Screening of some Sudanese Plant Extracts Against Common Gram Positive and Gram Negative Bacteria

By

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Abstract

*Acacia nilotica* (Garad), *Citrulls colocynthis* (Handal), *Nigella sativa* (Kamoun) and *Trigonella foenum greacum* (Helba) are plants, believed by Sudanese herbalists to have antimicrobial effect. These plants have been tested in the present study to investigate their *in vitro* potential effects against nine Gram positive and Gram negative bacteria. The selected organisms were *Bacillus cereus*, *Corynebacterium ovis*, *Staphylococcus aureus*, *Escherichia coli*, *pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Niesseria genorrhoeae*, *Porteus vulagaris* and *Salmonella typhi*.

The plants were extracted with two solvents, ethanol was used to extract the polar compounds, and the petroleum ether was used to extract the non-polar compounds, the extracts were tested by two methods Minimum Inhibition Concentration method (MIC) and the filter paper disc method. The MIC of the ethanolic extract of *Acasia nilotica*, inhibited all tested organisms at 8.3 mg/ml. The most sensitive organism was *S. typhi* which was completely inhibited at 8.3 mg/ml (no growth).

*S. aureus*, *P. vulgaris* and *N. gonorrhoeae* showed no growth at 3.8 mg/ml, 4.2 mg/ml concentration and 2.1 mg/ml concentration they showed variable degrees of inhibition to the tested organisms.

In disc method of ethanolic extract of *Acasia nilotica*, all tested Gram negative and Gram positive bacteria showed various inhibition zones at the concentration of 500 mg/ml.

The disc method of petroleum ether extract of the *A. nilotica* was found less effective. The inhibition zones of *S. typhi* were very narrow (9 mm in concentrations 500 mg / ml and 250 ml).

The MIC of the ethanolic extract of *C. colocynthis* was not effective. On the contrary, it enhances the growth of selected organisms.
In the disc method ethanolic, extract of *C. colocynthis*, *C. ovis* was most affected as it inhibited at 500 mg / ml and the inhibition zone was 11 mm., ethanolic extract of *C. colocynthis* was found to have some effect on *K. pneumoniae* and *N. gonorrhoeae* the inhibition zone were 10 mm at 62.5 mg / ml 31.25 mg / ml.

In the disc method of the petroleum ether of *N. sativa*, have a good result at 66.7 mg/ ml with *N. gonorrhoeae* and *E. coil*, which are shown no growth.

In the disc method of petroleum ether of *N. sativa*, *K. pneumoniae*, *P. aeruginosa*, *S aureus*, and *N. gonorrhoeae* were inhibited. However, *E. coil*, *P. vulgaris*, *S. typhi*, *B. cereus* and *C. vois* were resistant.

In the disc method of ethanolic extraction of *N. sativa*, all tested organisms were found resistant, except *S. aureus* and *N. gonorrhoeae*. This was inhibited at a concentration of 500 mg /ml.

In the MIC method of ethanolic extract of *T. foenum greacum* enhanced that the growth of all tested organisms. The growth on tested plant was more than that of the negative controls at these concentrations (66.7 mg/ml, 33.3 mg/ ml, 16.7 mg/ml, 8.3 mg/ml, 4.2 mg/ml and 2.1 mg/ml).

The disc method of ethanolic extract of *T. foenum greacum* at all six concentrations (15.625 mg/ml, 31.25 mg/ml, 62.5 mg/ml, 125 mg/ml, 250 mg /ml and 500 mg/ml) revealed no effect except on *C. ovis* which was inhibited at 500 mg/ml. In the disc method of petroleum ether extract of *T. foenum greacum*, *B. cereus* was inhibited at 500 mg/ml. However, other tested organisms at all these concentrations (15.625 mg/ml, 31.25 mg/ml, 62.5 mg/ml, 125 mg/ml, 250 mg/ml, and 00 mg/m) were resistant.
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Till the Seventeenth Century Botany and Medicine came down hand in hand, and then both arts became different sciences, and were separated. The botanical books ignored the medicinal properties of plants, and the medicinal books contained no plant lore. The essence of herbal was the combination of traditional plant lore, the medicinal properties of herbs, and their botanical classification (Grieve 1974).

Stories, and fairy tales of the past have often contained many references to the use of plants in medicine as treatment, many cultures around the world have strong believe in the use of many different plants as medicines (Stockwell, 1988). For examples In Africa and the Indian, the bark and seeds of *A. nilotica* are used as a source of tannins (Shetty, 1977). The species is also used for medicinal purposes. Bark of *A. nilotica* has been used for treating haemorrhages, colds, diarrhoea tuberculosis and leprosy while the roots have been used as an aphrodisiac and the flowers for treating syphilis lesions (Shetty, 1977). *Aregetona mexicana* is a plant used in Mexico as an anaesthetic during surgery, this plant is believed to be more powerful than opium, and it has an action as ematic, narcotic, purgative and sedative. It used to treat inflammation, and rheumatism (Tim, 2000). The Neem tree (Meliaceae, *Azadirachta indica*), has been studied in Asia for along time. The sun dried seeds of the plant are use by Indians to control pests in houses as well as stored cereal grains, and as a detergen resembling shampoo, for removal of lice from the head (Jotwani and Srivastova, 1984; Ibrahim, 1990).

For considerable time studying, and developing of traditional herbal medicine has been a main concern in Sudan. Thus, in 1973, The Medicinal and Aromatic Plants Research Unit was established by the National Council for Research. In 1992 this unit was up-graded to
become an institute. These developments indicates the increasing interesting in plants as a potential source of medicine.

It has long been believed in the Sudanese herbal, and medicinal plants culture that plants such as *Acacia nilotica* (Garad), *Citrullus colocynthis* (Handal), *Negella sativa* (Kamoun), and *Trigonella foenum graecum* (Helba), have antimicrobial effects. This plants are actually used by the so-called herbal doctors for treatment of many bacterial diseases but herbal doctors are traditional and not qualified Physicians. There are local uses of plants in the Sudan. Sudanese use mixture of Kamoun and Helba to treat abdominal pain and diarrhoea. Garad use as topical treatment for abscess and wounds in the Sudan. Handal use for treatment of mange.

These led us to study the effects of plants, against representative Gram negative and Gram positive bacteria. These types of pathogenic organisms and diseases they cause have been among the difficult to handle due to their resistance to many of the common antibiotics.

Organisms may develop resistance to many drugs, either rapidly or after long repeated course of therapy (Mary *et al.*, 2000). Resistance is avoided by controlling infection promptly, Inadequate doses promote the development of resistance, thereafter even greatly increased doses may fail to control the infection (http://www.merck.com/plus/manual/section13/chapter 153/153a.htm).

**Objective**

The objective of this study was to find out if some plants used in traditional medicine in Sudan, namely *Acacia nilotica* (Garad), *C. colosynthes* (Handal), *Negella sativa* (Kamoun), and *T. foenum* (Helba),
have potentials inhibitory effect \textit{(in-vitro)} against some gram positive and gram negative bacteria.

**Steps of Study**

The steps undertaken to accomplish the above objective were Firstly: collection, and extraction of plants with putative medicinal value. Secondly: cultivating, and preserving of selected pathogenic bacteria. Thirdly: the \textit{in vitro} testing of plant extracts against pathogenic bacteria, to determine their inhibitory effect and the Minimum Inhibition Concentration of extracts with antimicrobial potentials (MIC).
Chapter 1
Literature Review

1-1-1 Antimicrobial agents

Antimicrobial drugs are divided in two classes, based upon their general effects on bacterial population. These are bacteriocidal and bacteriostatic. Ehrlich 1907 found that the aserical compound, arsphenamine, was selectively toxic for Treponema pallidum, this was the first of a long series of drugs to be synthesized in the laboratory. A number of years later, Domagk 1935 showed that the red dye prontosil was effective in the treatment of Streptococcal infections. Later has been discover that it was due to sulfanomide derived from prontosil. The success of this drug stimulated a search for related compounds and resulted in the synthesis of effective compounds which is the sulfanomide group (Carter et al., 1986).

1-1-2 Antibiotics

The antibiotics are a group of complex organic chemicals which are produced initially by microorganisms during their growth and which in minute quantities have detrimental effects on other organisms (Brander and Bugh, 1977). Pasteur and Joubert 1877 first reported that air borne contaminants had lethal effect on culture of Bacillus anthracis. In 1929 Fleming observed that a fangus Penicillium Notatum, was strongly inhibitory to the growth of Staphylococci, when present on culture plate. Chain and associates 1940 succeeded in obtaining preparations from Penicillium. After the discovery of Penicillin an extensive search of antibiotics was began (Carter et al., 1997). Waksman and Lechevalier 1949 isolated a soil organism, Streptomyces fradiae which produced an
antibiotic that in crude contained an antifungal compound (Fradine) and group of antibacterial substances that were labeled neomycin. Other important antibiotics discover was ampicillin (Rolinson, and Steven, 1961).

Antibiotics act on organisms by inhibiting cell wall synthesis and activating enzymes that destroy the cell wall, increasing cell membrane permeability, interfering with protein synthesis, and interfering with nucleic acid metabolism (Mary et al, 2003).

1-1-3 Problems of antibiotics

Firstly, abroad spectrum antibiotics which uses orally affect microflora so it cause disturbance in digestion.

Secondly, the development of resistance among bacterial population exposed to antibiotics received great deal of attention both in human and Veterinary medicine (Brander and Bugh, 1977). Resistant can be cause by a variety mechanisms such as the peresece of enzymes that inactivate the antimicrobial agent; mutation in the antimicrobial agent’s target, which reduces the binding of the antimicrobial agent; the persence of an alternative enzyme for the enzyme that is inhibited by the antimicrobial agent; post transcriptional or post transcriptional modification of the antimicrobial agent; reduce uptake of the antimicrobial agent; active efflux of the antimicrobial agent; and overproduction of the target of the antimicrobial agent. In addition, resistant may be caused by unrecognized mechanisms (Tenover and Unger, 1999). Thirdly, antibiotics have been used in the therapy of animal and it may be harmful to human, and it may cause problem in the control of human diseases (Brander and Bugh, 1977). Finally, antibiotic some time is very
expensive so in recent years, medicinal plants represented a primary health source for the pharmaceutical industry. Large quantities are used for the preparation of infusions both in the countries where traditional medicine is still of great therapeutic, social, and economic importance, and in production of important pharmaceutical products a broad (Brander and Bugh, 1977).

1-1-4 Drugs resistance

Organisms may develop resistance to any drug, either rapidly or after long or repeated courses of therapy (Mary et al., 2000).

The determination of antimicrobial of a clinical isolate is often crucial for the optimal antimicrobial therapy of infected patients. This need is only increasing with increasing resistance and emergence of multidrug – resistant microorganisms. (Fluit et al., 2000). *Staphylococcus aureus* is an aggressive pathogen that represents a common cause of infections both in the community and in hospitals. Before the advent of antibiotics, *S. aureus* bacteraemia was associated with 80% mortality and the disease often occurred in young adults (Waldvogel, 1995). The advent of penicillin marked a major breakthrough in the treatment of serious *S. aureus* infections. However, many of these benefits were lost within 10 to 15 years of the introduction of Penicillin because of the widespread resistance that developed along with widespread dissemination of these resistant bacteria. *S. aureus* is a common pathogen that infects about 400,000 U.S. hospital patients a year. About one-quarter of them die. For decades, Scientists have been dreading but expecting *S. aureus* strain to emerge that is resistant to Vancomycin (EMILIA, 2002).

Production of extended-spectrum ß-lactamases (ESBLs) by *Klebsiella pneumoniae* is a widespread nosocomial problem. Appropriate infection
control and antibiotic management strategies are needed to stem the spread of this emerging form of resistance. Commonly encountered nosocomially acquired gram-negative bacteria, especially *K. pneumoniae*; produce ESBLs as an antibiotic resistance mechanism (David *et al.*, 2004).

One hundred clinical avian *E. coli* isolates were examined for their general susceptibility to a battery of antibiotics of human and veterinary significance. The prevalence of resistance to aminoglycosides range from 27% for Kanamycin to (97%) for Streptomycin among these isolates. Most *E. coli* isolates (86%) were resistant to the tetracyclin, and oxytetracyclin. Avian *E. coli* isolates were generally resistant to both streptomycin, and sulfonamides (97 of 100%). A high percentage of the *E. coli* isolates were also found resistant to Ambicillin 30% and Chloramphenicol (10%) (Lydia *et al.*, 1999).

Some of the *P. mirabilis* strains were multidrug resistant, which included some aminoglycosides and broad-spectrum cephalosporins.

The National Laboratory for Sexually Transmitted Diseases showed the distribution of antibiotic resistance of the strains of *N. gonorrhoeae* which was received and tested in 1998. They found that some strains have chromosomally mediated multiple resistance to the antibiotics tested, e.g. chromosomal resistance to Penicillin/Tetracycline/Erythromycin represents 3.2% (128) of the 4,001 strains tested. Rising ciprofloxacin resistance is associated with importation from Asia.

Emergence of Cephalosporin resistance is likely just around the corner (Canada Communicable Disease Repor, Population and Public Health Branch, 2000).
1-2 Uses of plants in medicine

"Eat leek in March and wild garlic in May, and all the year after the Physicians may play." Traditional Welsh rehme (Tyler, 1987). "An apple a day keep a Doctor away." Tradtional American rhyme. Finding healing powers in plants is ancient idea. People on all continents have long applied poultices and imbibed infusions of hunderds, if not thousands, of indigenous plants, dating back to prehistory. These plants are still widlly used in ethnomedicine around the world (Clark, 1996). Plants have been used for centuries to treat infections and other illnesses in humans, but controlled clinical studies are limited. In some cases, traditional hearlers working together with trained Scientists have begun keeping records of the safety and effectiveness of phytochemical treatments, but these are general uncontrolled studies (Norton and Addy, 1989).

1-2-1 History of the herbal medicine

Raji 2002, investigated that the in vitro activity of the extracts of *A. nilotica* and *Vitex doniana* against *Campylobacter jejuni*, *E. coli*, and *C. laridis* isolated from sheep in Zaria and Kaduna. Water and ethanol crude extracts of *Acacia nilotica* and *V. doniana* were tested on the thermophilic *Campylobacter* species. The results obtained show that ethanol extract of *A. nilotica* had Minimal Inhibition Concentration (MIC) of 80 mg/ml, while, water extract of the same plant gave an MIC of 250mg/ml. However, ethanol extracts of *V. doniana* had no inhibitory effects on the *Campylobacter* species tested. *A. nilotica* and *V. doniana* were used at concentrations ranging from 2 to 200 mg/ml of the extracts. Ethanol extract of *A. nilotica* at concentration of 200 mg/ml and 20 mg/ml had inhibitory diameters zone of 6mm and 4mm respectively. Water extract of the same plant at concentrations of 200 mg and 20 mg had diameters of only 2 mm and 1 mm, respectively. There was very little
or no inhibitions with *V. doniana* water and ethanol extracts. The emergence of *Campylobacter* strains resistant to most common antibiotics highlights the need to explore new methods for therapeutics against *Campylobacter* infections. This study has demonstrated that extracts of *A. nilotica* showed antibacterial activities against *Campylobacter* species isolated from sheep in Zaria and Kaduna. In Jordan the antimicrobial activity of ethanol extract of 15 plants used in traditional medicine there and in other countries were tested *in vitro* against 12 pathogenic bacteria. 25 mg/well of 12 plant extracts have antimicrobial activity. Three plants, exhibited broad spectrum antibacterial activity. These plants were *Puncia granatum*, *Quercum infectoria olive*, and *Rhus criarial*. The most susceptible bacteria were *P. aeruginosa*, *B. cereus*, and *S. pyogenes* (ATCC 12351) (Laila *et al*., 1999).

One hundred fifty-two methanol and water extract of different parts of 71 plants commonly used in Sudanese traditional medicine were screened for the inhibitory effects on hepatitis C virus (HCV) and protease (PR) using *in-vitro* as methods. Of these, methanol extracts of *A. nilotica*, *Boswellia carterii*, *Embeliaschimperri*, *Quercus infectoria*, *Trachyspermum ammi* and water extracts of *Piper cubeba*, *Q. infectoria* and *Syzygium aromaticum*, were the most active (Hussein, *et al*., 2000). *Cassia senna* and *C. italica*, were reported in literature as purgatives (Friedrich and Steften, 1973).

The aqueous extraction of *A. nilotica* and *Hibiscus sabderiffa* were tested for anti–inflammatory, analgesic and anti-pyretic activities in animal models. *A. nilotica* extract had an inhibitory effect on carrageenan induced paw edema and yeast induced pyrexia in rats. *H. sabdariffa* extract had no effect on paw edema but had an inhibitory effect on yeast induced pyrexia. Among the phytoconstituants found in both plants,
flavanoids, polysaccharides and organic acids may be mainly responsible for their pharmacological activities (Dafallah and al-Mostafa, 1996).

The Neem tree (Meliaceae, *Azadirachta indica*), has been studied in Asia for a long time. The sun dried seeds of the plant are used by Indians to control pests in houses as well as stored cereal grains, and as a detergent resembling shampoo, for removal of lice from the head (Jotwani and Srivastova, 1984; Ibrahim, 1990).

The Neem tree has also been found to have an antimicrobial effect (Khalid et al., 1989).

*Aregemona mexicana* is a plant used in Mexico as an anaesthetic during surgery, this plant is believed to be more powerful than opium, and it has an action as emetic, narcotic, purgative and sedative. It is used to treat inflammation, and rheumatism (Tim, 2000).

*Cucurbita maxima* and *Cucurbita pepo* seeds are used in all world for the treatment of tapeworms (Oliver, 1986).

Plant constituents possessing antimicrobial activity include flavonoids in *Polygonum sensgalense* (Polygonaceace), phenol chlorophrien (Lewis and Elvin Lewis, 1977) in *Chlorophora excelsa* (Moraceae), thymol and eugenol (Jain and Jain, 1972). The antimicrobial activity of 11 essential oils from aromatic plants against the strain INRAL2104 of the food borne pathogen *Bacillus cereus*, suggests that the use of cinnamon essential oil can be consider as an alternative to "traditional food preservative" (Valero and Salmeron, 2003).

Saponins in *Waburgia ugandensis* water extract elicited antimicrobial activity against *E. coli* and *S. aureus* in agar well assay, and antifungal activity against *Candida albicans*, in study do in Uganda (Olila et al, 2001). These are little from large kinds of medicinal plants used in the world.
1-2-2 Herbal medicine in Sudan

Sudan is the largest country in Africa with an area of 2496138 sq. km (Eltoham, 2003). Through it’s long history, the Sudan has witnessed the fusion of many cultures, Pharonic, Christian and Islamic along with the local indigenous cultures. With this unique history and vast variety of climate, terrain, fauna and flora, the people of the Sudan have developed their own unique traditional medical culture. They knew the secrets of herbal, mineral salts waters, animal products and extracts have all been in dispensable ingredient of traditional medicament. A wide array of health protective and curative measure are known. Not only for humans but also for animals some of these measures are useful, others are followed by custom and tradition they may be harmful. For a considerable time, studying and developing of traditional herbal medicine have been a main concern in Sudan. Thus, in 1973 Medicinal and Aromatic Plants research Unit was established by the National Council for research. During 1983, this unit was upgraded to become institute. In 1992, Traditional Medicine Research institute joined the medicinal and aromatic plants research institute (Mohammed Galal, 2003). The medicinal and aromatic plants found in Sudan are both wild (table 2) and cultivated (table 3).
Table 1: Wild Medicinal and Aromatic Plants commonly found and used in the Sudan*

<table>
<thead>
<tr>
<th>Scientific name</th>
<th>Local name(s)</th>
<th>الاسم المحلي</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Acacia nilotica</em> (linn.) Willd. ex Del</td>
<td>Sunt, Garad(fruit)</td>
<td>سنت، قرض</td>
</tr>
<tr>
<td>Subsp. Nilotoca Brenan</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subsp. Tomentosa (Benth.)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Acacia Senegal</em> (linn.) Willd var senegal</td>
<td>Hashb</td>
<td>شباب</td>
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<tr>
<td><em>Acacia syal</em> Del.</td>
<td>Talh</td>
<td>طلع</td>
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<tr>
<td>Var. fistula Brenan</td>
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<td>Var. fistula (SSchweinf.) Olive.</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Aloe spp</em></td>
<td>Sabbar</td>
<td>صبار</td>
</tr>
<tr>
<td><em>Agremone mexicana</em> L.</td>
<td>Agresone</td>
<td></td>
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<td><em>Ambrosia martina</em> L.</td>
<td>Damsisa</td>
<td>دمسية</td>
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<tr>
<td><em>Balanites aegyptiaca</em> Del.</td>
<td>Heglig, laloub</td>
<td>هجليج، تلاوب</td>
</tr>
<tr>
<td><em>Boswellia papyrfera</em> (Del.) Hochst.</td>
<td>Targ – targ, kakal, luban</td>
<td>طريق طرق، لبنان</td>
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<tr>
<td><em>Citrillus colocynthis</em> (L.) Schard</td>
<td>Handal</td>
<td>حنضل</td>
</tr>
<tr>
<td><em>Cymbopogon proximus</em> (Hochst.) Stsph.</td>
<td>Mahareb</td>
<td>محريب</td>
</tr>
<tr>
<td><em>Datura innoxia</em> Mill</td>
<td>Alsakran</td>
<td>السكراك</td>
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<tr>
<td><em>Daturametel</em> Mill</td>
<td></td>
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<tr>
<td><em>Dioscoerea</em> spp.</td>
<td>Dioscera</td>
<td>الداكرة</td>
</tr>
<tr>
<td><em>Hapolophyllum tuerculata</em> (Forssk.) A Juss</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Rauvolfia vomitoria</em> Afz</td>
<td>Rawolfia</td>
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<tr>
<td><em>Tamarindus indica</em> L.</td>
<td>Aradib.</td>
<td>العرطب</td>
</tr>
</tbody>
</table>

Table 2: Cultivated Medicinal and Aromatic Plants commonly found and used in the Sudan*

<table>
<thead>
<tr>
<th>Scientific name</th>
<th>Local name</th>
<th>الاسم المحلي</th>
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<tbody>
<tr>
<td><em>Azadircha indica</em> A. Juss</td>
<td>Neem</td>
<td>نيم</td>
</tr>
<tr>
<td><em>Brassica nigra</em> (L) Koch.</td>
<td>Khardal aswad</td>
<td>خردل اسود</td>
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<tr>
<td><em>Carica papya</em> L.</td>
<td>Babai</td>
<td>يا بي</td>
</tr>
<tr>
<td><em>Datura stramonium</em> L.</td>
<td>Sakran</td>
<td>سيكان</td>
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<tr>
<td><em>Foeniculum vulgare</em> Mill</td>
<td>Shamur</td>
<td>شمار</td>
</tr>
<tr>
<td><em>Grewia tenax</em> (Forssk.) Fiori</td>
<td>Godeim</td>
<td>قضيب</td>
</tr>
<tr>
<td><em>Hibiscus sabdariffa</em> L.</td>
<td>Karkadeh</td>
<td>كركدى</td>
</tr>
<tr>
<td><em>Hyoscyamus muticus</em> L.</td>
<td>Sakran musri</td>
<td>سيكان مصري</td>
</tr>
<tr>
<td><em>Nicotiana rustica</em> L.</td>
<td>Tuback, kamsha</td>
<td>توباك</td>
</tr>
<tr>
<td><em>Nigella sativa</em> L.</td>
<td>Kamoon Aswad</td>
<td>كمون اسود</td>
</tr>
<tr>
<td><em>Ocimum basilicum</em> L.</td>
<td>Reehan</td>
<td>ريحان</td>
</tr>
<tr>
<td><em>Ricinus communis</em> L.</td>
<td>Knirwi</td>
<td>خروع</td>
</tr>
<tr>
<td><em>Senna alexandria</em> Miller</td>
<td>Senna maka</td>
<td>سمكة</td>
</tr>
<tr>
<td><em>Solenostema arghel</em> (Del) Hayne</td>
<td>Hargel.</td>
<td>حرجل</td>
</tr>
</tbody>
</table>

1-3 Common Medicinal Plants

1-3-1 *Acacia nilotica* (Garad)

*Acacia nilotica* (L.) Del. (Leguminosae) is a thorny wattle native to India, Pakistan and much of Africa (Brendan, 1983). In Africa and the Indian subcontinent, *A. nilotica* is extensively used as a browse, timber and firewood species (Gupta 1970, Mahgoub 1979). The bark and seeds are used as a source of tannins (Shetty 1977). The species is also used for medicinal purposes. Bark of *A. nilotica* has been used for treating haemorrhages, colds, diarrhoea, tuberculosis and leprosy while the roots have been used as an aphrodisiac and the flowers for treating syphilis lesions. The gum of *A. nilotica* is sometimes used as a substitute for Gum Arabic (obtained from *A. senegal*) although the quality is inferior. The species is suitable for the production of paper and has similar pulping properties to a range of other tropical timbers (Gupta 1970).

The potential toxicity of *Acacia nilotica* was investigated in rats maintained 2 % and 8 % *Acacia nilotica* diet for 2 and 4 weeks. It is concluded that *Acacia nilotica*, at 2 % and 8 % levels, has low toxicity potential (Al-Mustafa and Dafallah, 2000). In China, they found that aqueous extract of *A. nilotica*, had an inhibitory effect on carrageen an induced paw edema and yeast- induced pyrexia in rats (Dafallah and Al-Mustafa, 1996).

1-3-2 *Citrullus colocynthis* (Handal)

The resin of *C. colocynthis* (Cucurbitaceae) is used as a gastro- intestinal stimulant and as a powerful purgative as well as hydrogogue therapeutic and anti-rheumatic cure in traditional medicine in Sudan (Eltohami 2003)
**C. olocynthis** is used to treat bronchitis, throat diseases, tubercular glands, ulcer and tumors (Ageel *et al.* 1987).

Ehab Mostafa Bakr 1996 investigated that, hexane extract of Bitter apple seed (**C. colocynthis**) proved to be the best repellent material to red spider mite

### 1-3-3 Nigella sativa (Black Seed, Kamoun)

**N. sativa** (Black Seed) has a history of 2500 years. This makes it one of the safest plant extracts for human consumption. It is an adaptogen which means an agent causing adaptive reactions. It increases the resistance of the human body, and protecting it against various diseases. Clinical trials have shown that Black seed Oil controls Blood Sugar and Cholesterol apart from many diseases and is considered to be one of the greatest healing herbs of all times. Nearly 70% of all traditional ayurvedic formulas contain a special blend of ingredients, which includes Black seeds for this purpose. Black seed is also known as Black Cummin, Black Caraway and many other names (Muhammad *et al.*, 2002). It belongs to the family Ranunculaceae the active ingredients are a glycoside (nigelline) and oil (nigellone) present in the seeds. Nigelline and nigellone have regulation effects on blood pressure and on blood sugar levels (Gamal *et al.*, 1998).

Topical application of **N. sativa** and **Corucus sativa** extracts, inhibited skin carcinogenesis in mice. A dose of 100 mg/kg body wt of this extract delayed the onset of papillomas formation and reduced the man number of papillmas per mouse, respectively (Salomi *et al.*, 1991). The plant also has immunomodulatory and interferon like active (Medicina *et al.*, 1997).

It is possible have a therapeutic use in some conditions of cough and bronchial asthma (Muhammad *et al.*, 2002). Clinical trials also indicate
its use for control of Blood Sugar and Cholesterol. The seeds of *N. savita* are considered carminative, stimulant, diuretic, emmenagogue, galactagogue, and are used in the treatment of mild cases of puerperal fever. They are externally applied for eruptions of skin. Alcoholic extracts of the seeds show antibacterial activity against *Micrococcus pyogenes var. aureus* and *Escherichia coli*. The Black Seed and its Oil is being used in many parts of the world for a long time, because of its usefulness in the treatment of several ailments a usefulness proved scientifically and by experience through ages of use in Asia and the Gulf and Middle East Countries. The Black Seed is commonly used for many Ayurvedic preparations in Sri Lanka and India and is highly recommended as a drug in Ayurvedic Scriptures (Muhammad *et al.*, 2002).

1-3-4 *Tigonalla foenum greacum* (Helba)

*Trigonella foenum greacum* (Leguminosae) Common name is Fenugreek. The medicinal uses of Fenugreek is much used in herbal medicine, especially in North Africa, the Middle East and India. It has a wide range of medicinal applications. The seeds are very nourishing and are given to convalescents and to encourage weight gain, especially in anorexia nervosa. The seeds should not be prescribed medicinally for pregnant women since they can induce uterine contractions. Research has shown that the seeds can inhibit cancer of the liver, lower blood cholesterol levels and also have an antidiabetic effect (Chevallier, 1996). The seed and leaves are anticholesterolemic, anti-inflammatory, antitumor, carminative, demulcent, deobstruent, emollient, expectorant, febrifuge, galactogogue, hypoglycaemic, laxative, parasiticide, restorative and uterine tonic (Bown 1995). The seed yields a strong mucilage and is
therefore useful in the treatment of inflammation and ulcers of the stomach and intestines (Chevallier, 1996). Taken internally, a decoction of the ground seeds serves to drain off the sweat ducts (Chiej 1984). The seed is very nourishing and body-building and is one of the most efficacious tonics in cases of physical debility caused by anaemia or by infectious diseases, especially where a nervous factor is involved (Phillips and Foy, 1990). It is also used in the treatment of late-onset diabetes, poor digestion (especially in convalescence), insufficient lactation, painful menstruation, labour pains etc (Bown 1995). The seeds freshen bad breath and restore a dulled sense of taste (Chevallier, 1996).

Externally, the seeds can be ground into a powder and used as a poultice for abscesses, boils, ulcers, burns etc, or they can be used as a douche for excessive vaginal discharge (Grieve, 1994). The leaves are harvested in the growing season and can be used fresh or dried. The seeds are harvested when fully ripe and dried for later use (Bown, 1995). Compounds extracted from the plant have shown cardiotonic, hypoglycaemic, diuretic, antiphlogistic and hypotensive activity (Duke 1985). One of its constituent alkaloids, called 'trigonelline', has shown potential for use in cancer therapy. The seed contains the saponin diosgenin, an important substance in the synthesis of oral contraceptives and sex hormones (Phillips and Foy, 1990).

1-4 Extraction

Water is almost universally the solvent used to extract activity. At home dried plants can be ingested as teas (plant steeped in hot water) or, rarely, tinctures (plant in alcoholic solutions) or inhaled via steam from boiling suspension of parts. Dried plant part can be added to oils or petroleum jelly and applied externally. Poultices can also be made from concentrated teas or tinctures (Brantner and Grein, 1994) (Thomson, 1978). Initial
screenings of plants for possible antimicrobial activities typically being by using crude aqueous or alcohol extractions and can be followed by various organic extraction methods (Zhang, and Lewis, 1997). The soxhelt extraction method was used (Chairman et al., 1965) in this study.

1-5 Bacteria and Diseases they cause

Bacteria are a group of prokaryotic microorganisms that cause a number of diseases in both man and animals. Some of these bacteria have the ability to retain the crystal violet even after decolorization, these appear blue-black when observed under the microscope and are called Gram-positive bacteria, and bacteria that loose the crystal violet when decolorized, because of the high lipid content of their cell wall, preserve the safranin counter Stain are Gram-negative bacteria. (Koneman et al., 1997).

1-5-1 Bacillus

Bacillus species is a large, Gram positive, genus with endospore-forming rod. Members of the genus Bacillus are catalase positive aerobic or facultatively anaerobic and motile with the exception of B. mycoides and B anthracis. Most species grow on nutrient agar but not on MacConkey agar (Quinn et al., 2000).

1-5-2 Diseases caused by Bacillus

Anthrax is one of the most important diseases caused by bacillus. It is peracute disease characterized by septicemia and sudden death. Also, it is characterized with the exudation of tarry blood from the body orifices
of the cadaver. This disease is caused by *Bacillus anthracis* it is a zoonotic disease worldwide in distribution but it is common in Africa, Asia, South America, Eastern Europe (Blood et al., 1985).

1- 5-3 *Corynebacterium*

The Corynebacteria are small pleomorphic Gram-positive, rods (about 0.5 Mm in width) they are occur in rod, coccoid, club and filamentous shapes. Stained smears from animal tissues often reveal groups of cells in parallel (Palisades) or cells at sharp angles to each other (Chinese letters) many have metachromatic granules (high-energy phosphate stores) and these are seen best in *Corynebacterium diphtheriae*. The Corynebacteria are non-spore forming, non-acid fast catalase-positive oxides negative, usually facultatively anaerobic and the animal pathogens are non-motile (Quinn et al., 2000). *Corynebacteria* spp. are Gram positive, generally non-motile, pleomorphic bacilli. With the exception of *C. diphtheriae*, they are generally part of the normal flora and they are widely distributed in the environment. *C. ulcerans* is a commensal of horses and cattl (Wallace et al., 2000).

1-5-4 Diseases caused by *Corynebacterium* species

*Corynebacterium pyogenes* causes mastitis in cattle, and is a secondary invader in calves and sheep pneumonia, and foot diseases of sheep. There are also a number of minor diseases causes by coryrebacteria, like perinatal deaths in lambs, pituitary abscess, arthritis and bursitis. *Corynebacterium pseudotuberculosis* causes orchitis in lamb, chronic pectoral abscess in horses. *Corynebacterium renalis* cause contagious bovine pyelonephritis, urinary tract infection of cattle, cystitis and pyelonephritis of pigs (Blood et al., 1985).
*Corynebacterium pseudotuberculosis*, which is a pathogenic toxin-producing species, is found mainly in lower animals. It causes caseous lymphadenitis, abscesses, and chronic purulent infections, especially in sheep and goats, and contagious acne of horses. Occasional human disease may form from contact with infected animals or food. In the past *C. pseudotuberculosis* was used to call *C. ovis* and *Nocard bacillus* (Senk, 1995).
1-5- 5 *Staphylococcus*

Members of the genus *Staphylococcus* are a spherical bacterium (coccus) which on microscopic examination appear in pairs, short chains, or bunched, grape-like clusters. These organisms are Gram-positive. Some species are capable of producing a highly heat-stable protein toxin that causes illness in humans (Christian and Greger, 1994). The bacterial cells achieve a diameter of 0.5 to 1.5 µm. They appear individually, in pairs, tetrades, short chains (three to four cells) or in the form of irregular clusters. They are not motile, non-sporogenic, catalase positive and do not form capsules. The majority of species are facultative anaerobes. On blood agar they grow in the form of circular colonies of a yellow, whitish or golden colour. The majority of *S. aureus* strains cause complete or incomplete (ß) haemolysis of red blood cells in the blood agar after 24 to 26 hours (Varaldo, *et al*., 1978). The pathogenicity of Staphylococci depends on the capacity to produce toxins, *Staphylococcus aureus* produces the following toxins and enzymes: hemolisin, dermotoxin, enterotoxin, toxin of toxic shock syndrome and coagulase. *S. aureus* produces in addition to the enumerated toxins, also leukocidines, which destroy leukocytes (Senk, *et al*., 1995).

1-5-6 Diseases caused by *staphylococcus* species

The pathogenic Staphylococci are: *Staphylococcus aureus*, *S. intermedius*, and *S. hyicus* (most strains) are coagulase-negative, *S. epidermidis*, and *S. saprophyticus*, occur as commensals and in the environment. They cause opportunistic infections in humans and, very occasionally, in animals although usually regarded as non-pathogenic. *Staphylococcus* species cause many diseases, notably, *S. aureus* cause udder impetigo of cattle, and staphylococcal septicemia.
of the newborn, S. hyicus cause tick pyemia of lambs, and exudative epidermitis of pigs’ (Blood et al, 1985). Staphylococcal food poisoning (staphyloenterotoxicosis; staphyloenterotoxemia) is the name of the condition caused by the enterotoxins which some strains of S. aureus produce. The onset of symptoms in staphylococcal food poisoning is usually rapid and in many cases acute, depending on individual susceptibility to the toxin, the amount of contaminated food eaten, the amount of toxin in the food ingested, and the general health of the victim. The most common symptoms are nausea, vomiting, retching, abdominal cramping, and prostration. Some individuals may not always demonstrate all the symptoms associated with the illness. In more severe cases, headache, muscle cramping, and transient changes in blood pressure and pulse rate may occur. Recovery generally takes two days, However, it is not unusual for complete recovery to take three days and sometimes longer in severe cases (Christian, and Greger, 1994). The S. aureus bacterium causes illnesses that range from minor skin infections and abscesses to life-threatening diseases such as pneumonia, meningitis, and septicemia. By changing its chemical makeup slightly to evade attack, S. aureus has become resistant to many commonly used antibiotics (Balaban, 1998). Udder infection with S. aureus can develop into clinical or subclinical forms of mastitis. Clinical mastitis caused by S. aureus can occur in acute, subacute, chronic or subclinical forms (Varaldo et al., 1978).

1-5-7 Escherichia coli

E. coli is medium-sized, Gram-negative, and rods. It is often motile aerobic, and facultatively anaerobic. It is catalase positive oxides
negative. It attacks sugars fermentatively, and gases normally produced. It is usually citrates negative, it is natural habitant of large and lower small intestine of all mammals. It is usually present in larger numbers in carnivores and omnivores than in herbivores. There are surface antigens of *E. coli* like, the capsular (K) antigens are determined by sugar side chains on the lipopoly saccharide molecules of the outer membrane. The flagellar (H), and fimbrial (F) antigens are protein. Some of the well known fimbrial antigens (K88 (F4) and K99 (F5) are adhesions that allow pathogenic *E. coli* strain to intestinal cells, and colonize in the small intestine. The O, H, and K antigens can be used to serotype strains of *E. coli*, each serotype designated by the number of the antigens it bears, for example: O157: K85: H19 (Barrow and Feltham, 1993).

1-5-8 Diseases caused by *E. coli*

The genus *Escherichia* is composed of several species, but only *E. coli* is an important pathogenic in animals. *E. coli* is comprising the normal flora of the gastrointestinal tract, but can be the cause of septicemic disease in foals, calves, piglets, puppies, and lambs; and a cause of enterotoxigenic diarrhea in newborn farm animals; *E. coli* cause edema disease of pigs. It may also be an opportunistitic in almost all animal species (e.g., in urinary tract disease, abscesses, and pneumonia.

Colibacillosis occurs in all species of new born farm animals, enteric Colibacillosis of feeder pigs and mastitis cause by *E. coli* are also important disease commonly caused by this organism, it also cause acute undifferentiated diarrhea of new born calves under 10 days of age.
Coliform gastroenteritis (post weaning *E. coli* diarrhea) is a disease of weaning pigs characterized by sudden death or severe diarrhea, dehydration, and toxemia (Blood *et al.*, 1985). In human, *E. coli* O157:H7 is an emerging cause of foodborne illness. An estimated 73,000 cases of infection and 61 deaths occur in the United States each year. Infection often leads to bloody diarrhea, and occasionally to kidney failure. Most illness has been associated with eating undercooked, contaminated ground beef. Person-to-person contact in families and child care centers is also an important mode of transmission. Infection can also occur after drinking raw milk and after swimming in or drinking sewage-contaminated water (CDC, 2004).

1-5-9 *Klebsiella*

It is Gram negative, rods, none motile. It is aerobic, and facultatively anaerobic. It is catalase positive, oxidase negative. It attack sugars, it is KCN and VP positive, (important exceptions) ornithine decarboxylase not produced urea, generally hydrolysed phenylanlanine negative, it grow in MacConkey, and blood agar (Barrow and Feltham, 1993).

1-5-10 Diseases caused by *Klebsiella* Species

Although members of this group are lacking pathogenicity, some of the types are associated with mastitis, with respiratory, and urogenital infections of animals. The organism *Klebsiella pnemoniae* var *genitalium* is found infrequently in the genital tract of mares, where it causes sever metritis. It can readily be transmitted from infected to healthy mares by the stallion at time of service and instruments of the individual who examines or treats the mares. It has also been found in
ureteritis, mastitis, and septicemia in the Scottish and also been found in the mastitis in cattle (Bruner and Gillespie, 1985).

1-5-11 Diseases caused by *Klebsiella pneumoniae*

An immunocompromised patient has been reported with a *K. pneumoniae* bacteremia admitted with endophthalmitis. The source of the infection was an asymptomatic left renal calculus associated with a perinephric abscess. Persistent bacteremia resulted in the development of ecthyma gangrenosum, which has not previously been associated with *Klebsiella* spp infection (Stotka and Rupp, 1991).

1-5-12 *Neisseria* species

*Neisseria* spp. is Gram negative cocci. It is aerobic, catalase positive, oxidase positive. It attack sugar by oxidation. *Niesseia* spp. grow better on the surface of solid media than in equivalent liquid media, most strains grow better in an atmosphere with increased CO2 and it is doubtful if it will grow under strictly anaerobic condition (Barrow and Feltham, 1993).

1-5-13 Disease caused by *Neisseria* species

*N. meningitis* is a cause of an acute purulent meningitis, variously called endemic cerebrospinal fever or cerebrospinal meningitis. It may also cause subacute septicemia with petechial rash but without meningococcal meningitis. The term meningococcal infection is used to embrace these two syndromes. *N. gonorrhoeae* cause a sexually transmitted or venereal disease, gonorrhoea, which is purulent infection of the mucous membrane of the urethra and also of the cervix uterus in
female they rarely be rectal infection secondary local and metastatic complication e. g. epididymitis, salpingitis, and arthritis may occur if the primary infection is not promptly treated. Purulent conjunctivitis of the new born, ophthalmic neonatorum, and avulvovaginitis in young girls also occur as primary gonococcal infection. The none pathogenic members of the genus *Niesseria* are common which are also reservoir of meningococcus. They included *N. cattarhlis*, *N. flava*, and *N. sicca* (Cruickshank *et al.*, 1973).

Gonorrhoea causes a severe urethritis, with green urethral discharge and dysuria. It causes proctitis in women and homosexual men, who may present with purulent discharge, bleeding and rectal pain. Gonorrhea is easily transmitted during oral, vaginal, or anal sex. The bacteria can infect the throat, producing a severe sore throat (gonococcal pharyngitis). It can infect the vagina, causing irritation with drainage (vaginitis), or the anus and rectum, producing a condition called proctitis. In addition, the organisms may spread up to the female reproductive tract, through the cervix and uterus, into the fallopian. The infection is called pelvic inflammatory disease (PID). This occurs in 10% to 15% of women with untreated gonorrhea. If the bacteria spread beyond the fallopian tubes, it can spread into the abdominal space and cause a severe infection, peritonitis. The bacteria can also spread to the blood stream causing gonococccemia and may settle in a joint causing gonococcal arthritis. On rare occasions gonorrhea can spread through non-sexual contact. An infected woman may transmit the infection to her newborn during childbirth. Infection of the newborn's eyes is called ophthalmia neonatorum (gonococcal conjunctivitis). Young girls who contract gonorrhea either from sexual abuse or intimate contact with recently
contaminated objects (such as a damp towel) develop a severe infection called vulvovaginitis (Sonya, 2002).

**1-5-14 Porteus species**

It is Gram negative bacteria, rods, motile. Aerobic, and facultatively anaerobic. It is catalase positive and oxidase negative. It attacked sugar fermentatively usually with gasis production. It is Phenylalanine positive, Urea hydrolyzed, and Gelatin hydrolyzed (Barrow and Feltham, 1993).

**1-5-15 Diseases caused by Porteus species**

*P. mirabilis* isolated from the faeces of healthy human and also from pathological specimens. There is still doubt as to the specific relationship of *P. morganii* to the syndrome known as summer diarrhoea infants. *Proteus* strain is often found as concomitants of *Shigella* and makes their appearance in the stool of the patient recovery from bacillary dysentery. In some cases of chronic otitis, *Proteus* strain is found alone or associated with pyogenic cocci, *Proteus* also occur as secondary invaders in wound, bedsores and the like where the infection is often endogenous. *Proteus* species are also intermitted urinary tract infections (Cruickshank etal., 1973).

**1-5-15 Pseudomonas species**

It is Gram negative rods, motile by polar flagella. It is aerobic, catalase positive, oxidase positive, produce alkaline in open tube of OF media, attacks few or no carbohydrates. The medium sized of it (0,1-0,5 × 1,5-
5.0 µm). Some species produce soluble pigments and most well grow on MacConkey agar (Quinne et al., 2000).

1-5-16 Disease caused by *Pseudomonas*

*Pseudomonas mallei* cause glanders or farcy (the skin form) in the equidae, Human and members of the cat family are susceptible with occasional infections. There are resistant to infection cause by *Pseudomonas mallei* In dogs, goats, sheep, camels, cattel, pigs, rats and birdsare (Quinne et al., 2000).

1-5-16-1 Glanders

Glanders is disease have two form, acute form with high fever, mucopurulent, nasal discharging, respiratory signs, septicemia, and death with in two weeks. Chronic form of gladers have two form, pulmonary form in this form there is nodules in lungs that break down and discharge in to bronchioles. An other form is cutaneous form or farcy, which is alymphangitis with ulcers along lyphatic vessels of the limbs and chest, the ulcer eventually heal leaving star shape scars. *P. mali* cause in humans, cats, and other disease (Quinne et al., 2000).

1-5-17 Diseases caused by *Pseudomonas aeruginosa*

*P. aeruginosa* is a Gram-negative rod that is ubiquitous in nature and an opportunistic pathogen in humans. *P. aeruginosa* is a particularly virulent pathogen that produces many exotoxins and enzymes that are virulence factors. *P. aeruginosa* produces a slime-enclosed biofilm that protects it from environmental elements and from host antibodies and phagocytes. Pneumonia caused by *P. aeruginosa* is associated with a poor
outcome with strikingly increased mortality rates compared with other pathogens (Jeremy et al., 2003).

1-5-18 *Salmonella* species

It is Gram negative, motile (a few acceptation). It is facultatively anaerobic, the medium sized rods attack sugar by produce of gasis, it is citrate positive usually KCN negative (except sub genus IV). It grows in blood agar and MacConkey agar. The colones *Salmonella* spp. are pale. It is Lysine decarboxylase usually positive except *S. paratyphi* A.

It is lactose negative, cattalase positive and oxidase negative. *Salmonella* species are divided in to over 2000 serotypes, more recently the genus hass been divided in 7 sub groups. The reservoir for salmonellae is the intestinal tract (Quinn et al., 2000).

1-5-19 Diseases caused by *Salmonella* species

Salmonellosis (paratyphoid) is disease of all animals cause by a number of different species of *Salmonella* and it manifested clinicaly by one of three major syndromes: a peracute septiceamia and acute enteritis. In Salmonellosis there are often dysentery with whole blood being passed in larg clots, and abdominal pain, in pregnant cows there are abortion. In ovine and capraine the form of disease is acute enteritis there may be some cause in septiceamia form. Abortion is common also, and is caused by *S. abortus ovis*. In equine Salmonellosis is sever acute, fulminating enterits with diarrhea, fever, dehydration, and neutropenia. Abdominal pain is common form of disease. Abortion in mare and septiceamia in foals cause by *S. abrtivoegidae* characterized by abortion in famle, testicular in male, and septiceamia in the newborn. Abortion in Ewes cause by *S. abortus ovis* which is uncommon cause of abortion in
ewes (Blood et al., 1985). Salmonella is also a bacterium that is widespread in the intestines of birds, reptiles and mammals. It can spread to humans via a variety of different foods of animal origin. The illness it causes is salmonellosis which is typically includes fever, diarrhea and abdominal cramps. In persons with poor underlying health or weakened immune systems, it can invade the bloodstream and cause life-threatening infections (CDC, 2003). Salmonella is also cause Typhoid fever which caused by S. typhi (Hassan and Gumaa, 1985).
Table 3: Some Disease caused by Some Gram positive and Gram negative bacteria

<table>
<thead>
<tr>
<th>Bacteria species</th>
<th>Disease</th>
<th>host</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Bacillus cereus</em></td>
<td>Food poisoning</td>
<td>All animals</td>
<td>Christian and Greger, 1994</td>
</tr>
<tr>
<td><em>corynebacteria ovis</em></td>
<td>caseous lymphadenitis</td>
<td>Sheep &amp; goats</td>
<td>Dorland's Illustrated Medical Dictionary 2004</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>udder impetigo</td>
<td>cattle</td>
<td>Blood <em>et al</em> 1985</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>Colibacillosis</td>
<td>Newborn farm animals</td>
<td>Blood <em>et al</em> 1985</td>
</tr>
<tr>
<td><em>Klebsiella pneumoniae</em></td>
<td>Respiratory and urogenital infection</td>
<td>All animals</td>
<td>Burner <em>et al</em> 1985</td>
</tr>
<tr>
<td><em>Neisseria gonorrhoeae</em></td>
<td>urethritis</td>
<td>In human</td>
<td>Sonya 2002</td>
</tr>
<tr>
<td><em>Proteus morganii</em></td>
<td>summer diarrhoea</td>
<td>Human</td>
<td>Cruickshank <em>et al</em>, 1973</td>
</tr>
<tr>
<td><em>Salmonella typhi</em></td>
<td>Typhoid fever</td>
<td>Human</td>
<td>Hassan and Gumaa, 1985</td>
</tr>
</tbody>
</table>
Chapter Two

Material and methods

Experiments of the present study were undertaken in three phases as follows:

1- Collection and cultivation of bacteria.
2- Collection and extraction of plants.
3- *In vitro* antimicrobial testing.

2-1 Collection and cultivation of bacteria

2-1-1 Blood Agar

Blood agar (OXOID) was used in the present study for the growth of most bacteria including many of the factitious species.

Composition:
Lab-lemco powder 1.0 g; yeast extract 0.2 g; peptone 5.0 g; sodium chloride 5.0 g; agar 15.0 g; DW. 1L.,

Preparation:
Blood agar medium was prepared following the manufacturer instructions as follows:
28 gram nutrient agar were dissolved in 1 liter of deionized water, and then sterilized in an autoclave at 121°C for 15 minutes. Cooled to 50 °C before sterile fresh 100 ml blood was added. Ovine blood was used in this preparation. Sample was collected from young healthy sheep containing fibrin. Defibrinated blood was stored in sterile bottle in a refrigerator (Quinn *et al.*, 1999).
Sterile blood at the rate of 10 percent (vol/vol) was added to the cooled nutrient agar, mixed well before the plates were poured. Bubbles which formed on the surface of the poured plates were removed by passing the plate quickly on a low Bunsen flame. Then Petri dishes were left for 30 minutes to solidify.

2-1-2 Nutrient agar
The nutrient agar medium was used for the in-vitro anti microbial testing.

Composition:
Lab-lemco powder 1.0 g; yeast extract 0.2 g; peptone 5.0 g; sodium chloride 5.0 g; agar 15.0 g; DW. 1L.

Preparation
28 g. of nutrient agar (OXOID) powder were dissolved in 1 liter deionized water and then sterilized in an autoclave at 121 °C for 15 minutes cooled to 50 °C and then poured into Petri dishes (20 ml volumes per dish).

2-1-3 Bacteria
nine Gram positive and Gram negative bacteria were used in this study.
Gram positive:
1- Bacillus cereus
2- Corynebacterium ovis
3- Staphylococcus aureus
Gram negative:
1- Escherichia coli
2- Pseudomonas aeruginosa
3- Klebsiella pneumoniae
4- *Niesseria gonorrhoeae*
5- *Proteus vulgaris*
6- *Salmonella typhi*

### 2-1-3 Maintenance and preservation of cultures

All organisms were cultured on blood agar. For long preserve, organisms were transferred to slope of blood agar and kept at 4°C in a refrigerator until used.

### 2-2 Collection and extraction of plants

*Acacia nilotica* (Garad), *Citrullus colocynthis* (Handal), *Nigella sativa* (Kamoun), and *Trigonella foenum greacum* (Helba), are commonly used in Sudanese herbal medicine. These plants were selected to examine their potential *in vitro* antimicrobial activities against representatives Gram positive and Gram negative bacteria.

#### 2-2-1 *Acacia nilotica*

The *Acacia* genus includes more than 1,200 species of flowering trees and shrubs. Many of them are used medicinally for their soothing properties. It belongs to the Family Leguminosae. The *Acacia* tree is indigenous to the Nile area, Ethiopia, East Africa, Angola, Mozambique, South Africa, Arabia, Iran, Afghanistan, and India. It grows to about seventy feet with hard, woody, rusty-brown coloured bark and feathery leaves. It produces small, bright yellow flower heads and pods up to six inches long. The fruits of *A. nilotica* are know in the Sudan as Garad. It is found in wet, moist, desert, semi desert, and Savanna regions in the Sudan, and other countries. The pods and bark are used to produce tannin materials. Tannin content of ranges between 25 to 33 percentages...
(Bebawi and Neugebohrn 1991). In the Sudan Garad is used for treatment of abdominal pain as antidiarrhoetic (Eltohami, 2003).

**2-2-2 Citrullus colocynthis**

*C. colocynthis* is known in the Sudan as Handal and the common name is bitter apple. It is a climbing or prostrate herb, its leaves are thick, ovate in outline, and deeply divided. Their flowers are creamy yellow. The fruit of Handal is globose and intensely bitter. The Fruit is edible to camels. The powdered pulp, either alone or mixed with black pepper is used against the clothes moth (Bebawi and Neugebohrn 1991). In traditional medicine *C. colocynthis* is used as a gastro-intestinal stimulant and as a powerful purgative, as well as hydrogogue cathartic and anti-rheumatic cure (Eltohami, 2003). In this study, seeds of fruit were used to investigate their inhibitory effects on bacteria.

**2-2-3 Nigella sativa**

*N. sativa* is known in the Sudan as Kamuon. It is a herbal, and a seasonal plant. It is about 1 meter in height. Their seed are small, black grains with a rough surface and oily whit interior. They are roughly triangulate (Gamal *et al.*, 1998). Stem is green, round, hairy, 2-5 mm diameter, the flowers are regular bisexual, terminal on branches, white or greenish white and about 3 cm in diameter, it has long stalk, the fruits has capsules with many black trigonal seeds (Muhammad *et al.*, 2002). The Seeds of this plant have a medicinal value. It is used against cough and chest diseases. In herbal medicine the plant is used for the treatment of diseases of liver and gallbladder (Abdalazzez *et al.*, 1988).
2-2-4 *Trigonella foenum greacum*

*T. foenum greacum* is known in the Sudan as Helba. This plant belongs to the family Leguminosae. It spreads in northern Africa and some Arab countries. It is a seasonal plant and it has white flowers. The plant seeds are used topically as ground material for the treatment of abscess, skin diseases and wound healing. (Abdalazzez *et al.*, 1988).

2-3 Extraction

2-3-1 Ethanolic extraction

The soxhelt extraction method was used in this study (Chairman *et al.*, 1965). 200 gram from each ground sample was accurately weight in an empty thimble covered with cotton wool and then placed in soxhelt-extraction apparatus. Ethanol was used as solvent, 350-400 ml were added, pre-weighed round bottomed flask full of 350-400 ml of solvent was fitted to the extractor. The apparatus was assembled, the extraction process was allowed to continue for 12 hours. The apparatus was then carefully dismounted, and the solvent was evaporated first at room temperature, then dried in an oven at 105 °C of the polar content:

The polar extract was calculated as follows:

\[ \text{Polar content \%} = \frac{\text{The weight of the polar content extract (gm)}}{\text{Weight of sample (gm)}} \times 100 \]

(Chairman *et al.*, 1965).

2-3-2 Petroleum ether extraction

Finely ground sample, from each plant was accurately weight, and put in an empty thimble. The thimble covered with cotton wool, then it was placed in soxhlet extraction apparatus. In this study petroleum ether was
used as solvent. Pre-weighed round bottomed flask full of 350-400 ml of petroleum ether was fitted to the extractor, the apparatus was assembled, the extraction continued for 12 hours. The apparatus was evaporated first at room temperature. Then dried in an oven at 105 °C. The oil constant weight was expressed as percentage of the oil content.

The non-polar extract was calculated as follows:

\[
\text{Oil \%} = \frac{\text{The weight of oil extracted (gm)}}{\text{Weight of sample (gm)}} \times 100
\]

(Chairman et al., 1965).

2-4 In-vitro antimicrobial testing

The two most commonly used methods to determine antimicrobial susceptibility are the minimum inhibition concentration method (Balair et al., 1970), and the disc or agar well diffusion assay (Navarro et al., 1996). Both methods were used in this study for both ethanolic and petroleum ether extracts of each of the four plants.

2-4-1 Minimum Inhibition Concentration method (MIC)

Four gram of ethanolic extracts of each plant was added to 20 ml sterile deionized water, which was put in sterile test tube. The tube was shaken until the contents were homogenized, the concentration at this stage was 200 mg/ ml. Then 10 ml of that homogenized solvent were taken, and added to another test tube, which contained 10 ml of sterile demonized water, the concentration was 100 mg/ ml. Then 10 ml was taken and added to a third tube which contained 10 ml sterile deionized water; the concentration was then 50 mg/ ml. Then 10 ml from the third tube which
contain 10 ml sterile deionized water, the concentration was 25 mg/ml. The serial dilution was repeated until the sixth dilution. The content of the six tubes were added to six plates each one contained 20 ml sterile nutrient agar. The plates were left to solidify. The concentrations used were as follows: 66.7 mg/ml, 33.3 mg/ml, 16.7 mg/ml, 8.3 mg/ml, 4.2 mg/ml, and 2.1 mg/ml medium. Plates contained C. colocyrrhisis extrat, and T. foenum greacum extract were solidified in all above six concentrations. Plates contained A. nilotica at 66.7 mg/ml medium, 33.3 mg/ml medium, and 16.7 mg/ml medium, did not solidified, so antimicrobial testing in that connection was not examined. Plates contained N. satva extract at 8.3 mg/ml medium, did not solidified, so antimicrobial effects in that concentration was not examined.

2-4-2 Antimicrobial testing

The negative control plates which contained nutrient agar, and the positive control were plates contained nutrient agar mixed with chlorotetracyclin at a concentration of 26.7 mg/ml medium. Tested plates contained extracts of plants under study, mixed with nutrient agar. They were each inoculated by tested organisms, and results were compared with negative and positive controls. The petroleum ether extracts for all tested plants, could not be examined by MIC method, because petroleum ether extract is oily, so that extracts of examined plants could not be homogenized. therefor, results had not been taken.

2-4-3 Disc Method

The discs was prepared from small filter paper of 5 mm diameter (Whatman Co.). Before used discs for antimicrobial testing, they were
sterilized by oven, then saturated by each of the six examined plants extract (Brander and Bugh, 1977).

Extraction concentration were as follows:
One gram from plant extract was dissolved in 2 ml solvents (ethanol or petroleum ether). One ml from that 2 ml was then taken and, added to another tube contained one ml solvent. This sterile dilution continued, for six concentrations (500 mg/ml, 250 mg/ml, 125 mg/ml, 62.5 mg/ml, 31.25 mg/ml, and 15.625 mg/ml.) Discs were saturated in all above concentrations, then left to dry at room temperature before antimicrobial testing was done. The antimicrobial test started by culturing tested organisms on nutrient agar surface. Prepared discs were then placed on surface of media to examine antimicrobial effects. The result were taken by measuring the diameter of inhibition zones around saturated discs after incubation period of 18 hours at 37 °C.

2-4-3-1 Ethanolic extract
Ethanolic extract of *A. nilotica*, *C. colocyntlis N. sativa*, and *T. foenum greacum*. were diluted to series of concentrations (500 mg/ml, 250 mg/ml, 125 mg/ml, 62.5 mg/ml, 31.25 mg/ml, and 15.625 mg/ml.). Filter paper discs were soaked into the extracts with the above concentrations, and then left to dry out. The selective Gram negative, and Gram positive bacteria were cultured on nutrient agar media free of extract. The discs were then placed on the surface of culture in a clockwise direction starting from low to high concentration.

2-4-3-2 Petroleum ether extract
Petroleum ether extract of *A. nilotica. C. colocyntlis, N. sativa*, and *T. foenum greacum* were diluted to a series of concentrations (500 mg/ml, 250 mg/ml, 125 mg/ml, 62.5 mg/ml, 31.25 mg/ml, and 15.625 mg/ml.).
Dry filter paper which were saturated firstly by the suitable concentrations, placed on the surface of nutrient agar, which had already been cultured by the selected Gram positive and Gram negative organisms. The cultures were incubated for 18 hours
Chapter three

Results

3-1 Ethanol extract

3-1-1 Acacia nilotica

3-1-1-1 Disc method

The results of ethanolic extract of *A. nilotica* against test strains using disc method are shown in Table 4. Figures 1, 2 and 3. At the concentration of 500 mg/ml all tested Gram negative, and Gram positive bacteria were inhibited with various inhibition zones. But there was good result with *C. ovis* the inhibition zone at 500 mg/ml was 21 mm. and *S. typhi* which inhibition zone was 26 mm. at 500 mg/ml. *N. gonorrhoea* was inhibited after 6 days post incubation at 38 °C and the inhibition zone at the concentration 500 mg/ml was 6 mm; 250 mg/ml was 8 mm and at 125 mg/ml was 11 mm. In case of *N. gonorrhoea* the inhibition zones were observed at 500 mg/ml, 250 mg/ml and 125 mg/ml figure 2 the inhibition zones increased when the concentration was decreased.

3-1-1-2 Minimum Inhibition Concentration method (MIC)

The effects of *A. nilotica* extract against tested strains using MIC method are shown in Table 5, Figure 4, 5, 6 and 7. *S. aureus, N. gonorrhoeae, P. aeruginosa*, and *B. cereus* were completely inhibited at the concentration 8.3 mg/ml. The growth of *P. vulgaris* in this concentration was similar to that in the positive control. The growth of *E. coli* and *S. typhi* was inhibited at 8.3 mg/ml, but the degree of inhibition of these organisms using *A. nilotica* extract was found less than that obtained by chlortetracycline, the negative growth control.
Table 4: Inhibition Zones (mm) of some Gram positive and Gram negative bacteria produced by ethanolic extract of *Acacia nilotica*

<table>
<thead>
<tr>
<th></th>
<th>500 mg/ml</th>
<th>250 mg/ml</th>
<th>125 mg/ml</th>
<th>62.5 mg/ml</th>
<th>31.25 mg/ml</th>
<th>15.625 mg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Bacillus cereus</em></td>
<td>15</td>
<td>11</td>
<td>8</td>
<td>8</td>
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<td>0</td>
</tr>
<tr>
<td><em>Corynebacteria ovis</em></td>
<td>21</td>
<td>17</td>
<td>17</td>
<td>16</td>
<td>14</td>
<td>12</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>12</td>
<td>11</td>
<td>8</td>
<td>6</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>18</td>
<td>20</td>
<td>13</td>
<td>10</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>Klebsiella pneumoniae</em></td>
<td>11</td>
<td>9</td>
<td>7</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>Neisseria gonorrhoeae</em></td>
<td>6</td>
<td>8</td>
<td>11</td>
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<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>Proteus vulgaris</em></td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>10</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td><em>Salmonella typhi</em></td>
<td>26</td>
<td>16</td>
<td>16</td>
<td>16</td>
<td>15</td>
<td>13</td>
</tr>
</tbody>
</table>
Figure 1: Inhibition zone (mm) of some gram positive and gram negative bacteria using ethanolic extract of *Acacia nilotica*
Figure 2: Sensitivity of some gram positive and gram negative bacteria to ethanolic extract of _Acacia nilotica_.

- _S. aureus_
- _N. gonorrhoeae_
- _P. aerогiосsa_
- _C. ovis_

Dosages: 500 mg/ml, 250 mg/ml, 125 mg/ml, 250 mg/ml, 500 mg/ml.
Figure 3: Sensitivity of *E. coli* to ethanolic extract of *Acacia nilotica*.
Table 5: Inhibition of growth of test organisms with ethanolic extract of *Acacia nilotica* using MIC method.

<table>
<thead>
<tr>
<th></th>
<th><em>A. nilotica</em> (8.3 mg/ml)</th>
<th><em>A. nilotica</em> (4.3 mg/ml)</th>
<th><em>A. nilotica</em> (2.1 mg/ml)</th>
<th>Positive Control Chlortetracycline (26.7 mg/ml)</th>
<th>Negative control</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Bacillus cereus</em></td>
<td>++++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>-</td>
</tr>
<tr>
<td><em>Corynebacteria ovis</em></td>
<td>++++</td>
<td>+++</td>
<td>+++</td>
<td>++</td>
<td>-</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>++++</td>
<td>++</td>
<td>++</td>
<td>+++</td>
<td>-</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>++++</td>
<td>+++</td>
<td>++</td>
<td>+++</td>
<td>-</td>
</tr>
<tr>
<td><em>Klebsiella pneumoniae</em></td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>+++</td>
<td>-</td>
</tr>
<tr>
<td><em>Neisseria gonorrhoeae</em></td>
<td>++++</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td><em>Proteus vulgaris</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>
<tr>
<td><em>Salmonella typhi</em></td>
<td>++++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>-</td>
</tr>
</tbody>
</table>

+++ No growth; +++ Poor growth; ++ Growth was more than positive control and little than negative control; + Growth equal with negative control
- Good growth; - - Growth more than negative control
Figure 4: Inhibition of growth of some gram positive and gram negative bacteria with ethanolic extract of *Acacia nilotica* using MIC method
Figure 5: Inhibition of growth of test organisms at concentrations 8.3 mg/ml of the ethanolic extract of *Acacia nilotica* using MIC method.
Figure 6: Inhibition of growth of test organisms at concentrations 4.3 mg/ml of the ethanolic extract of *A. nilotica* using MIC method.
Figure 7: Inhibition of growth of test organisms in concentrations 2.1 mg/ml of the ethanolic extract of *Acacia nilotica* using MIC method.
3-1-2 *Citrullus colocynthis*

3-1-2-1 MIC Method

The result of ethanolic extract of *C. colocynthis* against tested organisms using MIC method are shown in Table 6 and Figure 8. *N. gonorrhoeae* and *S. aureus*, showed poor growth (++++) at concentration 66.7 mg/ml, while others test organisms were found resistant.

3-1-2-2 Disc method

The results of ethanolic extract of *C. colocynthis* against test strain using disc method are shown in Table 7 and Figure 9. At the concentration 15.624 mg/ml, and 31.25 mg/ml *K. pneumoneia* was inhibited, the inhibition zones were 9 mm and 8 mm, respectively. At 62.5 mg/ml, and 31.25 mg/ml *P. vulgaris* was inhibited. The inhibition zones were 10 mm and 7 mm, respectively. At 62.5 mg/ml, and 31.25 mg/ml *S. aureus* was inhibited, the inhibition zones were 9 mm and 8 mm, respectively. The inhibition zones were *N. gonorrhoeae* was inhibited the inhibition zones were 10 mm and 9 mm, respectively. At the concentration 500 mg/ml, 250 mg/ml, 125 mg/ml, and 62.5 mg/ml *P. aeruginosa* was inhibited the inhibition zones were 6 mm, 6 mm, 7 mm, and 9 mm, respectively. At the concentration 500 mg/ml, 250 mg/ml, 125 mg/ml, and 62.5 g/ml *C. ovis* was inhibited The inhibition zones were 11 mm, 8 mm, 8 mm, and 6 mm, respectively.

3-1-3 *Nigella sativa*

3-1-3-1 MIC method

The results of ethanolic extract of *N. sativa* against tested strain using MIC method are shown in Table 8, Figure 10 and 11. At 66.7 mg/ml *N.
gonorrhoeae was inhibited (++++). E. coli was inhibited (++++) at concentration 66.7 mg/ml. All other test organisms were resistant.

3-1-3-2 Disc method

The results of ethanolic extract of N. sativa against test organisms using disc method are shown in Table 9, Figures 12 and 13. At 500 mg/ml, S. aureus was inhibited the inhibition zone was 16 mm, at 250 mg/ml inhibition zone was 10 mm; at 125 mg/ml the inhibition zone was 9 mm, and at 31250 µg/ml the inhibition zone was 8 mm. N. gonorrhoeae was inhibited at 250 mg/ml and 125 mg/ml, inhibition zone was 8 mm at the two concentrations. At all other concentrations (500 mg/ml, 250 mg/ml, 125 mg/ml, 62.5 g/ml, 31.25 mg/ml, and 15.624 mg/ml) other test organisms were resistant.

3-1-4 Trigonella foenum greacum

3-1-4-1 M I C method

The results of ethanolic extract of T. foenum greacum against test organisms using MIC method are shown in Table 10 figure 14 and 15. S. aureus and N. gonorrhoeae had low growth when they were compared with positive and negative control.

3-1-4-2 Disc method

The results of ethanol extract for T. foenum greacum against tested strain using disc method are shown in Table 12. Figure 16. All test organisms were resistant, except C. ovis which was inhibited at 500 mg/ml and the inhibition zone was 10 mm. The inhibition zones which are shown at 62.5
mg/ml and 31.25 mg/ml were very narrow. So it was not considered as a positive result.
Table 6: Inhibition of growths of test organisms with ethanolic extract of *Citrullus colocynthis* using MIC method.

<table>
<thead>
<tr>
<th></th>
<th>C. colocynthis 66.7 mg/ml</th>
<th>C. colocynthis 33.3 mg/ml</th>
<th>Positive Control Chlortetracycline (26.7 mg/ml)</th>
<th>Negative control</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Bacillus cereus</em></td>
<td>++</td>
<td>_</td>
<td>+++</td>
<td>_</td>
</tr>
<tr>
<td><em>Corynebacteria ovis</em></td>
<td>_ _</td>
<td>_</td>
<td>++</td>
<td>_</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>+++</td>
<td>++</td>
<td>+++</td>
<td>_</td>
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<tr>
<td><em>Escherichia coli</em></td>
<td>_ _</td>
<td>_</td>
<td>+++</td>
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<tr>
<td><em>Klebsiella pneumoniae</em></td>
<td>_ _</td>
<td>_</td>
<td>+++</td>
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</tr>
<tr>
<td><em>Neisseria gonorrhoeae</em></td>
<td>+++</td>
<td>++</td>
<td>+</td>
<td>_</td>
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<tr>
<td><em>Proteus vulgaris</em></td>
<td>_ _</td>
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<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>++</td>
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<tr>
<td><em>Salmonella typhi</em></td>
<td>++</td>
<td>++</td>
<td>+++</td>
<td>_</td>
</tr>
</tbody>
</table>

+++ No growth; +++ Poor growth; ++ Growth was more than positive control and little than negative control; + Growth equal with negative control
- Good growth; - Growth more than negative control
Figure 8: Inhibition of growth of test organisms with ethanolic extract of *Citrullus colothyncis* using MIC method.
Tabel 7: Inhibition Zones (mm) of some Gram positive and Gram negative bacteria produced by ethanolic extract *Citrullus colocynthis*

<table>
<thead>
<tr>
<th></th>
<th>500 mg/ml</th>
<th>250 mg/ml</th>
<th>125 mg/ml</th>
<th>62.5 mg/ml</th>
<th>31.25 mg/ml</th>
<th>15.625 mg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bacillus cereus</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td>6</td>
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</tr>
<tr>
<td><strong>Corynebacteria ovis</strong></td>
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<tr>
<td></td>
<td>11</td>
<td>8</td>
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<tr>
<td><strong>Staphylococcus aureus</strong></td>
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<td><strong>Escherichia coli</strong></td>
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<tr>
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</table>
Figure 9: Inhibition zones (mm) of some gram positive and gram negative bacteria produced by ethanolic extract of Citrullus colothyncis.
Table 8: Growths of test organisms at three different concentrations of of Nigella sativa extracts and MIC method.

<table>
<thead>
<tr>
<th></th>
<th>N. sativa 10.00µg/ml</th>
<th>N. sativa 100µg/ml</th>
<th>N. sativa 10µg/ml</th>
<th>Positive Control Chlortetracycline (26.7 mg/ml)</th>
<th>Negative control</th>
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<tbody>
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</tr>
<tr>
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<td>_ _</td>
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</tr>
<tr>
<td><strong>Staphylococcus aureus</strong></td>
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<td>++</td>
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<tr>
<td><strong>Salmonella typhi</strong></td>
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<td>_ _</td>
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</tr>
</tbody>
</table>

+++ No growth; +++ Poor growth; ++ Growth was more than positive control and little than negative control; + Growth equal with negative control
- Good growth; - - Growth more than negative control
Figure 10: Inhibition of growth of test organisms with ethanolic extract of *Nigella sativa* using MIC method.
Figure 11: Growth of tested organisms in concentration 10.00µg/ml of the mixture of *Nigella sativa* extracts and nutrient agar MIC method.
Table 9: Inhibition Zones (mm) of some Gram positive and Gram negative bacteria produced by ethanolic extracts of *Nigella sativa*.

<table>
<thead>
<tr>
<th></th>
<th>500 mg/ml</th>
<th>250 mg/ml</th>
<th>125 mg/ml</th>
<th>62.5 mg/ml</th>
<th>31.25 mg/ml</th>
<th>15.625 mg/ml</th>
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<tbody>
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Figure 12: Inhibition zones (mm) of some gram positive and gram negative bacteria produced by ethanolic extract of *Nigella sativa*.
Figure 13: Inhibition zone (mm) at different concentrations of ethanolic extract of *Nigella sativa*.
Table 10: Growth of test organisms at four different concentrations of *Trigonella foenum greacum* extracts MIC method.

<table>
<thead>
<tr>
<th></th>
<th>T. foenum (66.7) mg/ml</th>
<th>T. foenum (33.3) mg/ml</th>
<th>T. foenum (16.7) mg/ml</th>
<th>T. foenum (8.3) mg/ml</th>
<th>Positive Control Chlortetracycline (26.7) mg/ml</th>
<th>Negative control</th>
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<tbody>
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<td>Bacillus cereus</td>
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<tr>
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<tr>
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<tr>
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<tr>
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<td>_</td>
<td>_</td>
<td>_</td>
<td>++</td>
<td>_</td>
</tr>
<tr>
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<td>+++</td>
<td>++</td>
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<td>_</td>
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<tr>
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<td>_</td>
</tr>
</tbody>
</table>

++++ No growth; +++ Poor growth; ++ Growth was more than positive control and little than negative control; + Growth equal with negative control
- Good growth; - - Growth more than negative control
Figure 14: Inhibition of growth of test organisms with ethanolic extract of Trigonella foenum using MIC method
<table>
<thead>
<tr>
<th>Positive control</th>
<th><em>Trigonella foenum</em> extract at (66.6 mg/ml)</th>
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</table>

*K. pneumoniae*  
*S. aureus*  
*N. gonorrhoeae*  
*E. coli*

*Trigonella foenum* extract  
at 33.3 mg/ml  
*Trigonella foenum* extract  
at 16.7 mg/ml

Figure 15: Growth of test organisms at four different concentrations of *Trigonella foenum greacum* extracts MIC method.
Table 11: Inhibition Zones (mm) of some Gram positive and Gram negative bacteria produced by ethanolic extracts of *Trigonella foenum greacum*.

<table>
<thead>
<tr>
<th></th>
<th>500 mg/ml</th>
<th>250 mg/ml</th>
<th>125 mg/ml</th>
<th>62.5 mg/ml</th>
<th>31.25 mg/ml</th>
<th>15.625 mg/ml</th>
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<tr>
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<tr>
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</table>
Figure 16: Inhibition zones (mm) of test organisms produced ethanolic extract of *Trigonella foenum* 

<table>
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<th>Concentration (mg/ml)</th>
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<td>Staphylococcus aureus</td>
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<td>62.5</td>
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<tr>
<td>31.25</td>
<td>klebsiella pneumoniae</td>
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<tr>
<td>15.625</td>
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<td></td>
<td>Pseudomonas aeruginosa</td>
</tr>
<tr>
<td></td>
<td>Salmonella typhi</td>
</tr>
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</table>
3-2 Petroleum ether extracts.
MIC method could not be applied with petroleum ether extracts, because these extracts are oily, and in MIC method water was used as a solvent of plants extract, so the resulted mixture of extracts, with media was non homogeneous.

3-2-1 Disc method
3-2-1-1 Acacia nilotica
The results of petroleum ether extract of *A. nilotica* against test organisms using the disc method are shown in Table 12 and Figure 17. The results using filter paper discs saturated with petroleum ether extract against all selected organisms showed resistant patterns (*E. coli, K. pneumonia, N. gonorrhoeae, P. vulgaris, P. areuginossa, B. cerus and C. ovis*). Except *S. tiphi* and *S. aureus* which were found sensitive. *S. tiphi* was inhibited at 500 mg/ml, 250 mg/ml, inhibition zone were 9 mm. and 10 mm, respectively. At other concentrations *S. tiphi* was inhibited with equal inhibition zones. at 250 mg/ml *S. aureus* was inhibited, the inhibition zone was 11 mm.

3-2-1-2 Citrullus colocynthis
All tested organisms (*E. coli, K. pneumonia, N. gonorrhoeae, P. vulgaris, P. aeruginosa S. tiphi, B. cerus, C. ovis, and S. aureus*) were resistant at all concentration petroluem ether extract of *C. colocynthis* (500 mg/ml, 250 mg/ml, 125 mg/ml, and 625 mg/ml). Table 13.

3-2-1-3 Nigella sativa
*N. gonorrhoeae* was the most affected organisms it was inhibited at 500 mg/ml, 250 mg/ml, 125 mg/ml, and 625 mg/ml. The inhibition zones
were 13 mm, 10 mm, 10 mm, and 8 mm. *K. pneumonia* was found sensitive and showed inhibition zones at 62.5 mg/ml, 31250 µg/ml. The inhibition zones were 10 mm and 7 mm, respectively. *P. areuginossa* was inhibited at 125 mg/ml, and 62.5 mg/ml, 31.25 mg/ml. The inhibition zones were 11 mm, 10 mm, and 9 mm, respectively. *S. aureus* was inhibited at 125 mg/ml, and 62.5 mg/ml, 31.25 mg/ml. The inhibition zones were 11 mm, 10 mm, and 8 mm, respectively. *E. coli, P. vulgaris, S. typhi, B. cerus,* and *C. ovis,* were resistant at all concentration of petroleum ether extract of *N. sativa* (500 mg/ml, 250 mg/ml, 125 mg/ml, and 62.5 mg/ml, 31.25 mg/ml, and 15.624 mg/ml). Table 14. Figure 19.

3-2-1-4 Trigonella foenum greacum

*E. coli, K. pneumonia, N. gonorrhoeae, P. vulgaris, P. areuginossa S. typhi, C. ovis,* and *S. aureus* were resistant at all concentration of petroleum ether extract of *T. foenum greacum* (500 mg/ml, 250 mg/ml, 125 mg/ml, and 62.5 mg/ml, 31.25 mg/ml, and 15.624 mg/ml). Excep *B. cerus* was inhibited 500 mg/ml the inhibition zone was 10 mm. Table 15
Table 12: Inhibition Zones (mm) of some Gram positive and Gram negative bacteria produced by petroleum ether extracts of *Acacia nilotica*

<table>
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<tr>
<th></th>
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<th>62.5 mg/ml</th>
<th>31.25 mg/ml</th>
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<tr>
<td><strong>Bacillus cereus</strong></td>
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<td>0</td>
<td>0</td>
</tr>
<tr>
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Figure 17: Inhibition zone (mm) of test organisms produced by petroleum ether extract of *Acacia nilotica*
Figure 18: Inhibition zones (mm) of *Salmonella typhi* produced by petroleum ether extracts of *Acacia nilotica*. 
Table 13: Inhibition Zones (mm) of some Gram positive and Gram negative bacteria produced by petroleum ether extracts of *Citrullus colocynthis*

<table>
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<tr>
<th></th>
<th>500 mg/ml</th>
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<tr>
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<tr>
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Table 14: Inhibition Zones (mm) of some Gram positive and Gram negative bacteria produced by petroleum ether extracts of *Nigella sativa*.

<table>
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<th></th>
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Figure 19: Inhibition zones (mm) of some gram positive and gram negative bacteria produced by petrolum ether extract of *Nigella sativa*
Figure 20: Inhibition zones of Neisseria gonorrhoeae produced by petrolum ether extract of Nigella sativa.
Table 15: Inhibition Zones (mm) of some Gram positive and Gram negative bacteria produced by petroleum ether extracts of *Trigonella foenum greacum*.

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<th>500 mg/ml</th>
<th>250 mg/ml</th>
<th>125 mg/ml</th>
<th>62.5 mg/ml</th>
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Chapter four
Discussion

There has been a lot of talk about plants having medicinal value. Some plants have been used for centuries as a treatment of infections and other illness. Some Sudanese plants are commonly used for treatment of bacterial diseases, this is why it was important to investigate their antimicrobial activities or refute these claims. In the present study four plants namely *Acacia nilotica* fruit (Garad) *Citrullus colocynthis* seeds (Hanal) *Nigella sativa* seeds (Kamoun) *Trigonella foenum greacum* seeds (Helba) which are believed amongst Sudanese herbal therapists as antimicrobial agents, were examined. Tests were made to find their possible *in vitro* effects by observing the inhibition of growth of nine selected gram positive and gram negative bacteria.

Two solvents were used to extract the four plants, ethanol used to extract polar compounds, and petroleum ether (40- 60%) used to extract the fatty compounds of these plants. The plant extracts were tested using two methods, the Minimum Inhibition Concentration (MIC) method, and filter paper discs method. This latter method was found to be more reliable than the former. Because the results of the extract efficacy is very easy to read, the result had been taken by measuring the inhibition zone by transparent ruler in millimeter.

The present study revealed some successful result. Which was ethanolic extracts of *A. nilotica* against *S. typhi*, *C. ovis*, *E. coli*, and *B. cereus*. Although, experiments in the present study have shown that the disc method of the ethanolic extract of *A. nilotica* inhibited all tested organisms with various inhibition zones. So the ethanolic extract of *A. nilotica* was the most effective as antimicrobial agent among the four plants tested.
But ethanolic extract of *A. nilotica* has poor effect against *P. aeruginosa* and *N. gonorrhoea* which was inhibited after 6 days post incubation at 38 °C at 125 mg/ml and 250 mg/ml. The petroleum ether extract of *A. nilotica*, also has antimicrobial effect but it was less than that shown in ethanolic extract of the same plant. *S. aureus*, and *S. typhi* were mostly affected by petroleum ether extract of *A. nilotica*. The minimum inhibition concentration of them was 250 mg/ml, and 15.625 mg/ml, respectively. this means that the ethanolic extract of *A. nilotica* was more effective than petroleum ether extract of the same plant as antimicrobial fraction. Raji (2002) found that the ethanolic extract of *A. nilotica* had minimal inhibition concentration (MIC) of 80 mg/ml, while water extract of the same plant gave MIC of 250mg/ml Ethanol extract of *A. nilotica* at concentration of 200 mg/ml and 20 mg/ml. had inhibitory diameters zone of 6 mm and 4 mm, respectively when were used against *E. coli* and *C. laridis* (Raji et al., 2002).

Plants commonly used in Sudanese traditional medicine had been screened for their inhibitory effects on hepatitis C virus (HIV) and protease (PR) using *inviter* as methods. Of these, methanol extracts of *A. nilotica* was one of the most active extracts (Hussein, et al., 2000). In China they found that aqueous extract of *A. nilotica*, had an inhibitory effect on carrageenan induced paw edema and yeast- induced pyrexia in rats (Dafallah and Al-Mustafa, 1996). The potential toxicity of *Acacia nilotica* was investigated in rats maintained 2 % and 8 % *Acacia nilotica* diet for 2 and 4 weeks. It is concluded that *Acacia nilotica*, at 2 % and 8 % levels, has low toxicity potential (Al-Mustafa and Dafallah, 2000).

The ethanolic extract of *C. colocynthis* was very mild as antimicrobial agent, the affected organisms were *B. cereus*, which was inhibited at 500 mg/ml and 250 mg/ml. The inhibition zones in the two concentrations
were 6 mm, and at 125 mg/ml and at 62.5 mg/ml the inhibition zones were 7 mm, and 9 mm, respectively. That means the inhibition of ethanolic extract of *C. colocynthis* against *B. cereus* was increased when the concentration of the extract was decreased.

The petroleum ether extract of *C. colocynthis* did not show any inhibition effect. So, from this study I found that ethanolic extract of *C. colocynthis* have a mild effect as antimicrobial agent. Because inhibition zones which was shown in case of *B. cereus* were very narrow to consider ethanolic extract of *C. colocynthis* as antimicrobial agent. This result is there for considered not good result and not encouraging for further future investigation.

Petroleum ether extract and the ethanolic extract of *N. sativa* were effective as antimicrobial agent. *S. aureus* and *N. gonorrhoea* were inhibited. *S. aureus* was inhibited, the minimum inhibition concentration was 31.25 mg/ml. *N. gonorrhoea* inhibited the minimum inhibition concentration was 62.5 mg/ml. Ethanol extract of *N. sativa* was inhibited *N. gonorrhoea* and *S. aureus*. In case of *S. aureus* the inhibition zone was 16 mm. at 500 mg/ml, the minimum inhibition concentration was 31.25 mg/ml.

In case of *N. gonorrhoea* the Inhibition zones was 8 mm. at two concentrations 250 mg/ml and 125 mg/ml and 10 mm at 500 mg/ml. In this study the ethanolic extract of *N. sativa* was found less effective as antimicrobial agent when it compared with petroleum ether extract against this organisms.

Beside *N. gonorrhoea* and *S. aureus* the petroleum ether extract of *N. sativa* inhibited *K. pneumonia* and *P. aeruginosa*

the minimum inhibition concentration of them was 31.25 mg/ml (Table 14).
All above results show that *N. sativa* has good antibacterial effect, and *N. sativa* has antibacterial effect in two polar compound (Table 9) and the fatty one (Table 14). This result is in agreement with Fadadalla (2002). who found that, *N. sativa* is strong antimicrobial agent, and he also found methanolic extrac of *N. sativa* was less effective than the petroleum ether extract. Alcoholic seeds extract of *N. sativa* showed antibacterial activity against *Micrococcus pyogenes* var. *aureus* and *E. coli* (Muhammad *et al*., 2002). *N. sativa* have regulation effects on blood pressure and on blood sugar levels (Gamal *et al*., 1998).

Topical application of *N. sativa* and *Corcus sativa* extracts, inhibited skin carcinogenesis in mice (Salomi *et al*., 1991). The plant also has immunomodulatory and interferon like active (Medicina *et al*., 1997). The ethanolic extract and the petroleum ether extracts of *T. foenum greacum* were found very poor antimicrobial agent. they inhibited only one organism in the ethanolic extract of the plant. This organism was *K. pneumonia* it was inhibited at 125 mg/ml, and 62.5 mg/ml and the inhibition zones were 7 mm and 9 mm, respectively. These were relatively very narrow inhibition zones.

The petroleum ether extract inhibited *B. cereus* at 500 mg/ml, the inhibition zone was 10 mm. This result was considered not good and is not encouraging for further investigation. has shown that the seeds can inhibit cancer of the liver, lower blood cholesterol levels and also have an antidiabetic effect (Chevallier, 1996). The seed and leaves are anticholesterolemic, anti-inflammatory, antitumor, carminative, demulcent, deobstruent, emollient, expectorant, febrifuge, galactogogue, hypoglycaemic, laxative, parasiticide, restorative and uterine tonic(Bown, 1995). The seed yields a strong mucilage and is therefore useful in the treatment of inflammation and ulcers of the stomach and intestines (Chevallier, 1996). Taken internally, a decoction of the ground seeds
serves to drain off the sweat ducts (Chiej 1984). The seed is very nourishing and body-building and is one of the most efficacious tonics in cases of physical debility caused by anaemia or by infectious diseases, especially where a nervous factor is involved (Phillips and Foy, 1990). It is also used in the treatment of late-onset diabetes, poor digestion (especially in convalescence), insufficient lactation, painful menstruation, labour pains etc (Bown, 1995). The seeds freshen bad breath and restore a dulled sense of taste (Chevallier, 1996). Externally, the seeds can be ground into a powder and used as a poultice for abscesses, boils, ulcers, burns etc, or they can be used as a douche for excessive vaginal discharge (Phillips and Foy, 1990). The leaves are harvested in the growing season and can be used fresh or dried. The seeds are harvested when fully ripe and dried for later use (Bown, 1995). Compounds extracted from the plant have shown cardiotonic, hypoglycaemic, diuretic, antiphlogistic and hypotensive activity (Duke 1985). One of its constituent alkaloids, called 'trigonelline', has shown potential for use in cancer therapy. The seed contains the saponin diosgenin, an important substance in the synthesis of oral contraceptives and sex hormones (Phillips and Foy, 1990).

In this study the minimum inhibition concentration method (MIC) was found to be complicated, and the results were not consistent. Results in this method had been taken by comparison between growth of tested organisms in media containing plant extracts, with growth of the positive control, and negative control on the other side.

In MIC method, the petroleum ether extract could not be applied, because the fatty compounds can not be homogenized in distil water.

In the (MIC) method *A. nilotica* fruit extract at the concentrations 66.7 mg/ml, 33.3 mg/ml; and 16.7 mg/ml; did not allow the media medium to be solidify, so antimicrobial activity of *A. nilotica* extract in these concentrations were not taken.
Finally, it is considered that *A. nilotica* fruit, and *N. sativa* seeds are strong antimicrobial agents, and the belief of the Sudanese herbalist on them are true. The disc method is a good method to screening antimicrobial activities of plant extracts.
Conclusions:
The present study concluded that:

1- The ethanolic extractions of four tested plants have variable antimicrobial effect. But ethanolic extract of A. nilotica was found more effective antibacterial agent.

2- The petroleum ether extract of A. nilotica, also has antimicrobial effect but it was less than ethanolic extract

3- The ethanolic extract of C. colocynthis was found very mild as antibacterial agent.

4- The petroleum ether extract of C. colocynthis did not show any inhibitory effect.

5- N. sativa has antibacterial effect in the two fractions: the polar, and none-polar.

6- Ethanol extract and petroleum ether extracts of T. foenum greacum were very poor antimicrobial agent.

7- The disc method is a simple method and it give clear result when it compared with MIC method.
**Recommendations:**

The present study recommends for future works that:

1- *In vivo* studies to verify the antimicrobial effect of the ethanolic extract of *A. nilotica* and the petrolum ether extract of *N. sativa*. *In vivo* study in Lab animals would encourage subsequent application in the treatment of human and domestic animal diseases.

2- Fractionation and chemical characterization of the ethanolic extract components of *A. nilotica* and of the petrolum extract of *N. sativa* to determine the active ingredients and then testing these ingredients *in vivo*.

3- More Sudanese native plants and their seeds or fruits should be screened using the Disc method, as the present study revealed some potential materials.
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