Antibacterial Activity of *Acacia nilotica* and *Zizphus spina christi* Fruits Extracts on Aerobic Bacteria Associated with Throat Infection

By:

*Suhair Eiz Aldinn Azrag Ahmed*

(B.V.Sc. May ١٩٩٩)

University of Khartoum

Supervisor:

*Dr. Suliman Mohammed El Hassan*

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Facility of Veterinary Medicine
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DEDICATION

To my dear father

To my mother soul

To my brothers and sisters

To my colleague and friends

with love

Suhair
ACKNOWLEDGMENTS

First of all my thanks and praise is due to almighty Allah, the Beneficent, the Merciful, for giving me health and strength to accomplish this work.

I am most grateful to my supervisor Dr. Suliman Mohammed El Hassan for the superb assistance, continuous guidance, encouragement, and meticulous attention, patience throughout the study.

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ABSTRACT

Some Sudanese herbalists believed that the fruits of *Acaia nilotica* and *Zizohus spina christi* can cure sore throat infection. The two plants have been tested in the present study to investigate their *in vitro* potential effect against the bacteria associated with sore throat infection.

A total of 3 throat swabs were collected randomly in Khartoum state from patients of different ages and sexes suffering from sore throat infection during the period March 2002 to June 2002.

The Gram positive bacteria isolated were *Streptococcus pyogenes* (64, 7%), *Streptococcus pneumoniae* (0.3%), *Staphylococcus aureus* (31.3%), *Staphylococcus hemolyticus* (6.7%), *Actinomyces israelii* (6.7%), *Staphylococcus capitis* (3.3%), *Corynebacterium diphtheriae* (3.3%) and *Corynebacterium ulcerans* (3.3%). The Gram negative bacteria isolated was *Haemophilus influenzae* (0.3%).

Antibiotic sensitivity test were done for each isolate. The majority of the isolates were sensitive to Ampicillin (79.7%) and Penicillin (39.2%). However, some of them (0.3%) were resistant to Cloxacillin.

*Acaia nilotica* and *Zizohus spina christi* fruits were extracted by water then the extracts were tested for their antibacterial activity by
impregnated filter paper discs methods. Three different concentrations of the extracts were examined 1\%, 2\% and 3\%.

Water extract of *Acacia nilotica* inhibited the growth of many bacteria isolated in this study. The most effective concentration was 3\% concentration as 67.44\% of the isolates showed growth inhibition at this concentration while 58.13\% at 2\% and 33.88\% at 1\% concentration. The most sensitive species to 3\% concentration of *Acacia nilotica* were *Streptococcus pyogenes* and *Streptococcus pneumoniae*.

Water extract of *Zizphus spina christi* showed weak effect on the bacterial growth of the isolates in this study as 16.27\% of the isolates showed growth inhibition at 3\% concentration, 13.63\% of the isolates showed growth inhibition at 2\% concentration and 4.60\% at the concentration 1\%. The most sensitive species to 3\% concentration of *Zizphus spina christi* was *Streptococcus pyogenes*.

In the present study *Acacia nilotica* extract was found to be effective against the bacteria associated with sore throat infection while *Zizphus spina christi* had a weak effect.

In this investigation, *Acacia nilotica* was found to be more effective than Chloramphenicol, Erythromycin and Cloxacillin for treatment of sore throat infection in Khartoum state and equally effective
as Tetracycline but it was less effective than Ampicillin, Penicillin, Gentamycin and Streptomycin for treatment of sore throat infection in Khartoum state. While *Zizphus spina christi* was found to be less effective than Ampicillin, Erythromycin, Gentamycin, Chloramphenicol, Cloxacillin, Streptomycin, Tetracycline and Penicillin for treatment of sore throat infection in Khartoum state.
ملخص الأطرحة

يعتقد بعض العشائيين السودانيين أن ثمار القرض والسرد تعالج التهاب الظفر. اختبرت الدراسة الحالية هذه النباتات عملياً لمعرفة تأثيرها على البكتيريا المسببة للتهاب الظفر.

جمعت 30 مسحة من الظفر لأشخاص مصابين بتهاب الظفر من ولاية الخرطوم في الفترة من مايو 2004 إلى يوليو 2005، وُجدت البكتيريا التي عزلت اشتملت على بكتيريا موجبة لصبغة جرام وبكتيريا سالبة لصبغة جرام.

المكورات العنقودية وتشمل:

*Staphylococcus aureus* 13.3%, *Staphylococcus capitis* 6.3% and *Staphylococcus hemolyticus* 6.7%.

المكورات السببيه وتشمل:

*Streptococcus pyogenes* 46.7% و *Streptococcus pneumoniae* 3.0%.

والوندوات وتشمل:

*Corynebacterium diphtheriae* 3.3% و *Corynebacterium ulcerans* 3.3%.

والاكتينومايس وتشمل:

*Actinomyces israelii* 1.7%.

اما البكتيريا السالبة لصبغة جرام تشمل:

*Haemophilus influenzae* 3.0%.
وعند دراسة فاعلية المضادات الحيوية ضد الانواع التي عزلت من المرض وجد أن كل الانواع المعزولة حساسة لعقار الامبسلين 97.67% ومقاومة لعقار كلوكازاسلين 9.67%.

في هذه الدراسة تم استخدام الماء لاستخلاص الفقر والسود ثم اختبرت ثلاثة تراكيز من هذه المستخلصات 10%، 20% و30%. تم اختبار المستخلصات بطريقة إقراص ورق الترشيح المشبع النتائج أظهرت أن مستخلص الفقر ثبت نمو غالبية البكتريا وكان التركيز 30% هو الأكثر فاعلية حيث ثبت نمو 17% من البكتريا بينما التركيز 20% ثبت نمو 30.13% والتركيز 10% ثبت نمو 53.68%.

وجد أن أكثر أنواع البكتريا حساسية لمستخلص الفقر هي:

Streptococcus pyogenes 57.14% Streptococcus pneumoniae 66.66%.

اما مستخلص السدر فكان له تأثير ضعيف على البكتريا المسببة لإلتهاب الحلق حيث وجد أن التركيز 30% ثبت نمو 16.27% من البكتريا والتركيز 20% ثبت نمو 13.95% والتركيز 10% ثبت نمو 6.25%.

وقد وجد أن أكثر الأنواع حساسية لمستخلص السدر هي:

Streptococcus pyogenes 21.42%.

في هذا البحث وجد أن مستخلص الفقر أكثر فعالية من الكلورامفيكول الآريسرومايسين والكلوكاسلين ومساوي في الفعالية للنتراساكيتين واقل فعالية من الامبسلين، والبنسلين، الابرييتوميسين والجنتماسين في علاج إلتهاب الحلق. أما مستخلص السدر فقد وجد أنه أقل فعالية من الامبسلين، والبنسلين، الكلورامفيكول، الآريسرومايسين، والكلوكاسلين، النتراساكيتين، الابرييتوميسين والجنتماسين في علاج إلتهاب الحلق.
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sugars………………………………………………………………
γ,ε,ε Nitrate
broth………………………………………………………………
γ,ε,ο Glucose phosphate medium (MR-VP test medium)…………
γ,ε,ν Nutrient agar……………………………………………………
γ,ε,γ Blood
agar……………………………………………………………………
γ,ε,ω Chocolate
agar……………………………………………………………………
γ,ε,η MacConkey
agar……………………………………………………………………
γ,ε,ι Motility
medium………………………………………………………………
γ,ε,ι Hugh and leifson,s (O/F)
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INTRODUCTION

The throat infection is uniquely predisposed to infection by bacteria, viruses or fungi. Bacteria cause serious throat infection that can lead to many complications such as tonsillitis and rheumatic fever. Rheumatic fever is the most serious complication of sore throat infection because it results in damage to heart muscle and valves.

The extensive uses of antibiotic for treatment of sore throat infection lead to the emergence of strains of bacteria which are resistant to these antibiotics. To solve this problem we have to look for alternative medicine instead of the antibiotics that bacteria showed resistance to it. In folklore medicine, some Sudanese herbalist believed that some herbs such as *Acacia nilotica* and *Zizphus spina christi* fruits can cure throat infection.

The objectives of this study are:

i To isolate and identify the aerobic bacteria associated with sore throat infection.

ii To determine the antibiotic sensitivity of each isolates.
To examine the possibility of herbal medicine *Acacia nilotica* and *Zizphus spina christi* fruits for treatment of sore throat infection.
CHAPTER ONE

LITERATURE REVIEW

1.1 Normal flora of the mouth and upper respiratory tract:

The mucous membrane of the mouth and pharynx are often sterile at birth but may be contaminated by passage through the birth canal. The predominate organisms in the upper respiratory tract are non-hemolytic and alpha-hemolytic Streptococcus and Nisseria. Staphylococcus, Diphtherode, Haemophilus, Pneumococci, Mycoplasma and Prevotella are also encountered (Geo et al., 2002).

1.2 Respiratory tract infections:

1.2.1 Streptococcal throat infection:

Many streptococci are members of the normal flora of the human body. They produce disease only when established in parts of body where they don’t normally occur (Geo et al., 2002). Lancefield groups A, B, C and G streptococci are the major groups of beta hemolytic streptococci that cause bacteremia. The major reservoir for group A and B Streptococci is human (Woo et al., 2001). The most common infection due to B hemolytic streptococci is streptococcal sore throat. Virulent group A streptococci adhere to pharyngeal epithelium by means of lipotechoic acid covering surface pili (Geo et al., 2002).
Streptococcus pyogenes throat infection can lead to suppurative complication like peritonsillar cellulitis and abscesses (Bisno et al., ٠٠٠٢). Nonsuppurative complication of streptococcal pharyngitis, acute rheumatic fever and glomerulonephritis are rare. Streptococcus pyogenes can also colonize the throat of asymptomatic persons (Quinn, ٩٨٩١). From the throat streptococci may spread to surrounding tissue causing otitis media, mastotiditis and suppurative adenitis. It may also cause meningitis (Geo et al., ٠٠٠١).

Pharyngitis due Streptococcus pyogenes is one of the most common bacterial infections seen in general practice, accounting for a significant number of physician office visits per year (Tack et al., ٨٩٩١). Although group A beta hemolytic streptococci are responsible most frequently for Streptococcal pharyngitis, pharyngitis may result from infection with other lancefield Streptococcal groups, e.g. group C and G organisms have been implicated as cause of exudative pharyngitis (Benjamin and Perriello, ٦٧٩١; Hill et al., ٩٦٩١).

Streptococcal infection of the upper respiratory tract does not usually involve the lung. Pneumonia due to B-hemolytic streptococci is rapidly progressive and severe and is not commonly a sequel to viral infection (Geo et al., ٠٠٠١).
*Streptococcus pyogenes* is an important pathogen causing pharyngitis, scarlet fever, impetigo, erysipelas, cellulitis and nacrotizing fascitis (Jalava *et al.*, 2004). Although patients recover clinically without antibiotic therapy, treatment is recommended to hasten clinical resolution, to prevent rheumatic fever (Dajani *et al.*, 1991) and to reduce the incidence of locally invasive infection (Tack *et al.*, 1998).

The throat carriers shed the organism during speaking, coughing and sneezing on exposed surface and in the air. Nose carriers also shed the organism on their clothes, floor, dust, bedding, books and their vicinity; and the streptococci will remain alive for days, weeks and months if shielded from day light (Omer, 1994).

*Streptococcus pneumoniae* is a normal inhabitant of the upper respiratory tract of 5-20% humans (Geo *et al.*, 2001). *Streptococcus pneumoniae* remains a major cause of morbidity and mortality in underdeveloped and developed parts of the world, and resistance to common antibiotics is widespread (Tomaz, 1997; Crook and Sprat, 1998; Charpentier and Tuomanen, 2000; David *et al.*, 2003). In immuno-compromised people, the elder and young children, *Streptococcus pneumoniae* that initially colonized the nasopharynx may spread to distant sites such as the inner ear, lower respiratory tract, or
blood stream and causes diseases ranging from otitis media to pneumonia to meningitis (Gillespie and Balakrishnan, 2006; McCullers and Tuomanen, 2006; David et al., 2002). Death rate is high in old age (over 65 years) and young children (below 5 years) (Omer, 1991).

Diagnostic procedures for streptococcal pharyngitis have traditionally made use of culture methods in clinical laboratories. During the past several years, advance in immunology have led to the development of rapid detection methods. These new technologies have created a shift in the testing from the laboratory to the office of physician. Results are available in 5 to 30 minutes compared with the 18 to 42 hours required for traditional culture methods (Huck et al., 1989).

1.3 Staphylococcal infections:

Most human harbor staphylococci on the skin and in the nose or throat. Staphylococci particularly Staphylococci epidermidis, are members of the normal flora of the human skin, respiratory and gastrointestinal tracts (Geo et al., 2001).

Staphylococcus aureus is usually a secondary pathogen in patients with chronic lung disease (MacSween and Whaly, 1997). It is an opportunistic pathogen found in the nose and skin of healthy people (Omer, 1991). It causes infections in tissues and sites with lowered host-
resistance, e.g.: damaged skin and mucous membranes or haematoma. Staphylococcal disease may be classified as deep infection, acute tonsillitis, sinusitis, pneumonia and breast abscess (Satish, 1991).

1.2.3 Klebsiella infections:

Klebsiella pneumoniae is present in the upper respiratory tract, causes a small proportion of bacterial pneumonia (Geo et al., 2001). It is opportunist pathogen; it may produce pyogenic infection like abscess, infections of wound, or respiratory tract (Satish, 1991).

1.2.4 Corynebacterium infections:

Corynebacterium diphtheriae is localized on the mucous membrane of the nasopharynx and tonsils (Omer, 1991). Diphtheritic inflammation of the respiratory tract, results in inflammatory exudates and necrosis of the mucosal cells which causes sore throat and fever (Geo et al., 2001; Omer, 1991).

1.2.5 Haemophilus infections:

These are Gram-negative rods or coccobacili, often markedly pleomorphic, non-motile, aerobic and facultatively anaerobic, oxidase and catalase reaction vary between species and strains. Nitrate is reduced to nitrite. They are fastidious and require media containing X (Haemin or other prophyrlins) and Co-enzyme A (nicotinomide adenine dinucleotide
or its phosphate) factors and undefined constituents of the blood (Barrow and Feltham, 1993).

*Haemophilus influenzae* is found on the mucous membranes of the upper respiratory tract in humans. It is an important cause of meningitis in children and occasionally causes respiratory tract infections in children and adults (Geo et al., 2001).

\[\text{\textbf{1.7 Neisseria infections:}}\]

Humans are the only natural hosts for whom meningococci are pathogenic. The nasopharynx is the portal of entry (Geo et al., 2001).

Nasopharyngeal swabbing substantially underestimates carriage of *Neisseria meningitides* (Greiner et al., 2002). It was the leading cause of bacterial meningitis of children (MacLennan et al., 2002; Schuchat et al., 1997).

\[\text{\textbf{1.8 Virus infections:}}\]

The upper respiratory tract is an important site for hosts defense against invading pathogens, since it is the site at which inhaled antigens first come into contact with immune system (Kuper et al., 1997). Only half of the pneumonias are viral in origin, the others being secondary to bacterial or fungal super infections (Whimbey et al., 1996). Severe disease has also been reported in solid-organ transplant recipients,
particularly in the early post transplantation period (Apalsch et al., 1990). Most sore throat infections are due to viral infection. Many infections remain localized in the respiratory tract, although some viruses produce their disease symptoms following systemic spread (Geo et al., 2001).

1.3.1 Common cold:

Rhinoviruses and Corona viruses are responsible for the common cold in 40 to 40% and 10 to 10% of cases, respectively. Typical symptoms include coryza, sneezing, lacrimation, and chilliness that last for 2 to 7 days. No fatalities have been reported, but these infections may predispose individuals to more serious complications such, as sinusitis, otitis media and asthma (Aitken and Jeffries 2001). Human rhino virus cause an estimated one-third to one-half of all acute respiratory tract infections throughout the year (Couch, 1991; Monto, 1994) and account for the majority of respiratory illness during spring and fall (Gern and Busse, 1999).

Infection with rhino virus are usually limited to the upper respiratory tract, however, these viruses have also been shown to be involved in acute otitis media (Arola et al., 1988), sinusitis (Pitkaranta et al., 1997) and lower respiratory tract infections (Couch, 1997; Olive et al., 1990).
Influenza:

Influenza A and B virus infections are characterized by the sudden onset of fever, coryza, sore throat and headache. The symptoms typically last about 7 days with some patients developing protracted cough. During major epidemics, severe illness and death from primary viral or secondary bacterial pneumonia can occur, usually in the elderly and the immunocompromised (Aitken and Jeffries 2002). Influenza infections cause cellular destruction and desquamation of superficial mucosa of the respiratory tract but don’t affect the basal layer of epithelium (Geo et al., 2002).

Antimicrobial Drugs activity:

An ideal antimicrobial agent exhibits selective toxicity, this term implies that the drug is harmful to a parasite without being harmful to the host. Selective toxicity may be a function of specific receptor required for attachment, or it may depend on the inhibition of biochemical events essential to the parasite but not the host. Antimicrobial antibiotics and sulphanomids have no effect on viruses (Geo et al., 2001).

Antimicrobial resistance:
Many organisms produce mutant that are resistant to most drugs that they would ordinarily be susceptible to in the wild state (Carter, ١٩٨٦).

Most of antimicrobial resistance which is now making it difficult to treat some infectious disease, resistance might be due to the extensive use and misuse of antimicrobial drugs which have favoured emergence and survival of resistant strains of microorganisms (Cheesburgh, ٠٠٠٠). All beta haemolytic group A streptococci are sensitive to penicillin G (Geo et al., ١٠٠٢) and it has been the gold standard (or drug of choice) for treatment of streptococcal pharyngitis (Kaplan et al., ١٩٩٧; Dajani et al., ١٩٩٣; Jasir et al., ٠٠٠٠) and for many years it affected consistent microbiologic eradication rate of over ٩٠٪ (Feldman et al., ١٩٨٣).

Most of beta-hemolytic group A streptococci are sensitive to erythromycin (Geo et al., ٠٠٠٠) and some are resistant to tetracycline. The first reports of erythromycin-resistant isolates of *Streptococcus pyogenes* from human clinical sources appeared in ١٩٠٩ (Lowbury et al., ١٩٠٩).

The development of resistance by *Staphylococcus aureus* in response to penicillin and tetracycline is usually low, occurring at slow rates over considerable period of time and exposure (Carter, ١٩٨٧). Many
strains of *Haemophilus influenzae* are susceptible to ampicillin and most strains are susceptible to chloramphenicol. Antimicrobial drugs (penicillin, erythromycin) inhibit the growth of diphtheria bacilli (Geo *et al.*, ١٠٠٢).
Folkloric Medicine:

Plants normally grow on different soils which are extremely rich in microorganisms and infection remains a rare event. To keep out potential invaders, plant produces a wide range of selective antibacterial compounds either in a constitutive or an inducible manner (Cammue et al., 1991). Among these compounds several low molecular weight proteins or peptides with antibacterial or antifungal activity have been isolated in recent years from various plants (Terras et al., 1994; Hejgaard et al., 1997; Roberts and Selitrennikoff, 1997) and are believed to be involved in defense mechanism against phytopathogenic fungi by inhibiting microorganisms growth through diverse molecular modes, such as binding to chitin or increasing the permeability of the fungal membranes or cell wall (Dahot, 1998). Another strategy followed plants to thwart invaders is based on the localized production of antimicrobial known as phytoalexins (Van et al., 1994; Maher et al., 1994). Moreover, the synthesis of many presumed defense related proteins are induced when plants are confronted with pathogens (Linthorst, 1991).
Medicinal plants have been used for centuries as remedies for human disease because they contain components of therapeutic value (Nastra et al., 2000).

Recently, the acceptance of traditional medicine as an alternative form of health care and the development of microbial resistance to available antibiotics has led authors to investigate the antimicrobial activity of medicinal plants (Bisignano et al., 1991; Lis-Balchin and Deans, 1991; Maoz and Neeman, 1991; Hammer et al., 1999).

Moreover, the increasing use of plant extracts in the food, cosmetic and pharmaceutical industries suggest that, in order to find active compounds, a systematic study of medicinal plants is very important (Nostra et al., 2000).

In recent years several reports have been published on the scientifically confirmed antimicrobial activity of some natural products derivers from plants (Akendengue et al., 2003; Cosertino et al., 1999; Delorenzi et al., 2001; Delorenzi et al., 2003; Kayser et al., 2003; Mangena and Muyima, 1999; Neal et al., 1985; Oketch et al., 1997).

In the Sudan, medicinal folklore passed from one generation to another but has never been documented. The Medicinal Plant and Aromatic Plant Research Institute has drawn and urgent short-term
objective to issue an atlas of medicinal plants in Sudanese folklore medicine (El Ghazali et al., 1994).

1.5.1 Classification of *Zizphus spina christi*:

*Zizphus spina christi* is used for many medicinal purpose in folklore medicines and it belong to:

Family: Rhamnaceae.

Synonyms: *Rhamnus spina christi; Zizphus africana.*

Arabic name: Al sider (tree) (Figure 1), Al nabag (fruits) (Figure 2 and figure 3).

Distribution: wide spread throughout the Sudan.

1.5.2 Chemical constituent of *Zizphus spina christi*:

The fruits of *Zizphus species* have higher contents of protein and vitamins A and C than apples (Anonymous, 1974). The mineral content of calcium, phosphorus and iron in *Zizphus* fruits is also reported as being higher than in apples and even oranges (Jawanda and Bal, 1978).
Figure 1: The tree of *Zizphus spina christi*.
Figure 2: The fruits of *Zizphus spina christi*.
Figure 3: The fruits of *Zizphus spina christi*.

Classification of *Acacia nilotica*:

*Acacia nilotica* subsp *nilotica* is belong to:

Family: **Mimosaceae**.

Synonyms: *Mimosa nilotica; Acacia arabica*.

Arabic names: Sunot (tree) (Figure 4), Garad (fruit).

Distribution: wide spread throughout the Sudan.

Chemical constituent of *Acacia nilotica*:

Various flavonoids and phenolic compounds have been isolated from the flowers whereas tannins, gallic acid and m-digallic acids were reported from the pods, epoxy, and hydroxy fatty acids from the bark and dap respectively (El Ghazali *et al.*, 1994).
Uses of *Acacia nilotica* in folklore medicine:

*Acacia nilotica* is traditionally used to treat sore throat, colds, bronchitis, pneumonia, ophthalmia, diarrhea, dysentery, leprosy, venereal disease and hemorrhage because of its tonic, astringent and stimulant properties. Aqueous extracts of fruits showed activity against *Candida albicans*, Gram positive and Gram negative bacteria (El Ghazali *et al.*, 2002).
It was found that an extract from the fruits of *Acacia nilotica* inhibited the growth of many bacteria. This activity was not related to the
low pH of the extract. Different bacteria showed different degrees of sensitivity to the extract (El Ghazali et al., 2002).

The water extract of the bark is taken for treatment of diarrhea and dysentery (El Ghazali et al., 1981). The decoction of the leaves and fruits are used against cough (El Ghazali et al., 1994).
CHAPTER TWO

MATERIAL AND METHODS

1. Sterilization:

1.1. Sterilization of equipment:

Petri dishes, test tubes, forceps, flasks, Pasteur pipettes and graduated pipettes were sterilized in a hot air oven at 180°C for one hour. Bottles and plastic containers were sterilized by autoclaving at 121°C (15 lb/sq. inch) for 15 minutes.

1.2. Sterilization of culture media and solutions:

Media and solutions were sterilized by autoclaving at 121°C (15 lb/sq. inch) for 15 minutes, but carbohydrates media were sterilized by autoclaving at 115°C (10 lb/sq. inch) for 10 minutes.

1.4. Reagents and indicators:

1.4.1. Reagents:

1.4.4. Tetramethyl-p-phenylene diamine dihydrochloride:

This reagent was obtained from British Drug House, London (BHD), Ltd. The reagent was prepared as 3% aqueous solution. It was used for oxidase test.
٢٫١٫٢٫٢. **Hydrogen peroxide:**

This reagent was obtained from Agropharm limited, Buckingham. It was prepared as ٪۳ aqueous solution and it was used for catalase test.

٣٫١٫٢٫٢. **Methyl red:**

This reagent was prepared by dissolving methyl red (٤٠٫٠ g) in ethanol (٣٠ ml). The volume was made to ١٠٠ ml with distilled water. It was used for methyl red test.

٤٫١٫٢٫٢. **Alpha-naphthol solution:**

Alpha-naphthol is a product of (BDH). It was prepared as ٪۵ aqueous solution for Voges-Proskauer (VP) test.

٥٫١٫٢٫٢. **Potassium hydroxide:**

This reagent was prepared as ٪٤ aqueous solution. It was used for Voges-Proskauer test.

٦٫١٫٢٫٢. **Nitrate reagent:**

Nitrate test reagent consisted of two solutions and they were prepared according to Barrow and Feltham (٣٩٩١). Solution A composed of ٪٣٣ sulphanilic acid was dissolved by gentle heating in ٩٠٠ acetic acid. Solution B was composed of ٪٦ dimethyleamine-alpha-nephthylamine was dissolved by gentle heating in ٩٠٠ acetic acid.
٧.١.٢.٢. **Kovac’s reagent:**

This reagent contained ٥ g of para-dimethyl aminobenzaldehyde, ٥٧ ml amyl alcohol and ٥٢ ml concentrated hydrochloric acid. It was prepared as described by Barrow and Feltham (٣٩٩١) by dissolving the aldehyde in the alcohol by heating in water bath. It was then cooled and the acid was added. The reagent was stored at ٤٠ C for use in indole test.

٧.٢.٢. **Indicators:**

٧.٢.٣. **Andrade’s indicators:**

This indicator composed of acid fuchsin ٥ g, distilled water IL and N-NaOH ٠١٠ ml. The acid fuchsin was added, mixed and was allowed to stand at room temperature for ٤٢ hours with frequent shaking until the color changed from red to brown.

٧.٣.٢. **Bromothymol blue:**

Bromothymol blue indicator was obtained from (BDH), Ltd. The solution was prepared by dissolving ٢.٠ g of the bromothymol blue powder in ١٠٠ ml distilled water.

٧.٣.٣. **Phenol red:**

This reagent was obtained from Hopkins and William Ltd, London. It was prepared as ٢٪ solution.
Collection of blood for enriched media:

Defibrinated sheep blood was used in preparing blood agar medium. The blood was collected from the jugular vein in sterile flask containing glass beads and mixed gently during collection. The blood was distributed in 0.1 ml amount in sterile screw capped bottles and stored in refrigerator.

Preparation of media:

Nutrient broth:

Thirteen grams of nutrient broth (Oxoid) were added to one liter of distilled water, mixed well and distributed in 3 ml amount into clean test tubes, then sterilized by autoclaving at 121°C for 15 minutes.

Peptone water:

Fifty grams of peptone water powder (Oxoid) were added to one liter of distilled water, mixed well, distributed in 3 ml amount into clean test tubes and sterilized by autoclaving at 121°C for 15 minutes.

Peptone water sugars:

Peptone water sugars medium was prepared as described by Barrow and Feltham (1993). It contained peptone water 0.9 ml, Andrade’s indicator 1.0 ml, sugar solution 1.0 ml and distilled water 0.9
ml. The pH of peptone water was adjusted to \(\text{pH 1.7 - 3.7}\) before the addition of Andrade’s indicator. The complete medium was mixed well then distributed in \(\text{2 ml}\) volume into clean test tubes containing inverted Durham’s tube and sterilized by autoclaving at \(\text{110°C (1.1 lb/inch}^2)\) for \(\text{10 minutes.}\)

\(\text{Nitrate broth:}\)

The medium used was prepared as described by Barrow and Feltham (\(1993\)). Potassium nitrate \(\text{1 g}\) was dissolved in one-litter of nutrient broth, distributed in \(\text{5 ml}\) amount into clean test tubes and sterilized by autoclaving at \(\text{110°C}\) for \(\text{20 minutes.}\)

\(\text{Glucose phosphate medium (MR-VP test medium):}\)

This medium was prepared according to Barrow and Feltham (\(1993\)). Peptone powder \(\text{5 g}\) and \(\text{5 g phosphate buffer (K}_2\text{HPO}_4)\) were added to one litter of distilled water, dissolved by steaming then pH was adjusted to \(\text{5.6}\). Then \(\text{5 grams of glucose were added, mixed well, distributed into clean test tubes and sterilized by autoclaving at 110°C for 15 minutes.}\)

\(\text{Nutrient agar:}\)

To one litter of nutrient broth (Oxoid) \(\text{10 g}\) of agar were added, dissolved by boiling and sterilized by autoclaving at \(\text{121°C}\) for \(\text{15 minutes.}\)
minutes. Then cooled to about 5°C and distributed in 5 ml amount per plate. The poured plates were left to solidify at room temperature on leveled surface.

\(\text{\textbf{\textit{7.4.7 Blood agar:}}}\)

Forty grams of blood agar base NO.2 (Oxoid) were suspended in one liter of distilled water, dissolved by boiling, mixed and sterilized by autoclaving at 121°C for 15 minutes. Then cooled to about 5°C and sterile, defibrinated sheep blood was added aseptically to give final concentration 1%, then mixed gently and 5 ml of complete medium were poured into each sterile Petri dish. The poured plates were allowed to solidify at room temperature on flat surface.

\(\text{\textbf{\textit{7.4.8 Chocolate (heated blood) agar:}}}\)

Method of preparation was similar to that described for blood agar. The complete medium was heated to 70°C in water bath until it became brown in color. The medium was allowed to cool to about 55°C, remixed and dispensed into sterile petri dishes in 5 ml amount. The poured plates were allowed to solidify at room temperature on flat surface.
MacConkey agar:

Fifty two grams of MacConkey agar (Oxoid) were suspended in one litter of distilled water, dissolved by boiling, then sterilized by autoclaving at \(121\,^{\circ}C\) for \(51\) minutes and poured into sterile Petri-dishes in \(15\) ml amount. The plates were left to solidify at room temperature on a flat surface.

Motility medium – Gragie tube medium:

Thirteen grams of dehydrated nutrient broth (Oxoid) were added to \(5\) grams of Oxoid agar No. \(1\) and dissolved in one liter of distilled water. The pH was adjusted to \(4.7\). This medium was dispensed in volumes of \(5\) ml into \(20\) ml test tubes containing Gragie tubes, and then sterilized by autoclaving at \(121\,^{\circ}C\) for \(51\) minutes.

Hugh and leifson’s (O/F) medium:

This medium was prepared as described by Barrow and Feltham \(1991\). Two grams of peptone powder, five grams of sodium chloride, \(3.0\) g of potassium hypophosphate and three grams of agar were added to one liter of distilled water. Then heated in water bath at \(55\,^{\circ}C\) to dissolve the solids. The pH was adjusted to \(1.1\) and filtered. Then the indicator
bromothymol blue (\( \cdot, \cdot, \cdot 2\% \) aqueous solution) was added and the mixture was sterilized by autoclaving at \( 110^\circ \)C for 10 minutes.

Then filtered sterile glucose solution was added aseptically to give final concentration of \( 1\% \). Then the medium was mixed and distributed, aseptically in 10 ml amount into sterile test tubes of not more than 16 mm diameter.

\( \cdot, \cdot, \cdot, \cdot \) Diagnostic sensitivity test agar:

This medium was supplied by Oxoid. It consist of protease peptone, veal infusion solids, dextrose, sodium chloride, disodium phosphate, sodium acetate, adenine sulphate, guanine hydrochloride, uracil, xanthine and ion agar No.\( \cdot \).

Forty grams of medium were suspended in one liter of distilled water then brought to boil to dissolve completely and sterilized by autoclaving at \( 121^\circ \)C for 15 minutes. Then dispended into sterile Petri dishes in portions of 15 ml each. The poured plates were left to solidify at room temperature on leveled surface.

\( \cdot, \cdot, \cdot, \cdot \) Urea agar:

This medium composed of peptone, dextrose, sodium chloride, disodium hydrogen phosphate, potassium dihydrogen phosphate, phenol red and agar.
It was obtained in dehydrated form from Oxoid. The medium was prepared according to manufacturer’s instructions. $7.4 \text{ g}$ powder were dissolved in $90 \text{ ml}$ distilled water by boiling. After sterilization by autoclaving at $115^\circ C$ for $20 \text{ minutes}$, the basal medium was cooled to $50^\circ C$ and aseptically $5 \text{ ml}$ of sterile $4\%$ urea solution were added. The pH was adjusted to $7.8$ and distributed in $1\text{ ml}$ aliquots into sterile screw-capped bottles, which were allowed to solidify in slope position.

**Simmon’s citrate agar:**

The dehydrated medium of Oxoid consisted of sodium chloride, magnesium sulphate, ammonium dihydrogen phosphate, sodium ammonium phosphate, sodium citrate, bromothymol blue and agar.

Twenty three grams of the dehydrated medium were dissolved in $1000 \text{ ml}$ distilled water by steaming. The pH was adjusted to $7.0$ and the medium was then sterilized by autoclaving $121^\circ C$ for $51 \text{ minutes}$, distributed into sterile MacCarteny bottles, and allowed to set in slope position.

**Gelatin medium:**

The medium was prepared according to Barrow and Feltham (1993), by soaking $4 \text{ g}$ of gelatin in $20 \text{ ml}$ of distilled water. When thoroughly softened, it was added to $100 \text{ ml}$ of melted nutrient agar,
mixed and the medium was then distributed into volumes of 0 ml in screw capped bottles and sterilized by autoclaving at 121°C for 10 min.

Collection of samples:

A total of 9 throat swabs were collected randomly from people of different age and different sex during March 2002 to June 2002. The area of study was Khartoum state. Throat swabs were collected by medical officers or experienced nurses.

Infected throat was sampled by a sterile cotton wool swab, then the swab returned to its sterile tube and the tube was labeled then transported on ice in thermos flask for immediate culturing.

Culture of specimens:

The collected swabs were inoculated onto blood agar, chocolate agar and MacConkey agar. The inoculated plates were then incubated for 42 – 84 hours at 37°C.

Purification of culture:

All isolates were purified by several subculturing from single well-separated colony of each type on primary culture. The purification was carried out on nutrient agar or blood agar. The purity was checked by
examining Gram stained smear. The pure culture was then used for studying cultural and biochemical characteristics and sensitivity test.

**Microscopic examination:**

Smears were made from each type of colonies on primary culture and from purified colonies, fixed by heating and stained by Gram method (Barrow and Feltham, 1991). Then examined microscopically under high power. The smear was examined for cell morphology, arrangement and staining reaction.

**Identification of bacteria:**

The purified isolates were identified according to the criteria described by Barrow and Feltham (1991) this included staining reaction, organism morphology, growth condition, the colonies characteristics on different media, haemolysis on blood agar, motility and biochemical characteristics.

**Biochemical methods:**

**Oxidase test:**

The method of Barrow and Feltham (1991) was used. Strip of filter paper was soaked in ½ solution of tetramethyl–p-
phenylenediamine dihydrochloride and dried in hot-air oven and then placed on clean glass slide by sterile forceps. A fresh young tested culture on nutrient agar was picked off with sterile glass rod and rubbed on the filter paper strip.

If a purple color developed within 5–10 seconds, the reaction was considered positive.

\(2.02.3\) Catalase test:

The test was carried out as described by Barrow and Feltham (1991). A drop of \(3\%\) \(H_2O_2\) was placed on clean slide and then a colony of tested culture on nutrient agar was picked by glass rod and added to the drop of \(3\%\) \(H_2O_2\). Appositive reaction was indicated by production of air bubbles.

\(2.02.4\) Oxidation – Fermentation (O/F) test:

The test was carried out as described by Barrow and Feltham (1991). The tested organism was inoculated with straight wire into duplication of test tubes of Hugh and Leifeson’s medium. To one of the test tubes a layer of melted soft paraffin oil was added to the medium to seal it from air. The inoculated tubes were incubated at \(37^\circC\) and examined daily for fourteen days.
Yellow color in open tube only indicated oxidation of glucose. Yellow color in both tube showed fermentation reaction and blue or green color in open tube and yellow color in the sealed tube indicated production of alkali.

Sugar fermentation test:

This test was carried out as described by Barrow and Feltham (1993). The peptone water sugar was inoculated with organism under the test, incubated at $\cdot$C and then examined daily for several days. Acid production was indicated by appearance of reddish color, while gas production was indicated by appearance of empty space in the inverted Durham’s tubes.

Voges - Proskauer test:

The test was performed as described by Barrow and Feltham (1993). The test culture was inoculated into glucose phosphate medium (MR – VP medium) and incubated at $\cdot$C for $^\circ$ h. Three milliliter of $^\circ$ alpha-naphthol solution and one milliliter of $^\circ$ potassium hydroxide were added.

When bright pink color developed within $^\circ$ minutes, the reaction was regarded as positive.
Nitrate reduction:

The nitrate test was carried out as described by Barrow and Feltham (1991). The test culture was lightly inoculated into nitrate broth and incubated at 37°C for two days. Then 1 ml of solution (A) followed by 1 ml of solution (B) of nitrate test reagent were added.

Red colour indicated positive reaction which showed that nitrate had been reduced. If red colour did not develop, powdered zinc was added to see whether there was residual nitrate or not. Red color development indicated that nitrate in medium had been reduced to nitrite by zinc but not by organism, whereas unchanged colour indicated nitrate in original medium had been reduced completely and nitrite was further broken down by the organism.

Coagulase test:

The test was performed as described by Barrow and Feltham (1991). To 5 ml of 1:10 dilution of human plasma in saline, 0.1 ml of 18–24 h old culture of the tested organism was added, then incubated at 37°C and examined after 1–2 h for coagulation. Definite clot formation indicated positive result.

The test was also performed on slide. Two colonies of tested culture were placed on a clean slide, emulsified in drop of normal saline
and then a loop full of human plasma was added to the drop of bacterial suspension. Appearance of coarse visible clump was recorded as positive result.

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Indole production test:

Indole production test was carried out as described by Barrow and Feltham (٣٩٩١). The tested organism was inoculated into peptone water and incubated at ٧٣˚C for ٨٤h. One milliter of Kovac’s reagent was run down along side of the test tube. Appearance of pink color in the reagent layer within a minute indicated positive reaction.

٨٫٠١٫٢  

Methyl red (MR) test:

Methyl red test was carried out as described by Barrow and Feltham (٣٩٩١). The tested organism was inoculated into glucose phosphate medium (MR – VP medium) then incubated at ٧٣˚C for ٨٤h. Two drops of methyl red reagent were added, shaken well and examined. Appearance of red color indicated positive reaction, whereas orange or yellow color indicated negative reaction.

٨٫٠١٫٢  

Urease test:
A slope of urea agar medium was inoculated with the tested organism and incubated at 73°C. Change in color to red indicated positive reaction.

\[ \text{Citrate utilization:} \]

Simmon’s citrate medium was inoculated with tested organism and incubated at 73°C for up to 7 days and was examined daily for growth and color change. Blue color and streak of growth indicated positive citrate utilization.

\[ \text{Motility test:} \]

The Graigi tube in semi-solid nutrient agar prepared as described by Cruckshank et al. was inoculated by straight wire. A small piece of colony of the bacterium under test was picked by the end of the straight wire and stabbed in the center of semi-solid agar in the Graigi tube and then incubated at 73°C overnight. The organism was considered motile if it produced turbidity in the medium in and outside the Graigi tube.

\[ \text{Antibiotic sensitivity:} \]

Sensitivity of isolates, to a number of antibiotics was determined by disc diffusion technique (Cruckshank et al., 1970). The isolates were grown on peptone water and incubated at 73°C for two hours. About 2 ml
of culture was poured on a petri dish containing diagnostic sensitivity test (DST) agar medium and the inoculum was evenly distributed by rotation. Excess fluid was withdrawn using sterile Pasteur pipette and plate was left to dry at room temperature for 51 minutes.

Commercially prepared discs of Plasmatic Laboratory (England) were placed on the surface of the medium by sterile forceps, pressed gently to ensure full contacts with the surface of the culture medium. The plates were then incubated at 73°C for 42 hours and up to 84 hours. Zone of no growth around disc indicated inhibition of growth of tested organism by the antibiotic of that disc.

Herbal extract sensitivity:

Water extraction:

Water is almost universal solvent used for extraction of plant ingredient. An amount of 0.1, 0.2 and 0.3 grams of Acacia nilotica and Ziziphus spina christi were weighted and were soaked respectively in 100, 200 and 300 ml sterile water in a sterile flasks for 42 hour. The contents of the flasks were then filtered. The filtrates were kept at 4 °C for later use.

Antibacterial activity of the plants extracts:
The antibacterial activity of *Acacia nilotica* and *Zizphus spina christi* to various bacterial isolates obtained in this study were examined by disc method.

**Disc method:**

Diagnostic sensitivity test medium was prepared and left to solidify. The isolates were grown in peptone water and incubated at \(^{73} \degree C\) for two hours. About \(2\) ml of culture was poured on Petri dish containing Diagnostic sensitivity test agar medium and the inoculum was evenly distributed by rotation. Excess fluid was withdrawn using sterile Pasteur pipette and the plate was left to dry at room temperature for \(51\) minutes. Filter paper discs of \(5\) mm diameter were used. The discs were impregnated with \(0.1\), \(0.2\) and \(0.3\) concentration of water extract. The impregnated discs were then placed on the agar surface.

Inhibition zones around the discs was measured in centimeter and then scored as (+), when the inhibition zone was \(0.5\) cm; (+ +), \(1\) cm; (+ + +), \(1.5\) cm; (+ + + +), \(2\) cm and (-) when no inhibition was noticed.
CHAPTER THREE

\. RESULTS

\. \textbf{Isolation and identification:}

The bacterial isolates found in this study were identified according to their cultural characteristics, cell morphology, Gram stain reaction and their biochemical properties as described by Barrow and Feltham (\textsuperscript{1991}).

\. \textbf{Aerobic bacteria isolated from collected samples:}

The total number of bacterial isolates were \(34\) (\(\%3.41\)), thirty-four (\(\%3.31\)) were Gram-positive and nine (\(\%0.3\)) were Gram-negative.

The Gram-positive bacteria were \(\%6.67\) \textit{Streptococcus spp}, \(\%3.32\) \textit{Staphylococcus spp}, \(\%7.6\) \textit{Corynebacterium spp} and \(\%7.6\) \textit{Actinomyces spp}. The identified Gram-negative bacteria were nine \textit{Haemophilus influenzae} \(\%0.3\) as shown in table \textbf{1} and figure \textbf{8}.

\. \textbf{Staphylococcus spp:}

The seven (\(\%3.7\)) isolates of \textit{Staphylococcus spp} comprised as follows: four (\(\%3.7\)) \textit{Staphylococcus aureus}, two (\(\%3.7\)) \textit{Staphylococcus hemolyticus} and one (\(\%3.7\)) \textit{Staphylococcus capitis}.

These isolates were identified according to their cultural characteristics, Gram stain reaction, cell morphology and biochemical
properties. They were Gram-positive, non-motile, non-spore forming and ferment number of sugars as shown in table 2. When *Staphylococcus aureus* were cultured on blood agar and incubated aerobically at 37°C for 42 h, a wide zone of beta hemolysis was produced around the colonies.

**Streptococcus spp:**

From that twenty-three (6.67%) isolates of *Streptococcus spp* were obtained in this study, fourteen (7.64%) were *Streptococcus pyogenes* and nine (3%) were *Streptococcus pneumoniae*. These isolates were identified on bases of their cell morphology, Gram stain reaction and biochemical characteristics. They were Gram-positive cocci arranged in chains, non-motile, non-spore forming and ferment number of sugars. They required enriched media e.g. blood agar. These isolates were further classified on base of their action on blood agar to alpha hemolysis, beta hemolysis or non hemolysis as shown in table 2.

**Actinomyces spp:**

The two strains (7.6%) of *Actinomyces israelii* obtain in this study were Gram positive rods, non-motile, non-spore forming, aerobic and ferment number of sugar as shown in table 2.

**Haemophilus influenzae:**

The nine (3%) strains of *Haemophilus influenzae* obtained in this study were identified according to their morphology, Gram stain reaction
and cultural characteristics. They were Gram-negative coccobacilli, non-motile, oxidase positive and catalase positive as shown in table ٢.

When it were grown on chocolate agar and incubated at ٧٣°C overnight they produced mucoid colonies.

٣,٤ Mixed bacterial infection:

Mixed bacterial infection was observed in ٩٣ (٣٩٥٪) cases of sore throat swabs examined in this study. Haemophilus influenzae was isolated from ٤ (٤٠٪) cases mixed with Streptococcus pyogenes, ٤ (٤١٪) cases mixed with Streptococcus pneumoniae and ٤ (٤٪) cases mixed with Staphylococcus aureus.

٣,٥ In vitro antimicrobial sensitivities:

The forty-three bacterial isolates found in this study were examined for their sensitivity to different antimicrobial agents. Forty-two isolates (٧٦٪) were sensitive to Ampicillin, ٤ isolates (٢٠٪) were sensitive to Penicillin, ٨٣ isolates (٤٤٪) were sensitive to Tetracycline, ٧١ isolates (٦٠٪) were sensitive to Gentamicin, ٧١ isolates (٤٨٪) were sensitive to Streptomycin, ٨٣ isolates (١١٪) were sensitive to Chloramphenicol, ٧١ isolates (٥٪) were sensitive to Erythromycin and ٧١ isolates (٣٪) were sensitive to Cloxacillin as shown in table ٣ and figure ١٣.
Ampicillin was most effective drug as 97.79% of the isolates were sensitive to this drug while Penicillin was the second drug of choice as 20.39% of the isolates were sensitive to this drug. However Cloxacillin was least effective drug as 90.27% of the isolates were resistant to it as shown in table 3.

3.4 **In vitro antibacterial activity of plants extracts:**

Three different concentrations of water extract of *Acacia nilotica* and *Ziziphus spina christi* were prepared to study the effect of these plants on the bacterial isolates found in throat swabs collected from patients with sore throat.

3.4.1 **In vitro antibacterial activity of *Acacia nilotica* water extract:**

Water extract of *Acacia nilotica* inhibited the growth of many bacteria examined in this study. Twenty-nine (76.44%) isolates showed growth inhibition at 3% concentration, twenty-five (85.31%) isolates showed growth inhibition at 2% concentration, twenty-three (35.84%) isolates showed growth inhibition at 1% concentration.

When the sensitivity of *Streptococcus pyogenes* isolates obtained in this study to *Acacia nilotica* extract was examined it was found that, the
growth of 8 isolates was inhibited by 3.0% concentration *Acacia nilotica* extract and zone of inhibition of growth measured 7, 1.5 and 1 cm for 3, 7 and 8 isolates respectively. While 3 isolates were not inhibited (ie. no zone) by 3.0% concentration *Acacia nilotica* extract.

The examination sensitivity of *Streptococcus pneumoniae* isolates found in this study to *Acacia nilotica* extract revealed that, the growth of 8 isolates was inhibited by 3.0% concentration *Acacia nilotica* extract and the zone of inhibition of growth measured 7 and 1.5 cm for 3 and 8 isolates respectively. While 3 isolates were not inhibited by 3.0% concentration *Acacia nilotica* extract.

The sensitivity testing of *Staphylococcus aureus* isolated in this study to *Acacia nilotica* extract, showed that the growth of four isolates was inhibited by 3.0% concentration *Acacia nilotica* extract and the zone of inhibition of growth measured 1.5 cm and 1 cm for 3 isolates for each of them, figure 5 and 6.

The growth of the two isolates of *Staphylococcus hemolyticus* found in this study were inhibited by 3.0% concentration *Acacia nilotica* extract and the zone of inhibition growth measured 1.5 cm and 1 cm.
The growth of *Staphylococcus capitis* obtained in this study was inhibited by 0.3% concentration *Acacia nilotica* extract and the growth inhibition zone measured 1 cm.

The growth of *Corynebacterium spp* obtained in the present study was inhibited by 0.3% concentration *Acacia nilotica* extract and the growth inhibition zone measured 1 cm for *Corynebacterium ulcerans* and 1.5 cm for *Corynebacterium diphtheriae*. 
Figure ٥: Sensitivity of *Staphylococcus aureus* to three different concentrations of water extract of *Acacia nilotica* and *Zizphus spina christi* determined by disc method.

A : ١٪ , B : ٢٪ , C : ٣٪ concentration of *Acacia nilotica* extract.

D : ١٪ , E : ٢٪ , F : ٣٪ concentration of *Zizphus spinachristi* extract.
Figure ٦: Sensitivity of *Staphylococcus aureus* to three different concentrations of water extract of *Acacia nilotica* and *Zizphus spina christi* determined by disc method.

A : ٪٠١, B : ٪٠٢, C : ٪٠٣ concentration of *Acacia nilotica* extract.

D : ٪٠١, E : ٪٠٢, F : ٪٠٣ concentration of *Zizphus spinachristi* extract.
The growth of *Actinomyces israelii* was inhibited by 3% concentration *Acacia nilotica* extract and the growth inhibition zone measured 1.5 cm for the two isolates obtained in the present study, figure 7.

The growth of six isolates of *Haemophilus influenzae* was inhibited by 3% concentration *Acacia nilotica* extract and the growth inhibition zone measured 1 cm for five isolates and 0.5 cm for one isolates. While three isolates were not inhibited (ie. no zone) by 3% concentration *Acacia nilotica* extract as shown in table 4.

**In vitro antibacterial activity of Zizhus spina christi water extract:**

The three concentration of water extract of *Zizphus spina christ* showed weak effect on the bacterial growth of isolates examined in this study. Seven isolates (72.61%) showed growth inhibition zones at 3% concentration, five (26.11%) isolates showed growth inhibition zones at 2% concentration, and two isolates (56.4%) showed growth inhibition zones at 1% concentration as shown in table 7.

The sensitivity of the isolates obtained in this study at 3% concentration *Zizhus spina christ* was examined and it was found that the growth of three isolates of *Streptococcus pyogenes* was inhibited and
the growth inhibition zone measured \( 2 \) cm, \( 1.5 \) cm, and \( 0.5 \) cm. While eleven isolates were not inhibited by \( 3\% \) concentration.

When the sensitivity of \textit{Haemophilus influenzae} to \( 3\% \) concentration \textit{Zizphus spina christi} was examined it was found that the growth of three isolates was inhibited and the growth inhibition zone measured \( 1 \) cm for one isolates and \( 1.5 \) cm for two isolates. While six isolates were not inhibited (ie .no zone) by \( 3\% \) concentration of \textit{Zizphus spina christi}. The growth of the all isolates obtained in this study was not inhibited by \( 3\% \) concentration of \textit{Zizphus spina christi} extract as shown in table 5.

When \( 3\% \) concentration water extract of \textit{Acacia nilotica} was compared with \( 3\% \) concentration water extract of \textit{Zizphus spina christi}, it was found that \textit{Acacia nilotica} extract was more effective than \textit{Zizphus spina christi} extract as \( 44.76\% \) of the isolates showed growth inhibition at \( 3\% \) concentration of \textit{Acacia nilotica} while only \( 72.61\% \) of the isolates showed growth inhibition at \( 3\% \) concentration of \textit{Zizphus spina christi} extract.
Table 1: Bacterial species isolated from throat swab samples collected randomly from patients in Khartoum state:

<table>
<thead>
<tr>
<th>Bacteria species</th>
<th>No of samples examined</th>
<th>No of isolates</th>
<th>Isolation percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Strepto. pyogenes</em></td>
<td>30</td>
<td>14</td>
<td>46.7%</td>
</tr>
<tr>
<td><em>Strepto. pneumoniae</em></td>
<td>30</td>
<td>9</td>
<td>30%</td>
</tr>
<tr>
<td><em>Staph. aureus</em></td>
<td>30</td>
<td>4</td>
<td>13.3%</td>
</tr>
<tr>
<td><em>Staph. hemolyticus</em></td>
<td>30</td>
<td>2</td>
<td>6.7%</td>
</tr>
<tr>
<td><em>Staph. capitis</em></td>
<td>30</td>
<td>1</td>
<td>3.3%</td>
</tr>
<tr>
<td><em>Coryne. diphtheriae</em></td>
<td>30</td>
<td>1</td>
<td>3.3%</td>
</tr>
<tr>
<td><em>Coryne. ulcerans</em></td>
<td>30</td>
<td>1</td>
<td>3.3%</td>
</tr>
<tr>
<td><em>Actiomy. israelii</em></td>
<td>30</td>
<td>2</td>
<td>6.7%</td>
</tr>
<tr>
<td><em>Haemoph. influenzae</em></td>
<td>30</td>
<td>9</td>
<td>30%</td>
</tr>
<tr>
<td><strong>Mix culture</strong></td>
<td>30</td>
<td>13</td>
<td>43.3%</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>30</td>
<td>43</td>
<td>143.3%</td>
</tr>
</tbody>
</table>
Table 2: Characters and biochemical reactions of bacteria isolated from sore throat patients Khartoum state.

<table>
<thead>
<tr>
<th>Characters</th>
<th>Strepto. pyogenes</th>
<th>Strepto. pneumoniae</th>
<th>Staph. aureus</th>
<th>Staph. hemolyticus</th>
<th>Staph. capitis</th>
<th>Coryne. diphtheriae</th>
<th>Coryne. ulcerans</th>
<th>Actino. israelii</th>
<th>Haemophilus influenzae</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gram stain</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Shape</td>
<td>cocci</td>
<td>cocci</td>
<td>cocci</td>
<td>cocci</td>
<td>cocci</td>
<td>coccobacilli</td>
<td>Cocobacilli</td>
<td>Polymorphic</td>
<td>Coccobacilli</td>
</tr>
<tr>
<td>Motility</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Oxidase</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Catalase</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>O/F</td>
<td>F</td>
<td>F</td>
<td>F</td>
<td>F</td>
<td>F</td>
<td>F</td>
<td>F</td>
<td>F</td>
<td>F</td>
</tr>
<tr>
<td>Lactose</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
<td>-</td>
<td>-</td>
<td>ND</td>
<td>ND</td>
<td>-</td>
</tr>
<tr>
<td>Maltose</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>-</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Mannitol</td>
<td>ND</td>
<td>ND</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Xylose</td>
<td>ND</td>
<td>ND</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>ND</td>
<td>ND</td>
<td>+</td>
</tr>
<tr>
<td>Sucrose</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>ND</td>
</tr>
<tr>
<td>Raffinose</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Nitrate</td>
<td>ND</td>
<td>ND</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Coagulase</td>
<td>ND</td>
<td>ND</td>
<td>+</td>
<td>-</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Haemolysis</td>
<td>Beta</td>
<td>Alpha</td>
<td>Beta</td>
<td>-</td>
<td>ND</td>
<td>ND</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>VP</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>ND</td>
</tr>
<tr>
<td>Urease</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Gelatin</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>-</td>
<td>-</td>
<td>ND</td>
</tr>
<tr>
<td>Casein</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>-</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

F: Fermentative. ND: NOT DONE
Table: Antimicrobial sensitivity of bacteria isolated from throat swab samples collected randomly from patients in Khartoum state.

<table>
<thead>
<tr>
<th>Antimicrobial drug</th>
<th>No of bacterial isolates examined</th>
<th>No of sensitive isolates (percent)</th>
<th>No of resistant isolates (percent)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin</td>
<td>43</td>
<td>42 (97.77%)</td>
<td>1 (2.23%)</td>
</tr>
<tr>
<td>Penicillin</td>
<td>43</td>
<td>40 (93.02%)</td>
<td>3 (6.98%)</td>
</tr>
<tr>
<td>Cloxacillin</td>
<td>43</td>
<td>12 (27.91%)</td>
<td>31 (72.09%)</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>43</td>
<td>21 (48.83%)</td>
<td>22 (51.17%)</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>43</td>
<td>31 (72.09%)</td>
<td>12 (27.91%)</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>43</td>
<td>31 (72.09%)</td>
<td>12 (27.91%)</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>43</td>
<td>29 (67.44%)</td>
<td>14 (32.56%)</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>43</td>
<td>28 (60.11%)</td>
<td>10 (39.89%)</td>
</tr>
</tbody>
</table>
Table 4: Sensitivity of Gram positive and Gram negative bacteria isolated randomly from sore throat patients in Khartoum state to water extract of *Acacia nilotica*:

<table>
<thead>
<tr>
<th>Bacterial species</th>
<th>Number of isolates examined</th>
<th>% concentration 3%</th>
<th>% concentration 2%</th>
<th>% concentration 1%</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Strept. pyogenes</em></td>
<td>4</td>
<td>1 (8 cm)</td>
<td>0 (0.5 cm)</td>
<td>0 (0 cm)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 (1 cm)</td>
<td>0 (0 cm)</td>
<td>0 (0 cm)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3 (0.5 cm)</td>
<td>0 (0 cm)</td>
<td>0 (0 cm)</td>
</tr>
<tr>
<td><em>Strept. pneumoniae</em></td>
<td>9</td>
<td>2 (1 cm)</td>
<td>0 (0 cm)</td>
<td>0 (0 cm)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3 (0.5 cm)</td>
<td>0 (0 cm)</td>
<td>0 (0 cm)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.5 (0.5 cm)</td>
<td>0 (0 cm)</td>
<td>0 (0 cm)</td>
</tr>
<tr>
<td><em>Staph. aureus</em></td>
<td>4</td>
<td>1 (8 cm)</td>
<td>0 (0.5 cm)</td>
<td>0 (0 cm)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 (1 cm)</td>
<td>0 (0 cm)</td>
<td>0 (0 cm)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3 (0.5 cm)</td>
<td>0 (0 cm)</td>
<td>0 (0 cm)</td>
</tr>
<tr>
<td><em>Staph. hemolyticus</em></td>
<td>2</td>
<td>1 (8 cm)</td>
<td>0 (0.5 cm)</td>
<td>0 (0 cm)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 (1 cm)</td>
<td>0 (0 cm)</td>
<td>0 (0 cm)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.5 (0.5 cm)</td>
<td>0 (0 cm)</td>
<td>0 (0 cm)</td>
</tr>
<tr>
<td><em>Staph. capitis</em></td>
<td>1</td>
<td>1 (8 cm)</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td><em>Coryne. diphtheriae</em></td>
<td>1</td>
<td>1 (8 cm)</td>
<td>1 (0 cm)</td>
<td>0 (0 cm)</td>
</tr>
<tr>
<td><em>Coryne. ulcerans</em></td>
<td>1</td>
<td>1 (8 cm)</td>
<td>1 (0 cm)</td>
<td>0 (0 cm)</td>
</tr>
<tr>
<td><em>Actino. israelii</em></td>
<td>2</td>
<td>1 (8 cm)</td>
<td>1 (0 cm)</td>
<td>0 (0 cm)</td>
</tr>
<tr>
<td><em>Haemoph. influenzae</em></td>
<td>9</td>
<td>2 (8 cm)</td>
<td>1 (0 cm)</td>
<td>0 (0 cm)</td>
</tr>
</tbody>
</table>

(+ + + +), γ cm growth inhibition zone diameter; (+ + +), 1.5 cm; (+ +), 1 cm; (+), 0.5 cm; (-) none.
Table 5: Sensitivity of Gram positive and Gram negative bacteria isolated randomly from sore throat patients in Khartoum state to water extract of *Ziziphus spina christi*.

<table>
<thead>
<tr>
<th>Bacterial species</th>
<th>Number of isolates examined</th>
<th>( % ) concentration</th>
<th>( % ) concentration</th>
<th>( % ) concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Strept. pyogenes</em></td>
<td>( % )</td>
<td>((1\text{ cm}))</td>
<td>((1.5\text{ cm}))</td>
<td>((1.0\text{ cm}))</td>
</tr>
<tr>
<td></td>
<td></td>
<td>((2\text{ cm}))</td>
<td>((1.5\text{ cm}))</td>
<td>((1.0\text{ cm}))</td>
</tr>
<tr>
<td><em>Strept. pneumoniae</em></td>
<td>( % )</td>
<td>((0.5\text{ cm}))</td>
<td>((1.0\text{ cm}))</td>
<td>((0.5\text{ cm}))</td>
</tr>
<tr>
<td><em>Staph. aureus</em></td>
<td>( % )</td>
<td>((0.5\text{ cm}))</td>
<td>((1.0\text{ cm}))</td>
<td>((0.5\text{ cm}))</td>
</tr>
<tr>
<td><em>Staph. hemolyticus</em></td>
<td>( % )</td>
<td>((0.5\text{ cm}))</td>
<td>((1.0\text{ cm}))</td>
<td>((0.5\text{ cm}))</td>
</tr>
<tr>
<td><em>Staph. capitis</em></td>
<td>( % )</td>
<td>((0.5\text{ cm}))</td>
<td>((1.0\text{ cm}))</td>
<td>((0.5\text{ cm}))</td>
</tr>
<tr>
<td><em>Coryne. diphtheriae</em></td>
<td>( % )</td>
<td>((0.5\text{ cm}))</td>
<td>((1.0\text{ cm}))</td>
<td>((0.5\text{ cm}))</td>
</tr>
<tr>
<td><em>Coryne. ulcerans</em></td>
<td>( % )</td>
<td>((0.5\text{ cm}))</td>
<td>((1.0\text{ cm}))</td>
<td>((0.5\text{ cm}))</td>
</tr>
<tr>
<td><em>Actino. israelii</em></td>
<td>( % )</td>
<td>((0.5\text{ cm}))</td>
<td>((1.0\text{ cm}))</td>
<td>((0.5\text{ cm}))</td>
</tr>
<tr>
<td><em>Haemoph. influenzae</em></td>
<td>( % )</td>
<td>((0.5\text{ cm}))</td>
<td>((1.0\text{ cm}))</td>
<td>((0.5\text{ cm}))</td>
</tr>
</tbody>
</table>

\( (+ + + +) \), \( \% \) cm growth inhibition zone diameter; \( (+ +) \), \( 1.0 \) cm; \( (+) \), \( 1 \) cm; \( (-) \) none.
Table 7: Sensitivity of bacteria species isolated from sore throat patients in Khartoum state to three different concentration water extract of *Acacia nilotica*.

<table>
<thead>
<tr>
<th>Bacteria species</th>
<th>Number of isolates examined</th>
<th>10% concentration</th>
<th>20% concentration</th>
<th>1% concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Strepto. pyogenes</em></td>
<td>14</td>
<td>8 (57,14%)</td>
<td>8 (57,14%)</td>
<td>8 (57,14%)</td>
</tr>
<tr>
<td><em>Strepto. pneumoniae</em></td>
<td>9</td>
<td>6 (66,66%)</td>
<td>6 (66,66%)</td>
<td>4 (44,44%)</td>
</tr>
<tr>
<td><em>Staph. aureus</em></td>
<td>4</td>
<td>3 (75,00%)</td>
<td>3 (75,00%)</td>
<td>3 (75,00%)</td>
</tr>
<tr>
<td><em>Staph. hemolyticus</em></td>
<td>2</td>
<td>1 (50,00%)</td>
<td>1 (50,00%)</td>
<td>1 (50,00%)</td>
</tr>
<tr>
<td><em>Staph. capitis</em></td>
<td>1</td>
<td>1 (100,00%)</td>
<td>ND</td>
<td>1 (100,00%)</td>
</tr>
<tr>
<td><em>Coryne. diphtheriae</em></td>
<td>1</td>
<td>1 (100,00%)</td>
<td>1 (100,00%)</td>
<td>1 (100,00%)</td>
</tr>
<tr>
<td><em>Coryne. ulcerans</em></td>
<td>1</td>
<td>1 (100,00%)</td>
<td>1 (100,00%)</td>
<td>1 (100,00%)</td>
</tr>
<tr>
<td><em>Actiomy. israelii</em></td>
<td>2</td>
<td>2 (100,00%)</td>
<td>2 (100,00%)</td>
<td>2 (100,00%)</td>
</tr>
<tr>
<td><em>Haemoph. influenzae</em></td>
<td>9</td>
<td>6 (66,66%)</td>
<td>3 (33,33%)</td>
<td>3 (33,33%)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>43</strong></td>
<td><strong>29 (67,44%)</strong></td>
<td><strong>20 (46,13%)</strong></td>
<td><strong>23 (53,86%)</strong></td>
</tr>
</tbody>
</table>
Table v: Sensitivity of bacteria species isolated from sore throat patients in Khartoum state to three different concentration water extract of *Zizphus spina christi*:

<table>
<thead>
<tr>
<th>Bacteria species</th>
<th>No of isolates examined</th>
<th>No. of isolates sensitive (percent) at:</th>
<th>3.3%concentration</th>
<th>2.2%concentration</th>
<th>1.1%concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Strepto. pyogenes</em></td>
<td>4</td>
<td></td>
<td>3 (21.42%)</td>
<td>3 (21.42%)</td>
<td>2 (14.28%)</td>
</tr>
<tr>
<td><em>Strepto. pneumoniae</em></td>
<td>6</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Staph. aureus</em></td>
<td>4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Staph. hemolyticus</em></td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Staph. capitis</em></td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Coryne. diphtheriae</em></td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Coryne. ulcerans</em></td>
<td>1</td>
<td></td>
<td>1 (100%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Actiomy. israelii</em></td>
<td>4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Haemoph. influenzae</em></td>
<td>3</td>
<td></td>
<td>3 (33.33%)</td>
<td>1 (11.11%)</td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>43</td>
<td></td>
<td>7 (16.27%)</td>
<td>0 (11.62%)</td>
<td>2 (4.65%)</td>
</tr>
</tbody>
</table>
Table 8: Sensitivity of bacteria species isolated from sore throat patients in Khartoum state to different antimicrobial drugs and % concentration of water extract of *Acaci nilotica* and *Zizphus spina christi*.

<table>
<thead>
<tr>
<th>Bacterial species</th>
<th>No of isolates examined</th>
<th>Amp</th>
<th>Erythro</th>
<th>Gent</th>
<th>Chlor</th>
<th>Cloxa</th>
<th>Strepto</th>
<th>Tetra</th>
<th>Penic</th>
<th>Acacia nilotica extract</th>
<th>Zizphus spina christi extract</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Strept. pyogenes</em></td>
<td>14</td>
<td>100%</td>
<td>97.1%</td>
<td>98.6%</td>
<td>42.8%</td>
<td>98.0%</td>
<td>100%</td>
<td>94.1%</td>
<td>91.1%</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Strept. pneumoniae</em></td>
<td>9</td>
<td>100%</td>
<td>11.2%</td>
<td>75.7%</td>
<td>44.4%</td>
<td>11.2%</td>
<td>88.8%</td>
<td>44.4%</td>
<td>100%</td>
<td>66.7%</td>
<td>88.8%</td>
</tr>
<tr>
<td><em>Staph. aureus</em></td>
<td>4</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
<td>50.0%</td>
<td>100%</td>
<td>50.0%</td>
<td>100%</td>
<td>50.0%</td>
<td>75.0%</td>
<td>50.0%</td>
</tr>
<tr>
<td><em>Staph. hemolyticus</em></td>
<td>3</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
<td>50.0%</td>
<td>100%</td>
<td>50.0%</td>
<td>100%</td>
<td>50.0%</td>
<td>75.0%</td>
<td>50.0%</td>
</tr>
<tr>
<td><em>Staph. capitis</em></td>
<td>1</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
<td>75.0%</td>
<td>100%</td>
</tr>
<tr>
<td><em>Coryne. diphtheriae</em></td>
<td>1</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
<td>75.0%</td>
<td>100%</td>
</tr>
<tr>
<td><em>Coryne. ulcerans</em></td>
<td>1</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
<td>75.0%</td>
<td>100%</td>
</tr>
<tr>
<td><em>Actiomy. israelii</em></td>
<td>2</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
<td>75.0%</td>
<td>100%</td>
</tr>
<tr>
<td><em>Haemoph. influenzae</em></td>
<td>9</td>
<td>88.8%</td>
<td>77.7%</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
<td>77.7%</td>
<td>88.8%</td>
<td>77.7%</td>
<td>33.3%</td>
<td>43.3%</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>43</strong></td>
<td>97.7%</td>
<td>84.4%</td>
<td>77.7%</td>
<td>72.1%</td>
<td>77.7%</td>
<td>77.7%</td>
<td>77.7%</td>
<td>77.7%</td>
<td>77.7%</td>
<td>77.7%</td>
</tr>
</tbody>
</table>

Amp=Ampicillin; Erythro=Erythromycin; Gent=Gentamicin;
Chlor=Chloramphenicol; Cloxa=Cloxacillin; Penic=Penicillin;
Strepto=Streptomycin; Tetra=Tetracycline.
Figure 7: Bacterial species isolated from sore throat patients in Khartoum state

- **Streptococcus pyogenes**
- **Streptococcus pneumoniae**
- **Staphylococcus aureus**
- **Staphylococcus hemolyticus**
- **Staphylococcus capitis**
- **Corynebacterium diphtheriae**
- **Corynebacterium ulcerans**
- **Actinomyces israelii**
- **Haemophilus influenzae**
- **Mix culture**
Figure 8. Antimicrobial sensitivity of bacteria isolated from throat swab sample collected from sore throat patients in Khartoum state.

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Sensitive</th>
<th>Resistant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chloramphenicol</td>
<td>65.10%</td>
<td>34.80%</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>67.40%</td>
<td>32.50%</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>72.00%</td>
<td>27.90%</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>72.00%</td>
<td>27.90%</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>48.80%</td>
<td>51.10%</td>
</tr>
<tr>
<td>Cloxacillin</td>
<td>27.90%</td>
<td>72.00%</td>
</tr>
<tr>
<td>Penicillin</td>
<td>93.00%</td>
<td>6.90%</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>97.60%</td>
<td>2.32%</td>
</tr>
</tbody>
</table>
Figure 9. Sensitivity of bacteria isolated from throat swab samples collected from sore throat patients in Khartoum state to different antimicrobial drugs and 30% concentration of water extract of *Acacia nilotica* and *Zizphus spina christi*.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acacia nilotica</td>
<td>67.40%</td>
</tr>
<tr>
<td>Penicillin</td>
<td>93.00%</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>67.40%</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>72.00%</td>
</tr>
<tr>
<td>Cefoxitin</td>
<td>27.90%</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>65.10%</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>72.00%</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>48.80%</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>97.60%</td>
</tr>
<tr>
<td>Zizphus spina christi</td>
<td>16.20%</td>
</tr>
</tbody>
</table>
In this study thirty-swabs were collected randomly from sore throat patients in Khartoum state. These swabs were cultured, incubated at 37°C and examined for the presence of bacterial growth.

Twenty-seven samples showed bacterial growth and the rest three samples did not show any bacterial growth. These results showed that 90% of sore throat in Khartoum state were caused by bacteria, while 10% of these cases were caused by others agents which may be viral or fungal.

Out of thirty samples examined in this study, twenty-three strains of *Streptococcus spp* were isolated. These results revealed that 77% of sore throat cases in Khartoum state were caused by *Streptococcus spp*. These results agreed with the results obtained by El amin (2002).

*Streptococcus pyogenes* was the most frequent isolate found in this study as it was recovered from 77% of the sore throat examined. These results confirmed the findings of Turnidge (2001) and Colle *et al.* (1991) who reported that *Streptococcus pyogenes* was the main cause of sore throat.
In this study *Staphylococcus* spp were isolated from 3.3% of the swabs examined and 3.4% of *Staphylococcus* spp isolated were *Staphylococcus aureus*. These finding agrees with those observed by McSween and Whaly (1989), Staish (1990) and ELamin (1993). *Staphylococcus hemolyticus* was isolated from 7.6% and *Staphylococcus capitis* was isolated from 3.3% of the swabs examined and these results were not reported by other investigators.

*Haemophilus influenzae* was isolated from 0.3% of the throat swabs examined in this investigation. This results confirm the results obtained by Kumar (1991) and Elamin (1993) who reported that *Haemophilus influenzae* infection of the throat have world wide distribution.

*Corynebacterium* spp were isolated from 6.6% of the swabs examined and 3.3% of this investigation isolates were *Corynebacterium diphtheriae*. This result agrees with Omer (1994) who reported that *Corynebacterium diphtheriae* is localized on the mucous membrane of the nasopharynx and tonsils. Also 3.3% of this investigation isolates were *Corynebacterium ulcerans* and this result was not reported by other investigators.
In this study *Actinomycess israelii* was isolated from ٥٦٪٦ of the swab examined. The isolation of *Actinomycess israelii* from sore throat cases was not reported by previous study.

In the present investigation ٤٣٪٣ of sore throat cases showed the presence of mixed bacterial infections. These results revealed that ٤٣٪٣ of sore throat in Khartoum state may be caused by more than one pathogenic bacterium.

The results of this study showed that the common organisms associated with sore throat in Khartoum state were *Streptococcus pyogenes* ٥٧٪٦, *Streptococcus pneumoniae* ٤٪٢, *Haemophilus influenzae* ٤٪٢, *Staphylococcus aureus* ٢٣٪٣ and *Corynebacterium spp* ٢٣٪٣. This finding agrees with those observed by Elamin (٤٠٤٢) who reported that *Streptococcus pyogenes*, *Streptococcus pneumoniae*, *Staphylococcus aureus* and *Haemophilus influenzae* were the main causes of sore throat in Khartoum state.

The results of antibiotics sensitivity testing of the isolates found in this study to different antibiotics showed that ٧٦٪٧٩ and ٢٠٪٣٩ of the isolates were sensitive to Ampicillin and Penicillin respectively while Erythromycin, Chloramphenicol, Gentamicin, Streptomycin and Tetracycline were moderately effective for treatment of sore throat caused by bacteria.
In this study all *Streptococcus pyogenes* isolates were sensitive to Penicillin and Ampicillin; these results agree with Geo *et al.* (2001) who reported that all beta hemolytic *Streptococci* were sensitive to Penicillin. While most of *Streptococcus pyogenes* isolates were less sensitive to Erythromycin (41.75%), Chloramphenicol (58.24%) and Streptomycin (5.82%). Lowbury *et al.* (1991) reported that *Streptococcus pyogenes* isolates from human clinical sources were resistant to Erythromycin and this study confirms this findings.

All isolates of *Streptococcus pneumoniae* isolated in this study were sensitive to Ampicillin (100%) and Penicillin (100%) while most of isolates were less sensitive to Erythromycin (11.11%), Chloramphenicol (4.44%), Cloxacillin (11.11%) and Tetracycline (4.44%). These results agree with Geo *et al.* (2001) who reported that pneumococci was sensitive to Penicillin and resist to Tetracycline and Erythromycin.

All isolates of *Staphylococcus aureus* isolated in the present investigation were sensitive to Ampicillin (100%), Erythromycin (100%), Gentamicin (100%), Tetracycline (100%), Chloramphenicol (100%) and Streptomycin (100%) while some of the isolates were sensitive to Cloxacillin (5%) and Penicillin (5%). The results of this
work confirm Geo et al. (١٠٠٢) findings as (٥·٧٪) of Staphylococcus aureus were found resistant to Penicillin in this investigation.

Haemophilus influenzae isolates of the present study were sensitive to Gentamicin (٩٠·١٪), Chloramphenicol (٩٠·١٪) and Streptomycin (١٠·١٪). These results agree with those of Geo et al. (١٠٠٢).

These results illustrate that Ampicillin could be considered the drug of choice for treatment of sore throat infection as most of isolated bacteria (٧٦·٧٪) were sensitive to it and Penicillin is the second drug of choice as many of the isolated bacteria (٣١·٩٪) were sensitive to it.

The response of different isolates found in this study to the three different concentration of water extract of Acacia nilotica and Zizphus spina chrisiti were varied. It was found that ٣·١٪ concentration of water extract of Acacia nilotica was most effective concentration when compared with the other two concentrations as ٤٤·٧٪ of the isolates were sensitive to it while ٣١·٨٪ and ٨٤·٣٪ of the isolates were sensitive to ٢·١٪ and ١·٠٪ concentrations respectively.

On the other hand the three concentrations of water extract of Zizphus spina christi showed weak effect on the growth of bacterial isolates examined in this study as ١٦·٨٪, ٦٣·٩٪ and ٤·٦٪ of the
isolates were sensitive to 90%, 80% and 10% concentration respectively.

The results shown in table reveal that *Acacia nilotica* extract was more effective against *Streptococcus pyogenes*, *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Staphylococcus hemolyticus*, *Staphylococcus capitis*, *Corynebacterium dithpheriae*, *Corynebacterium ulcerans*, *Actinomycess israelii* and *Haemophilus influenzae* when compared with *Zizphus spina christi* extract.

*Acacia nilotica* inhibited the growth of 76.4% of the isolates examined, *Zizphus spina christi* 72.2%, Ampicillin 77.6%, Penicillin 93.5%, Gentamycin 72.9%, Streptomycin 72.4%, Tetracycline 77.4%, Chloramphenicol 70.1%, Erythromycin 88.8% and Cloxacillin 72.9%. This results indicates that *Acacia nilotica* is less effective than Ampicillin, Penicillin, Gentamycin and Streptomycin and equally effective as Tetracycline and more effective than Chloramphenicol, Erythromycin and Cloxacillin for treatment of sore throat infection in Khartoum state. While *Zizphus spina christi* is less effective than Ampicillin, Erythromycin, Gentamycin, Chloramphenicol, Cloxacillin, Streptomycin, Tetracycline and Penicillin for treatment of sore throat infection in Khartoum state as shown in table and figure.
The sensitivity of *Streptococcus pyogenes* isolates when examined to Gentamycin, Cloxacillin, Streptomycin, Chloramphenicol and Erythromycin was 28.2%, 32.8%, 28.2%, 32.8% and 6.1% respectively while their sensitivity to 30% concentration *Acacia nilotica* water extract was 81.1% and *Zizphus spina christi* extract was 41.4%. This indicates that 30% concentration *Acacia nilotica* water extract is equally effective as Erythromycin and more effective than Gentamycin, Cloxacillin, Streptomycin and Chloramphenicol for treatment of sore throat caused by *Streptococcus pyogenes*. However 30% concentration *Acacia nilotica* water extract is less effective than Ampicillin and Penicillin for treatment of sore throat caused by *Streptococcus pyogenes*. Also this study indicates that 30% concentration *Zizphus spina christi* extract is less effective than Ampicillin, Penicillin Erythromycin, Cloxacillin and Chloramphenicol but almost similarly effective as Gentamycin and Streptomycin in inhibition of growth of *Streptococcus pyogenes* causing sore throat in Khartoum state.

The sensitivity of *Streptococcus pneumoniae* to Gentamycin, Erythromycin, Chloramphenicol, Cloxacillin and Tetracycllin was 77.7%, 44.4%, 44.4%, 44.4%, 44.4% respectively while their sensitivity to *Acacia nilotica* extract was 68.7% and *Zizphus spina christi* extract was 0.0%. This indicates that 30% concentration *Acacia nilotica*
extract is more effective than Erythromycin, Cloxacillin, Chloramphenicol, and Tetracycllin for treatment of sore throat infection caused by *Streptococcus pneumoniae* also this study indicate that *Zizphus spina christi* extract is less effective than Gentamycin, Erythromycin, Chloramphenicol, Cloxacillin and Tetracycllin in inhibition of *Streptococcus pneumoniae* causing sore throat in Khartoum state.

The sensitivity of *Staphylococcus aureus* isolates to Cloxacillin and Penicillin was $\%0.05$ while their sensitivity to *Acacia nilotica* extract was $\%0.07$ and *Zizphus spina christi* extract was $\%0.01$. This results indicate that $\%0.7$ concentration *Acacia nilotica* water extract is more effective than Cloxacillin and Penicillin for treatment of sore throat infection caused by *Staphylococcus aureus* and it is also indicate that $\%0.7$ concentration *Zizphus spina christi* extract is less effective than Gentamycin, Erythromycin, Chloramphenicol, Cloxacillin, Tetracycllin, Streptomycin and Ampicillin in inhibition of the growth of *Staphylococcus aureus* causing sore throat in Khartoum state.

The sensitivity of *Staphulococcus hemolyticus* isolates to Ampicillin was $\%1.0$, Erythromycin $\%1.0$, Gentamycin $\%1.0$, Chloramphenicol $\%1.0$, Tetracycline $\%0.7$, Penicillin $\%0.7$. While their
sensitivity to *Acacia nilotica* extract was 75%. This indicates that 70% concentration of *Acacia nilotica* extract is less effective than Ampicillin, Erythromycin, Gentamycin and Chloramphenicol for treatment of sore throat caused by *Staphylococcus hemolyticus*. However 70% concentration of *Acacia nilotica* extract is more effective than Tetracycline and Penicillin. Also this study indicates that 70% concentration of *Zizphus spina christi* extract has no effect on *Staphylococcus hemolyticus*.

The sensitivity of *Staphylococcus capitis* to Ampicillin was 100%, Erythromycin 70%, Gentamycin 100%, Chloramphenicol 100%, Cloxacillin 70%, Streptomycin 70%, Tetracycline 100%, Penicillin 100%, *Acacia nilotica* extract 100% and *Zizphus spina christi* extract was 70%. This result indicates that 70% concentration of *Acacia nilotica* extract is equally effective as Ampicillin, Gentamycin, Chloramphenicol, Tetracycline and Penicillin and more effective than Erythromycin, Cloxacillin and Streptomycin. Also this study indicates that *Zizphus spina christi* extract has no effect on treatment of sore throat caused by *Staphylococcus capitis*.

The sensitivity of *Corynebacterium diphtheriae* to Ampicillin was 100%, Erythromycin 70%, Gentamycin 100%, Chloramphenicol 70%, Cloxacillin 70%, Streptomycin 100%, Tetracycline 100%,
Penicillin, Acacia nilotica extract and Zizphus spina christi extract was 80%. This results indicates that 90% concentration of Acacia nilotica extract is equally effective as Ampicillin, Gentamycin, Streptomycin, Tetracycline and Penicillin and more effective than Erythromycin and Cloxacillin. 90% concentration Zizphus spina christi extract has no effect on treatment of sore throat caused by Corynebacterium dithpheriae.

The sensitivity of Corynebacterium ulcerans examined in this study to Ampicillin was 80%, Erythromycin 80%, Gentamycin 80%, Chloramphenicol 80%, Cloxacillin 80%, Streptomycin 80%, Tetracycline 80%, Penicillin 80%, Acacia nilotica extract 80% and Zizphus spina christi extract was 80%. These results indicates that 90% concentration of Acacia nilotica extract is equally effective as Ampicillin, Erythromycin, Gentamycin, Streptomycin, Tetracycline and Penicillin for treatment of sore throat caused Corynebacterium ulcerans. Also this results indicates that 90% concentration of Zizphus spina christi extract has no effect on treatment of sore throat caused Corynebacterium ulcerans.

The sensitivity of Actinomyces israrlii isolates examined in this study to Ampicillin was 80%, Erythromycin 80%, Gentamycin 80%, Chloramphenicol 80%, Cloxacillin 80%, Streptomycin 80%,
Tetracycline 99.7, Penicillin 99.1, *Acacia nilotica* extract 99.7 and *Zizphus spina christi* extract was 99.7. This results indicate that 99.7 concentration of *Acacia nilotica* extract is equally effective as Ampicillin, Erythromycin, Gentamycin, Chloramphenicol, Cloxacillin, Streptomycin and Penicillin but it is more effective than Tetracycline for treatment of sore throat caused by *Actinomycetes israrlii*. While 99.7 concentration of *Zizphus spina christi* extract has no effect on *Actinomycetes israrlii* isolated from sore throat infection in Khartoum state.

The sensitivity of *Haemophilus influenzae* isolates to Cloxacillin was 99.9, Erythromycin 99.7, Tetracycline 99.7, Ampicillin while their sensitivity to *Acacia nilotica* extract was 99, 99 and *Zizphus spina christi* extract was 99, 99 as shown in table 8. This indicates that 99.7 concentration *Acacia nilotica* water extract is more effective than Cloxacillin and Erythromycin and is equally effective as Tetracycline for treatment of sore throat caused by *Haemophilus influenzae*. Also this study indicates that 99.7 concentration *Zizphus spina christi* extract is more effective than Cloxacillin and Erythromycin for treatment of sore throat caused by *Haemophilus influenzae* in Khartoum state as shown in table 8.
The antibacterial effect of *Acacia nilotica* extract observed in this investigation may be due to the presence of tannic acid in *Acacia nilotica* which inhibit the growth of the bacteria (Smith and Mackie ٤٠٠٢; Smith *et al.*, ٣٠٠٢) or may be attributed to other active component in *Acacia nilotica* extract.

These results illustrated that *Acacia nilotica* is effective for treatment of sore throat, also these results support that *Acacia nilotica* var *nilotica* is tradionally used for treatment of sore throat (ElGhazali *et al.*, ٠٠٠٢).

**Conclusion and Recommendations**
Conclusion:

It can be concluded that:

1) It is probably that 91.6% of sore throat cases in Khartoum state are caused by bacterial infection.

2) The most common organisms associated with sore throat in Khartoum state were *Streptococcus* spp, especially *Streptococcus pyogenes*.

3) Ampicillin is the drug of choice as most of the isolated bacteria (79.76%) were sensitive to it and Penicillin is the second drug of choice as 39.20% of the isolates were sensitive to it.

4) Water extract of *Acacia nilotica var nilotica* may be used for treatment of sore throat infection.

5) Water extract of *Zizphus spina christi* was less effective for treatment of sore throat infection as most of the isolates did not show any response to it. However, it is recommended that the fruit (nabag) to be eaten by throat infected children as it is moderately effective and inhibited the growth of some bacteria isolated in this study and moreover it is rich in vitamins especially vitamin C.
This investigation reported for the first time the isolation of *Staphylococcus hemolyticus*, *Staphylococcus capitis* and *Actinomyces israelii* from sore throat patient.

**Recommendations:**

1) Further study using methanol, ethanol and petroleum ether extracts of *Acacia nilotica* and *Zizphus spina Christi* using the other two methods, cup plate method and agar dilution method is recommended.

2) Phytochemical screening of water, methanol, ethanol and petroleum ether extracts of *Zizphus spina christi* and *Acacia nilotica* should be carried out to identify their active ingredients.
REFERENCES


