.0 \)

PH 7.4 0.2
Direction:

Suspend 38g in 1 liter of distilled water. Bring to boil to dissolve completely. Sterilize by autoclaving at 121°C for 15 minutes. Cool to 50°C, and pour into sterile Petri dishes. Dry the surface of the medium before inoculation.

Preparation of Mcferland turbidity standard:

1. Prepare 1% (v/v) solution of sulphuric acid by adding 1ml of concentrated sulphuric acid to 99 ml of water and mix well.
2. Prepare 1.175% (w/v) solution of barium chloride by dissolving 2.35g of di-hydrate barium chloride (Bacl₂·2H₂O) in 200ml of distilled water.
3. Add .5ml of barium chloride solution to 99.5 ml of sulphuric acid solution and mix.