ADDIS ABABA UNIVERSITY

GRADUATE STUDIES PROGRAMME

DEPARTMENT OF BIOLOGY

MASTER'S THESIS

INVESTIGATION OF ANTIMICROBIAL ACTIVITIES OF *ALBIZIA GUMMIFERA* AND *FERULA COMMUNIS* ON *STREPTOCOCCUS PNEUMONIAE* AND *STREPTOCOCCUS PYOGENES* CAUSING UPPER RESPIRATORY TRACT INFECTIONS IN CHILDREN

BY

ABAYNEH UNASHO

A THESIS PRESENTED TO THE GRADUATE STUDIES PROGRAMME, A.A UNIVERSITY IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE DEGREE OF MASTER OF SCIENCE IN BIOLOGY (BIOMEDICAL SCIENCES)

JAN. 2005

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Declaration

I the undersigned, declare that this is my own work and it has not been presented in any other institution/university for a similar degree or other purpose

Abayneh Unasho	_____	_____
Name	Date	Signature

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TABLE OF CONTENTS

PAGE

LIST OF TABLES

PAGE

LIST OF FIGURES

PAGE

LIST OF APPENDICES

PAGE

Abstract

A study involving the collection and isolation of the common Gram-positive bacterial pathogens and the antimicrobial susceptibility test of traditionally used plant species was conducted over a period of 5 months (January – August, 2004) at the Ethiopian Health, and Nutrition research institute. Among the specimens collected from the patients of URTIs visiting the OPD of Black Lion hospital, Kirkos and Tekle Haimonot Health centers, a total of 191 infants and children (aged≤ 14 Years) were included as subjects of the study of which 60 specimens of ear discharge were collected from those with clinically symptomatic for acute otitis media and 131 specimens of throat swab were from those with confirmed pharyngotonsillitis clinical symptoms. Out of the total 191 specimens collected, 12% (22 to 191) were positive for clinical isolates of S. Pyogenes ((3.3% (2 of 60) from the specimens of ear discharge, and 15% (20 of 131) from the throat swabs)). Among 60 specimens, of ear discharge collected from the patients of AOM, 10% (6 of 60) of the sample were found positive for S. Peneumonia isolates. The in vitro antibacterial activites of 80% methanol crude extracts prepared from the seeds of Ablizia gummifera and, roots of Ferula communis as well as their respective hydroalcoholic solvent fractionates of both plant species were tested for inhibitory activity against the clinical isolates of six S. pneumonae and twenty two S. pyogenes using agar dilution method. 80% ethanol solublized fractions of both plants were found to have antibacterial effects to all assayed bacteria while aqueous solublized fractions did not exhibit any effect. Minimum inhibitory concentration (MIC of the 80% ethanol solubilized fractions was determined and the MIC of the fractions ranged from 500µg/ml t to 1000µg/ml for both plants showing the extracts may contain bioactive compounds of the therapeutic interest.

1. GENERAL INTRODUCTION

Acute Respiratory tract infections (ARTIs) are infections of the respiratory tract and the alveoli of the lungs caused by invasion of viruses, bacteria and fungi (Bermann, 1983). According to world Health organization, (1991) ARI is clinically categorized, as acute upper respiratory tract infections (AURTIs) and acute lower respiratory tract infections (ALRTIs).

Respiratory Tract infections (RTIs) continue to be a major cause of morbidity and mortality world wide in general and developing countries in particular according to WHO(1999). More recent review also indicated that most of those who die from ARTIs are young children (Ostroff, 1999). Besides this, in developing countries, in every 7 seconds, a child under 5 years of age dies because of acute respiratory infection usually pneumonia. (WHO, 1995). Out of the 12.9 million deaths of children under 5 years that occurred in 1990, some 4.3 million were attributed to ARTIs (WHO, 1994). Moreover, very recent review by Mulholland (2003) estimated that 1.9 million children deaths per year are due to ARTIs, expressing global burden of respiratory infections and the need for intervention for immediate solution.

Two of the AURTIs are acute pharyngitis (pharyngotonsillities) and acute Otitis media (AOM). Although, majority of pharynogotonsillities are due to viral, bacterial infection is also possible and the major etiological bacterial agent is *Streptococcus pyogenes* (Mekane and Kandel, 1986;Sleigh and Timbury, 1994) while the etiological agents associated with AOM are *Streptococcus pneumoniae, Haemophilus influenzae* and less often *Moraxella catarrhalis*, and beta (β) haemolytic *S. pyogenes*. However, more recent review by WHO (2003) reported that *S. pneumoniae* is the cause of approximately 40% AOM and needs grater attention and intervention.

In Ethiopia, the problems of ARI in terms of morbidity and mortality have not been sufficiently described to health planners. However, reports of ARIs are limited to hospitals and a few community based surveys (Lulu Muhe, 1997). Besides, reports have indicated that community acquired pneumonia (CAP) is the most common cause of adult medical admission in Addis Ababa and the leading cause of respiratory admission with mortality rate of 17% (Getachew Aderaye, 1994).

Since their discovery, antimicrobial drugs have proved remarkably effective in the control of bacterial infection. However, it was soon realized that bacterial pathogens were unlikely to surrender unconditionally to antibacterial agents, because some pathogens rapidly became resistant to many of the first effective drugs (Gold and Moellering, 1996) and gradually became widespread throughout the world.

According to world medical Journal (WMJ, 2000), there is a failure to treat respiratory infections due to the emergence of antibiotic resistant strains among the most common respiratory pathogens. Accordingly, *S. pneumoniae* is resistant to penicillin, cephalosporins, macrolides, tetracyclines, chloramphenicol, and some of the Co-trimoxazole drugs. *S. pyogenes* are resistant to erythromycin and other macrolides while *Haemophilus influenze* are resistant to Co-trimoxazole, amphicillin and amoxicillin (WMJ, 2000). However, penicillin has been the treatment of choice for group A. Streptococcal infections, but it is now discouraging that recent studies by Akoacherf and colleagues (2002) in Cameroon, showed that *S. pyogenes, S. aureus,, S. penumoniae* and *Haemophilus influenzae* are resistant to penicillin, erythromycin, clindamycin, chloramphenicol and tetracycline.

Reports of different studies conducted in Ethiopia have also shown that there is a high prevalence rate of antibiotic resistant bacterial pathogens which have created immense clinical problems in the treatment management of most infectious diseases like pneumonia (Ashenafi Belhu and Lindtjorn, 1999; Solomon Gebre Selassie, 2002).Resistance to antimicrobial agents erodes the efficacy of antimicrobial agents (WMJ, 2000). Thus, the development of new and more powerful antibiotics (WMJ, 2000) and use of integrated modern and traditional medicines against such resistant pathogens (WHO, 1978) are some of the major solutions. Therefore, there is a need to search for antimicrobial agents, from medicinal plants originated from traditionally used plants.

Within this context, the aim of the present study was, therefore to evaluate the *in vitro* antibacterial activities of two traditionally used plants: *Albizia gummifera* (Ambabesa-Muka, oromifa, Sessa- Amh.) and *Ferula communis* (Doge-oromifa, Dog-Amh.) against clinical isoaltes of *S. pyogenes* and *S. pneumoniae*.

2. LITERATURE REVIEW

Respiratory tract is a channel that leads to the lungs. Its functions include exchange of gases, filtering and humidifying air. It is also responsible for the expression of speech and consists of sense of taste, smell and hearing. This channel is divided into two systems: the upper and lower respiratory tracts (URT & LRT). Upper respiratory tract (URT) consists of naosopharynx, tonsils, oropharynx, epiglottis, eustachian tube and middle ear while the lower respiratory tract (LRT) is made up of trachea, bronchi, bronchioles and the alveoli (Mckane and Kandel, 1986).

The warm and moist surfaces of the human respiratory tract provide ideal conditions for the growth of disease causing pathogens and normal flora. The upper respiratory tract is abundantly populated by microorganisms of which most are harmless members of the normal flora that contribute to the respiratory defences by bacterial competition and producing antibactericidal agents against the pathogens (Mckane and Kandel, 1986; Tortora *et al.*, 1989;Murry *et al.*, 1998). However, some of these micro organisms are opportunistic pathogens that can cause diseases if predisposing factors compromise the resistance of an individual or when they reach to lower respiratory tract, hence the lower respiratory tract is usually sterile (Mckane and Kandel, 1986; Tortora *et al.*, 1989).

Most air born human pathogens are acquired from infected person or from the inanimate environment containing microorganisms or infected animals. But usually person to person transmission by contaminated respiratory droplets is the most common mechanism for

transmitting respiratory diseases (Mckane and Kandel, 1986; Sleigh and Timbury, 1994; Webber, 1996; Murry *et al.*, 1998).

Mechanism of the bacterial pathogenesis involves the processes that bacteria acquire genetic traits that enable them to enter (invade) the environment of the human body, remain in niche (adhere or colonize), gain access to food sources (possess degradative enzymes) and escape from the host protective immune responses (Murry *et al.*1998).

Many of these genetic factors are virulence factors which enhance the ability of a bacterium to cause diseases, directly destroying the tissues or some release toxins which are then disseminated by the blood to cause system wide illness. Some bacteria still evade host defence mechanisms. *S. pyogenes* for example, has a capsule made up of hyaluronic acid, which mimics human connective tissue, thereby making the bacteria protected, and keeping them free from being recognized by the host immune system (Shanson, 1995; Murry *et al.,* 1998).

2.1 Acute Respiratory tract infections

AURTI is an acute infection, involving the nose, throat, epiglottis and the middle ear, while ALRTI consists of an acute infection involving the larynx, trachea, bronchi, bronchioles or the lungs. Common clinical symptoms of AURTIs are the common cold, epiglotitis, pharyngitis (inflammatory infection of the pharynx), meningitis, sinusitis, and acute otitis media, while that of clinical syndromes of ALRTIs are bronchitis, bronchiolitis and pneumonia.

Epiglotitis is an acute inflammatory infection of the epiglottis and the adjacent cells. Acute bronchitis is an inflammatory infection of the bronchi while bronchiloitis is an acute infection of bronchioles which causes swelling and narrowing leading to the development of pneumonia (lower airway obstruction). Pneumonia is an inflammatory infection of the lungs and has more degree of severity (WHO, 1991).

Respiratory Tract Infections (RTIs) continue to be a major cause of morbidity and mortality world wide infections, which are prevalent during the cold months and are of particular importance in children, elderly people and in those persons with reduced immune responses (Sanford and Finch, 1992; cited in Akoacherf *et al.*, 2002).

Moreover, Acute Respiratory Tract infections are prevalent world wide, and responsible for many deaths and 99% occurs in developing countries (Fig. 1) according to world Health Organization report (WHO, 1999).. Furthermore, WHO (1999) has also reported that the six deadly infectious diseases are now the world's biggest killers Of children and young adults of which Acute Respiratory Tract Infection is the leading one (Figure 1). Two of the major acute upper respiratory tract infections (AURTI) are reviewed in more detail below.

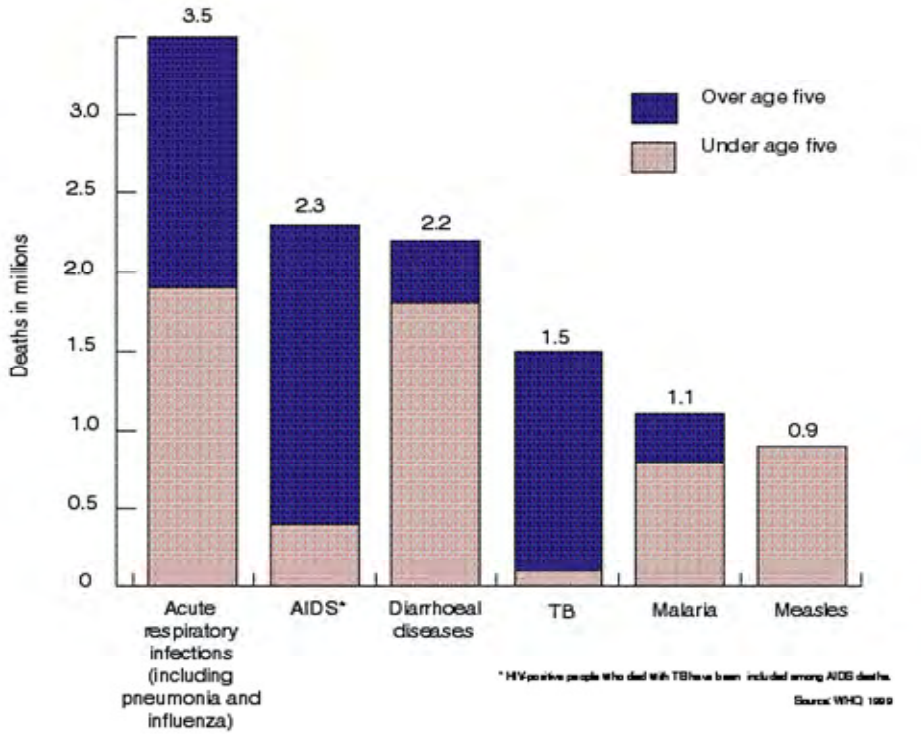


Fig. 1 Leading infectious Killers, millions of deaths, worldwide, in all ages, 1998.(WHO,1999)

2.1.1 Acute pharyngitis (Sore throat or phanyngotonsillitis)

Pharyngotonsillitis: - is an inflammatory syndrome of the pharynx with clinical symptoms of fever, redding and swelling of the mucous membrane and lymph nodes and soft palate with exudates (acute follicular tonsillitis) (Mckane and Kandel, 1986; Sleigh and Timbury, 1994).*Streptococcus pyogenes* being the major bacterial etiological agent of Streptococcal sore throat, extension of the infection involves the sinuses and the middle ear infection producing sinusitis and otitis media respectively. But, late complications include rheumatic fever and acute gromerulonephritis (Klein, 2000).

2.1.2 Acute Otitis media (AOM)

Acute otitis media (AOM) is defined by convention as the first three weeks of a process in which the middle ear shows the sign and symptoms of acute inflammation (Donaldson, 2004). Moreover, acute otitis media is also an inflammation, of the middle ear, that presents with a rapid on set of signs and symptoms such as pain, fever, irritability, anorexia or vomiting (Bermann, 1995). Otoscopic finding of inflammation of AOM may include bulging of the tympanic membrane and a yellow or red colour or both, exudates and a purulent discharge from the ear.

Many studies of the bacteriology of AOM have been performed and the results are remarkably consistent in demonstrating the etiological importance of *S. pneumoniae* and *H. influenzae* in all age groups while *S. pneumoniae* being the most important bacteria causing AOM (Klein, 2000). Recent studies also support that the most common bacteria associated with AOM, are *Streptococcus penumoniae, Hemophilus influenzae* and less often, *Moraxella catarhalis* and β haemolytic *Streptococcus pyogenes*, which originate in the nasophanynx and spread to the middle ear cavity via the Eustachian tube (Roos *et al.* 2001). Furthermore, recent studies made by Centres for Disease Control (CDC) and WHO (2003), reported that *S. pneumoniae* is the cause of approximately 40% AOM, which needs public attention to control the pathogen.

In Ethiopia the problem of ARI, in terms of morbidity and mortality has not been sufficiently described to health planners. However, reports of ARIs are limited to hospitals and a few community based surveys (Lulu Muhe, 1997). It has been known that among 21,853 ambulatory patients in Ethio-swedish children Hospital over a year, ARTI was the principal problem in 20% (Lulu Muhe, 1997) and 35% of 25,000 annual child attendances outside Addis Ababa (Freijis and Streky 1973; cited in Lulu Muhe, 1997).

2.2 The Biology of *Streptococcus pyogenes* and *Streptococcus pneumoniae*

2.2.1 Classification

The genus *Streptococcus* includes three medically important pathogens of humans: *namely S. pyogenes, S. agalactia* and *Streptococcus pneumoniae* (Kenneth and Falkow, 1994). Classification of the genus is based on at least three different schemes that are still used to classify the organisms. i.e.

1. **Serological properties (Lancefield groupings**) :- on the basis of the serological properties, these organisms are organized into A-H, K-V, a total of 20 serogroups (Sleigh and Timbury, 1994). This serological classification was developed by R. Lancefield in 1933. The serogroups possess group specific antigens, most of which are cell wall carbohydrates. However, not all streptococci possess these group specific cell wall antigens (*S. Pneumoniae*, viridans streptococci).

2. **Haemolytic pattern**: - Based on their action on blood agar i.e. beta (β) haemolysis (complete haemolysis), alpha (α) haemolysis (Incomplete or partial haemolysis) and gamma (γ) haemolysis (non- haemolytic groups). Haemolytic pattern is of the basis for the classification of streptococci (Tortora *et al.* 1989).

3. **Biochemical or physiological properties**: - of the group in which all give a negative catalase reaction and each group has their own physiological features (Murry *et al*., 1998; Bison and Stevens, 2000).

S. pyogenes*:* - known as group A *Streptococcus pyogenes* (GAS) is also called group A (β) Haemolytic streptococci (GABHS) based on the Lancefield antigen grouping and beta haemolysis. In a positive throat culture (most reliable and gold standard method for diagnosing streptococcal pharyngitis), the organism appears as (β) haemolytic colonies among the other normal throat flora which are usually α or γ haemolytic on 5% sheep blood agar (Bisno and Stevens, 2000; Cunningham, 2000).

GAS is also classified into M protein serotypes (M_1, M_2, M_3…) on the basis of surface protein (M protein) and there are more than 100 different GAS M protein serotypes (Ma *et al.* 2002). Besides serological classification, M protein is a major virulence factor (Kotarsky *et al* .2000) which plays a pivotal role in the pathogenesis of GAS infection. Furthermore, presumptive identification of GABHS relies on susceptibility test of bacitracin Test (Faklam, 1997; cited in Cunningham, 2000). Bacitracin(an antibiotics whose action is similar to penicillin) test differentiates GABHS from group B,C,G (β haemolytic Lancefied grouping (Wedding and Toenjes,1998).

S. pneumoniae:- is also known as *pneumococcus* or *Diplococcus pneumoniae* because it usually forms cell pairs (found in pairs) (Tortora *et al.,* 1989; Sleigh and Timburry, 1994).The organism is also catalase negative and appears as α haemolytic on the blood agar or chocolate agar (WHO, 2003). Moreover, the organisms produce a dense polysaccharide capsule which is the basis for serological classification of pneumococci into more than 84 serotypes (Tuomanen *et al.* 1995).

However, recent studies by Donaldoson (2004) indicated that there are 90 identified serotypes of pneumococci based on capsular polysaccharide of the bacteria. Capsular polysaccharide also protects the bacteria from phagocytosis (Tuomanen *et al*.1995). Pneumococci can be further distinguished from other Gram positive alpha, haemolytic streptococci by observing the inhibition zone of growth next to Optochin (ethylhydrocuprein hydrochlride) or by performing on bile solubility test. They can also react with antiserum of their capsules, which appears to swell (Swelling= quellung reaction) (Tortora *et al.* 1989).

2.2.2 Streptococcus pyogenes

2.2,2,1 Historical Background

Streptococcus pyogenes*:* - was first described by Billorth in 1874 in a patient with erysipelas and wound infection and in the blood of a patient with puerperal sepsis by Pasteur in 1979 (Bisno and Stevens, 2000). Rosenbach named the organism, *S. pyogenes* in 1984 (Sharma, 2003). Fehleisen isolated chain forming organism in pure culture from erysipelas lesion and showed that these organisms could induce erysipelas in humans (Bisno and Stevens, 2000; Sharma, 2003).

Furthermore, studies by Schotmuller and Brown, 1903;cited in Sharma,(2003) led to the knowledge of different pattern of haemolysis described as α , β, and γ haemolysis, and the later development was, the Lancefiedl classification of β hemolytic streptococci by serograouping on the basis of carbohydrate and M protein (M Serotypes) (Bisno and Stevens, 2000;Cunningham, 2000).

2.2.2.2 General features

Group A *Streptococcus pyogenes* (GAS):- is a Gram-positive, a non motile, non spore forming cocci in short chains to moderate sized or pairs of cells and also single in clinical specimen. Individual cells are round to ovoid cocci. (Bisono and stevens, 2000) and the colonies also appear, as white to grey having 1 to 2 mm in diameter. Besides these, strains of GAS may appear highly mucoid to non mucoid on freshly prepared media, but wrinkled on dry media (Bisno and Stevens, 2000; Cunningham, 2000).

Furthermore, GAS are nutritionally fastidious, thus they are usually cultivated in complex media often supplemented with blood to grow. Metabolism of GAS is fermentative, but the organism is naturally catalase negative (deficient) and aerotollerant anaerobes (facultative anaerobes), because *S. pyogenes* has adapted mechanisms for defence against reactive species of oxygen (King *et al*., 2000; Wilson *et al.*2002).

2.2.2.3 Epidemiology

Group A *Streptococcus pyogenes* (GAS):- is a natural pathogen for human being and is only found in humans (Editorial, 1998; Wilson *et al.*, 2002). Although GAS is not considered as a normal flora of pharynx, pharyngeal carriage of the group A *Streptococcus pyogeness* can occur without clinical symptoms of the disease (Cunningham, 2000).

GAS frequently colonize the throats of symptomatic persons,and pharyngeal carriage rate among normal school aged children (5 to 15 years) vary with the geographical location and seasons of the year (Bisno and Stevens, 200). Moreover carriage rate of 15-20% have been noted in several studies among health children and somewhat lower in adults (Murry *et al.*, 1998; Bisno and Stevens, 2000; Wilson *et al.*, 2002).

All age groups are susceptible to the spread of organisms under crowded conditions such as schools, military barrack and Hospitals (Cunningham, 2000). But the peak

incidence of GABHS pharyngitis is in children aged 5-10 years old (Murry *et al.* 1998). It is usually transmitted by direct contact from person to person via aerosols created from respiratory nasal secretions or droplets produced by coughing and sneezing or by direct infected skin to uninfected skin contact (Murry *et al.*, 1998; Wilson *et al.*, 2002) and these are the most common mechanism of transmitting upper respiratory diseases (McKane and Kandel, 1986), but contamination of dust, clothing, blankets or other fomites do not have a significant role in a contagion (Bisno and Stevens, 2000).

Recurrent infections are sometimes seen in families when prompt antimicrobial therapy has prevented the development of type specific immunity. This situation allows reinfection from other infected or colonized siblings, when antimicrobial treatment is stopped. Such "ping pong" infection reinfection cycles sometimes require simultaneous treatment of the entire family to prevent continued transmission (Kenneth and Falkow, 1994).

2.2.2.4 Pathogenesis and virulence factors

Group A streptococci (GAS):- provoke a wide spectrum of human diseases, ranging from uncomplicated non- invasive to severe invasive infections, giving a total of 90% streptococcal diseases (Boyed, 1995; Teglund *et al.*, 1997; Cunningham, 2000; Alouf *et al.*, 2001). Non-invasive common GAS infections include diseases such as strep throat (pharyngotonsillitis), scarlet fever, impetigo, ear infections and pneumonia and

these infections are less severe and more contagious than invasive GAS infection (Cunningham, 2000; Wilson *et al.*, 2002).

Invasive GAS infections are more aggressive and may cause a condition known as STSS (Streptococcal toxic shock syndrome), rheumatic fever, meningitis puerperal sepsis, and necrotizing fasciitis (hospital gangrene or flesh eating disease) (Cunningham, 2000; Wilson *et al.*, 2002).

Multiple risk factors influence pathogenesis of the bacterium and influence the initiation of the infection in the host (Sharma, 2003). These risk factors include host factors such as age, genetic factors, and host immune response, various streptococcal virulence factors (e.g. M protein, C5a peptidase, hyaluronic acid etc and factors such as wound/sore are associated with streptococcal pathogenesis (Basma *et al.* 1999)

.Besides, lack of protective immunity to specific virulence factors produced by the bacteria is likely to affect host susceptibility to infection (Sharma, 2003). Moreover, studies have also shown that low level of antibodies directed to specific SPE (Streptococcal Pyrogenic Exotoxins) or to M protein may cause host susceptibility to invasive infections (Holm *et al.* 1992).

Virulence factors- can be defined as a component of a pathogen that damage the host, and can include components that either is essential for the survival of a pathogen or not. The components can comprise microbial products that permit the pathogen to enhance pathogenesis and cause disease (Casadevall and Pirofski, 1999).

Moreover, virulence factors are genetically encoded substances and the acquisition of such genes is sufficient for a bacterium to become a pathogen. Thus, *Streptococcus pyogenes* possess several components of virulence factors/ antigenic components/ that are believed to be important in pathogenesis and classification (Cunningham, 2000). Virulence factors produced by strains of *S. pyogenes* that contribute to its invasiveness and pathogencity are:-

A. **M protein** (antigen):- is the major virulence factor of group A streptococci. It's effects appear to result from a variety of biological activities which include inhibition of opsonization, and phagocytosis (antiphagocytic effect), colonization and adhesion (attachment) and stimulating tissue cross reactive immune reaction causing autoimmune diseases such as rheumatic heart disease and glomerulnephritis (Horstmann *et al.*, 1992;Murry *et al.*, 1998)

B. **Hyaluronic acid capsule** (non immunogenic):- surrounds the organism making it slippery. and is required to resist phagocytosis and the mechanism may be due to the physical barrier of the capsule in preventing access to phagocytes (Cunningham, 2000).Besides this the capsule may be an important adherence factor in the epithelial cells of the pharynx (Schrager *et al.,* 1998; cited in Cunningham, 2000).

C. **C5a peptidase**: - It is a proteolytic enzyme (endopeptidase) found on the surface of group A streptococci (GAS). The peptidase destroys the chemotactic signals by cleaving the complement derived C5a at its PMN

binding site (Cleary *et al.* 1992). This event then inhibits the recruitment of phagocytic cells to the site of infection, because complement C5a mediates inflammation by recruiting and activating phagocytic cells, however, C5a peptidase disrupts this process by degrading C5a (Murry *et al.*, 1998;Cunningham, 2000).

D. **Streptolysin**s: - toxins that haemolyse red blood cells and are toxic to a variety of cells include polymorphonuclear leukocytes, platelets, and tissue culture cells (Sharma, 2003). Streptolysins can be streptolysin S (non-immunogenic), which is active aerobically and cause β haemolysis on the blood agar, while streptolysin "O" haemolyses RBC under anaerobic condition. It is also immunogenic, stimulating the production of antistreptolysin "O" antibody (ASO).

E. **Streptokinase:-** it is a protease that lyses fibrin

F. **Leucocidin: -** toxin that causes lysis of white blood cells by damaging the cell membrane of phagocytes and possibly its lysosomes.

G. **Lipoteichoic acid**:- component of Gram positive bacterial cells which facilitates adherence to pharyngeal epithelial cells

H. **NADASE** (Nicotinamide Adenine Dinucleotide); - kills leucocytes, antibody is formed after infection against NADASE.

I. **DNASES** (Deoxyribonucleases):- Include A, B, C, and D that breaks down DNA and stimulate an antibody response particularly against DNASe B.

J. **Erythrogenic toxin**:- Responsible for the rash seen during scarlet fever and is also associated with STSS (streptococcal toxic shock syndrome (Cheesbroughs, 2000)

K. **Pyrogenic exotoxins**: - GAS produce 3 different types of exotoxins (i.e A, B, C). These toxins are responsible for causing fever, and scarlet fever rash, increasing susceptibility to endotoxic shock, cause dysfunction of the reticiulo endothelial system and depress antibody synthesis. These toxins serve as immunomodulators of the host defence system because they stimulate T cells to proliferate, and are referred to as supper antigens (Sharma, 2003).

Thus, pathogenic mechanisms of GAS largely depend upon their virulence factors. Therefore, mechanism by which GAS becomes pathogenic involves their capacity to:-

- Adhere to the host surface using their virulence factors such as M protein, LTA etc
- Cause host tissue cross reactivity using M protein
- Become anti phagocytic
- Destroy chemotactic factors using C5a peptidase
- Evade the host defences (Medina *et al.* 2003)

2.2.2.5 Incidence of the group A streptococcal infection

Studies have shown that group A streptococcal infection and their complications constitute a considerable public health problem particularly in developing countries (Kaplan, 1997). Furthermore, it has been reported that in Egypt, the incidence of streptococcal pharyngitis is 31 per hundred child years and between 0.4% -1.0% of school children have clinical evidence of cardiac valvular damage due to acute rheumatic fever, (El-Kholy, 1973; 1980; cited in Steinhoff *et al.* 1997). Besides, an estimate of 12 million have acute rheumatic or rheumatic heart disease and these disorders account for about 400,000 deaths world wide and disability in hundreds of thousands of young adults and children (Bull. WHO, 1995; cited in Steinhoff *et al.* 1997).

Furthermore, it has been indicated that rheumatic fever is a major cause of acquired heart disease in children worldwide (Stollerman, 1997). It occurs most frequently in under developed counties where access to medical care is limited and children live in poverty and unsanitary crowded conditions. Stollerman (1997) also pointed out that the inadequate prevention of streptococcal infection and deprivation of children in communities near Johannesberg, South Africa, and aborigines of Northern Australia have a very high rate of rheumatic fever ($\geq$ 20 cases per 1000).

On the otherhand, compared to communities in the developed world with adequate access to medical care show very low rate of rheumatic fever (0.2 to 0.5 cases per 1000). In addition to, a recent epidemiological survey, in a rural north India has also indicated 210 cases of rheumatic heart disease per 100,000 school children aged 6 to 15 years (Kaur *et al.*, 1998; cited in Cunningham, 2000). Apart from this, Kaur and his colleagues as cited by Cunningham (2000) have calculated that the incidence of rheumatic heart disease worldwide ranges from 0.55% to 11% per 1,000.

Unlike rheumatic fever, the out breaks of acute glomerulonephritis have continued to decline. This may be due to changes in the streptococci or the host factors (Silva, 1998; cited in Cunningham, 2000). Moreover, Cunningham in his review also indicated that regions of the world which still exhibit a high incidence of post streptococcal acute glomerulonephritis include Africa, New Zealand, the Caribbean South America and Kuwait (Cunningham, 2000).

Since the mid- 1980, there has been an unexplained resurgence of group A streptococcal infections observed. I.e. there have been an increase in the out break of rheumatic fever, streptococcal toxic shock syndrome and severe invasive and soft tissue infections (Wilson *et al***.** 2002), including in well developed countries such as USA, but whether, these types of GAS infections will decline, stay the same or increase is not known (Stevens, 1995; Wilson, *et al.*, 2002). It may be due to the emergence of certain M protein serotypes including M types, 1,3,5,6 and 18 of the

GAS associated with the new out breaks of the rheumatic fever and invasive disease (Wilson *et al*. 2002).

One study of glomerulonephritis strains in Ethiopian children was carried out, but did not associate certain genotypes with specific diseases such as acute glomerulonephritis (Tewodros *et al.*, 1993; cited in Cunningham, 2000).

2.2.3. Streptococcus pneumoniae: - (The Major causative agent of Acute Otitis Media)

2.2.3.1 **Historical Background**

In 1888, the organism was first identified concurrently by L. Pasteur in France, who named it "Microbe septicemique du saliv" and by Stenberg in the United States who called it Micrococcus Pasteuri. By late 1880,s the term pneumococcus was generally used because this bacterium had come to be organized as the most common cause of labour pneumonia. The name *Diplococcus* was also used/or assigned in 1926 because of its appearance in Gram stained sputum and in 1974 the organism was renamed once again as *Streptococcus pneumoniae* because of its morphology during growth in liquid medium (Musher, 2000). *S. pneumoniae* is also named as *Diplococcus pneumoniae* because it usually forms cell pairs (found in Pairs) (Tortora *et al*. 1989; Sleigh and Timbury, 1994).

Early in the 20th century, three serotypes were distinguished and called serotypes, 1.2, and 3. All other pneumococci were called group 4 (Musher, 2000). However, there are

90 serotypes of *S. pneumoniae*, identified on the basis of the antigenic differences in their capsular polysaccharides (Musher, 2000; Donaldson, 2004). The serotypes are represented by numbers of letters based on American or Danish numbering system.

In the American numbering system serotypes are numbered from 1 to 90 in which they were identified, but the more widely accepted Danish numbering system of groups is according to antigenic similarities. For example Danish serogroup 19 includes type 19F, 19A, 19B and 19C (the letter F indicates the first number of the group to be identified followed by A,B,C etc) which in the American system would be types 19, 57, 58 and 59 respectively (Musher, 2000). Stereotyping was clinically relevant in 1930s when antisera were administered for therapy and is of great interest from the epidemiologic and public health standpoints today, especially as new vaccines are being developed.

2. 2 .3.2 **General features**:-

S. pneumoniae :- is a Gram- positive, elongated (lance late), diplococci or short chains of cocci, non motile, typically have a ringed (draughtsmen's) appearance with sunken center due to autolysis of older colonies, but younger colonies may resemble dew drops due to large capsules before autolysis (Cheesbrough, 2000; WHO, 2003). On blood and chocolate agar following overnight incubation at 35 to 37 ^{0}C, colonies of *S. pnenmoniae* also appear as small greyish and translucent or mucoid (e.g serotype 3, produces large mucoid colonies) with 1-2 mm in diameter, surrounded by a greenish

zone of α haemolysis (Cheesbrough 2000;WHO, 2003). Nearly every clinical isolates of *S. pneumoniae* are capsulated (Cheesbrough, 2000; Musher, 2000).

The organism is nutritionally fastidious and can grow, under atmospheric conditions ranging from aerobic to caponophilic and a few are obligately anaerobic. Pneumococci produce pneumolysin (formerly called haemolysin) which breaks down haemoglobin into a green pigment, as the result pneumococcal colonies are surrounded by a green zone during growth on blood agar plates (Musher, 2000; Cheesbrough, 2000).

Growth of pneumococci is inhibited by ethyl hydrocupreine hydrochloride (optochin/ and the organisms are lysed by bile salts. Thus, the organisms are identified in the microbiology laboratory by four reactions: (1) α haemolysis of blood agar, (2) catalase negativity, (3) susceptibility to optochin, and (4) solubilitiy in bile salts. (Cheesbrough, 2000; Musher, 2000).

2.2.3.3 Epidemiology

S. pneumoniae :- is a member of the normal pharyngeal flora of most people and is important human pathogen. It is frequently carried in throat without causing diseases. The nasopharyngeal carriage of pneumococcus is highly prevalent among young children and predisposes, the carrier to his or her siblings and others in close contact to carriers to pneumococcal infection (Giebinks, 2001). The rates of nasopharyngeal carriage are 44% among all children six years older or younger (Zenni *et al.* 1995),

60% to 80% among children attending care centres outside home (Bokem *et al.*1995) and more than 70% among children younger than three years of age who have acute otitis media (Dagam *et al.* 1998).

Moreover, at any given time, upto 60% of the people in the community may carry pneumococci in the nasopharynx, which is the normal ecological niche of a bacterium in humans. However, the carriage rate in a given community may be high but the incidence of invasive disease usually is comparatively low (Obaro *et al.* 1996).

Pneumococci spread easily from person to person by respiratory droplets or through direct inoculation of secretions (Gold and Moellering, 1996). According to Munford and Murrphy (1994); cited by Gold and Moellering (1996), the organism may also spread from patients to hospital staff, and therefore, the carriage rates among nurses caring for patients with pneumoccal pneumonia can be high

Pneumococcal infections usually spread by colonization of the human pharynx. This is an important step towards infection. Consequently, pneumococcal colonization is an important risk factor besides viral predisposition in developing the disease. For example, young children who are frequently colonized with pneumococci, more often develop Acut Otitis Media (AOM) than do children who are not or less frequently colonized (Faden *et al.* 1997).

2.2.3.4 Pathogenesis and Virulence factors

S. pneumoniae:- is the major cause of pneumonia, empyema, Otitis media, septic arthritis, septicaemia and meningitis (Tuomanen *et al.* 1995) Furthermore, *Streptococcus pneumoniae* and *Hemophilus influenzae*, are the leading cause of HIV associated community acquired bacterial pneumonia (Street, 1996). Moreover, recent reviews made by Donaldson (2004) have also showed that *S. pneumoniae* remains the most common pathogen responsible for acute otitis media in all age groups including neonates and is the major cause of morbidity and mortality causing childhood illness and death.

Pneumococcal infection and its complications result partly from the direct actions of pneumococcal virulence factors and the corresponding immune responses to various pneumococcal components resulting in adhesion, invasion, inflammation and shock (Gillespi and Balakrishnan, 2000). Pneumococcal virulence factors mainly include:-

Capsular polysaccharide: The pneumococcus is encapsulated, which is the main virulence factor of the bacterium. Capsular polysaccharide protects pneumococci from phagocytosis by polymorphonuclear leukocyts, following opsonization by polysaccharide specific antibodies and complements proteins (Vidarsson *et al.*, 1994; Tuomanen *et al.*, 1995) i.e. it inhibits phagocytosis and protects the pathogens from the engulfment by alveolar macrophages. Besides, more than 80 distinct serotypes have been identified based on differences in antigenic composition of their capsule (Obaro

et al. 1996), but recent reviews have indicated that there are 90 identified serotypes based on differences in their capsular polysaccharide antigens enabling the bacterium to show heterogenicity (Weiser and Kapoor, 1999; Donaldson, 2004). Serotyping of pneumococci can be determined by the quellung reaction in which specific antibodies react with capsular antigens on the surface of the organism being tested, causing capsular swelling (Obaro *et al.* 1996).

Furthermore, more than 80% of the severe, pneumococcal infections are caused by 23 of the 90 different serotypes and the current 23 valent polysaccharide vaccine was formulated to include the serotypes that cause 85 to 90% of the bacteraemic infection in the united states (Broome *et al.* 1980).However, this vaccine is very effective in young adults (at low risk factors) and about 60% effective in elderly but ineffective in children less than 2 years of age (Jedrzeejas, 2001) indicating the need for further investigation of the pathogen .

Cell wall: - The components of pneumococcal cell wall stimulates the recruitment of leukocytes into the lungs and subarachnoid space (Tuomanen *et al*. 1985), induce production of cytokines (Heumann *et al.* 1994), stimulate the production of platelet activating factor (PAF) (Cabellos *et al.* 1992) and causes direct damage to neurons and cerebral blood flow. The presence of techoic acid in a cell wall component also enhances its inflammatory activity (Tomasz and Saukkonen, 1989).

Surface protein: - the surface proteins of pneumococci are largely undefined and their specific functions are not known (Pear *et al.* 1993). However, surface proteins such as pneumolysin released during lysis, is active in pore formation and it is also cytotoxic to virtually every cell in the lungs which enhances pathogenecity of the bacterium (Boulnois *et al.* 1991). Pneumolysin is a 53 Kda, produced by all clinically isolated pneumococci which have a cytolytic activity involving interaction with cholesterol of a cell membrane and insertion into the lipid layer. This is followed by oligomerization to form pores of the membrane which eventually brings about host cell lysis (Berry *et al* .1999).

Hyaluronidase: - an enzyme which facilitates pneumococcal invasion by degrading connective tissue. The importance of this enzyme in the pathogenic mechanism has been shown that pneumococcal strain with higher hyaluronidase activity breach the blood-brain barrier and disseminates more effectively (Gillespi and Balakrishnan, 2000). Besides this, it was concluded that high hyaluronidase activity is the most important predisposing factor, contributing to the development of pneumococcal meningitis (Kostyukova *et al.* 1995).

2.2.3.5 Incidence of pneumococcal infections

Streptococcus pneumoniae remains an important cause of morbidity and mortality worldwide. Despite the advent of powerful antibiotics and other medicinal advances, the incidence of mortality associated with bacteraemic pneumonia has remained at 25% over the past 40 years (Obaro *et al.* 1996). Moreover, globally the pneumococci infection accounts for over million deaths each year in children under 5 years of age (Obaro *et al.* 1996). Furthermore it has also been reported that *S. pneumoniae*, the leading cause of childhood pneumonia and meningitis is responsible for 20 to 40% of the global total of 4 million deaths from pneumonia per year (Samir *et al.* 1997) and preventing such deaths should be a high priority in the health developing policies of developing countries.

However, it has been also reported that the majority of children in the United States have at least one pneumococcal infection of the middle ear by the age of five years and between 15% - 25% community acquired cases of pneumonia caused by pneumococci with a case fatality rate of 5 to 7% in treated patients (Broome *et al.* 1980).

Besides this, in United Kingdom, it has also been reported that pneumococci is responsible for 30%-50% community acquired pneumonia and 8% nosocomial pneumonia (Obaro *et al.* 1996). Acute Otitis media mainly caused by *S. pneumoniae*, is also one of the common infections in children less than 2 years of age and is the common reason for a paediatric sick visit and may take up to one third of the visit a

paediatrician devotes to ill children (Howie and Schwartz, 1983; Diriba Muleta *et al.* 2004).

In developing countries such as Gambia the attack rate of pneumococcal disease is high particularly in children and an estimated 60%-90% of the lower respiratory infection in children less than 5 years of age is caused by *S.* pneumoniae. Similar or even higher attack rates have been recorded in communities of adults such as the mining communities of South Africa in whom the rate may be as high as 100% per 1000 population per year (Obaro *et al.* 1996).

In Ethiopia, reports have indicated that community acquired pneumonia (CAP) is the most common cause of adult medical admission in Addis Ababa and the leading cause of respiratory admission with mortality rate of 17% (GetachewAderaye,1994). In general, there are several cases of upper respiratory tract infections and otitis media among children; however, there is very little information about the isolation (Solomon Gebresellasie, 1998) and characterization of potential bacterial pathogens from the ear infections in Ethiopia (Diriba Muleta *et al.* 2004). Hence there is a strong need to generate data on prevalence, drug resistance and susceptibility test of bacterial pathogens of the upper respiratory tract infections.

2 .3 Susceptibility of *S. pneumoniae* and *S. pyogenes* to Antimicrobial agents and management of infections.

2.3.1 Susceptibility to antimicrobial agents

Since their discovery, antimicrobial drugs have proved remarkably effective for their control of bacterial infections. However, it was soon realized that bacterial pathogens were unlikely to surrender unconditionally to antibacterial agents, because some pathogens rapidly became resistant to many of the first effective drugs (Gold and Moellering, 1996).

Moreover, antimicrobial resistant microorganisms in general have been known from the early days of chemotherapy (Finland, 1995) and even predate the discovery of antibiotics. Resistance is also an inevitable result of the rapid replication and evolution of microbes, since a single genetic mutation may lead to resistance altering the pathogenicity or the viability of bacterial strains (Gold and Moellering 1996). This mechanism enhanced rapid development of resistant strains of most bacterial population.

In 1940's all *Streptococcus pneumoniae* were exquisitely susceptible to penicillin with the concentration of less than 0.1 mg/L which killed them rapidly (Gold and Moellering, 1996). However, resistant strains emerged progressively with more and more resistant pneumococci strains, developed requiring minimum inhibitory concentration of penicillin up to 4 to 8 mg/L and often resistant to other drugs were described in South Africa in the mid 1970's (Jacob's *et al.* 1978). Subsequently

penicillin resistant *Streptococcus pneumoniae* have been found virtually widespread throughout the world.

Studies carried out by Ling and colleagues (1983) at the university of Hong Kong showed that among 119 respiratory isolates of *Hemophilius influenzae* and 87 respiratory isolates of *Streptococcus pneummoniae*, 58% and 23% of these isolates tested were found to be resistant to tetracycline (MIC = 4-12 mg/L) and (4-6 mg/L) respectively. Dejsirilet and Colleagues (1999), have also reported that the prevalence of penicillin resistant *S. pneumoniae* in Thailand has dramatically increased over the last decade.

Moreover, another study showed that among Finish children, *S. pneumoniae* strains caused about 39% of acute otitis media of which all isolates were sensitive to penicillin, 3% were resistant to erythromycin and 12% resistant to co-trimoxazole which is indicating strain and geographical variations (Eskola *et al.* 1992). In addition to, another studies made by Whitnney and colleagues (2000) in the USA, showed that over, 24% isolates of *S. pneumoniae* from children under 5 years of age were resistant to penicillin and these isolates were also resistant to other antimicrobial agents. They also showed that the proportions of isolates that were resistant to 3 or more classes of drug increased from 9 to 14%, i.e. proportion of isolates that were resistant to penicillin (from 21% to 25%), cerfotaxine (10% to 14%) Meropenem (10% to 16%), erythromycin (11 to 15%) and Trimethoprim sulfamethoxazole (25% to 29%).

The study by Giebinks (2001) showed that the overuse of antibiotics, has contributed to the Group A *Streptococcus pyogenes* (GAS) and *S. pneumoniae* which are reported to be resistant to macrolids and penicillin respectively (Kaplan, 1997). Furthermore, World Medical Journal (WMJ, 2000) has reported that there is a failure to treat respiratory infections due to antibiotic resistant strains of the most common respiratory pathogens.

According to WMJ (2000) report: *Streptococcus pneumoniae* is resistant to penicillin, cephalosporins, macrolides, tetracyclines chloroamphenicol and some co- trimoxazole, Group A *Streptococcus pyogenes* is resistant to erythromycin and other macrolids (azithromycin, clarithromycin and roxithromycin), *Hemophilus influenzoae* are resistant to co- trimoxazole, ampicillin and amoxycillin. However, penicillin has been the treatment of choice for group A Streptococcal infection, but it is discouraging that recent studies by Akoacherf and colleagues (2002) in Cameron, showed that *S. pyogenes, S. aureus, S. pneumoniae and H. influenzae* are resistant to penicillin, erythromycin, clindamycin, chloramphenicol and tetracycline.

Reports of different studies conducted in Ethiopia revealed that there are high prevalence rates of antibiotic resistance of bacterial pathogens, which have created immense clinical problem in the treatment of most infectious disease like pneumonia (Ashenafi Belhu and Lindtjorn, 1999; Solomon Gebreselassie, 2002). Solomon Gebreselassie (2002) has also reported that the resistance pattern of *S. aureus,* CoNS,

S. pneumoniae and enterococci to penicillin was 91.5%, 94.4% 7.7%, and 100% respectively.

He also showed that strains of *S. aureus* were susceptible to vancornycin, clindamycin and gentamycin, but relatively higher resistance of *S. pyogenes* to cephalothin, trimethoprim and clindamycin. He has also reported the existance of multidrug resistance (i.e. to 3 or more drugs) in most of the isolated bacteria i.e. *S. aureus* showed resistance in 9 of the 13 drugs tested, CoNS in 12 of the 13 antibiotic tested, *S. pneumoniae* in 10 of 11., *S. pyogenes*, in 5 of 11 and enterococci spp showed resistance in 6 of 11 antibiotics.

Resistance to antimicrobial drugs is a global problem, and the situation has now reached at a crisis stage world-wide (Kunnin, 1993) and the problems are immense: Resistance to antimicrobial agents erode the efficacy of antimicrobial agents (WMJ, 2000) and as the result, it increases: - 1. The burden of illness causing morbidity and mortality. 2. The need for repeated courses of antibiotic use and its side effects. 3. Compromised social and working life. 4. The possibility of hospital admissions and increased overall treatment cost.5. Decrease in labour productivity, and the cost of incidence of diseases that result from the transmission of infected individuals whose infection due to resistant organism are not treated in a timely manner (WMJ, 2000; Howard *et al.,* 2003). Moreover emergence of antimicrobial resistance is a worrisome trend in developed and developing nations, but developing nations face much greater threat from the growth of resistance in pathogens causing community acquired diseases such as malaria pneumonia etc (Howard *et al.* 2003). Thus a search for overall solutions that is sustainable for drug resistant bacterial pathogens are urgently needed.

2.3.2 Management of Bacterial infections

Serious health problem due to diseases caused by bacterial pathogen on one hand and drug resistance development against antimicrobial agents on the other hand are threats to public health and social life throughout the world (Zeleke Wold Tenssae, 2001). Thus, there is a need for immediate solutions to curve the direction of the problems. It is widely accepted that, in order to reduce morbidity and mortality due to drug resistant bacterial pathogens, the development of either new and more powerful antibiotics (WMJ, 2000) or use of integrated modern and traditional medicines against resistant pathogens (WHO; 1978) are proposed solutions. Furthermore, measures like public education, proper management of the drugs, development of vaccines against resistant bacterial pathogens etc are the necessary parameters to avert and control drug resistant pathogens.

2.3.2.1. Public education

Primary health education is the key step to public awareness about the health and the methods of disease transmission. For these objectives the public, at large and patients, in particular need to be educated about the risks of infectious diseases, and seeking medicinal help at the onset of illness (Obaro *et al.* 1996). Furthermore, patients need to know treatment procedures because it is well known that many patients do not know complete antibiotic dosage and course. They often stop once they feel better due to few side effects. This leads to the

development of resistant strains in course of time which may spread through the population (WMJ, 2000).

2.3.2.2. Susceptibility test and appropriate prescriptions

Inappropriate prescriptions, particularly under dosing whereby antibiotics may be prescribed at a low concentration for long periods of time or they may be taken at an infrequent time intervals, may give chances for the development of resistant strains, and impacted an increase in antibiotic resistance worldwide (WMJ, 2000). Thus, this practice should be improved and avoided. Besides, laboratory practices such as drug susceptibility testing must be given serious attention because it provides more information about patient's infections, can reduce morbidity and mortality rates (Solomon Gebreselassie, 2002) and diminishes the impacts of community dependence on physician's choice of therapy (Howard *et al.* 2003). Physicians in developed countries may be quicker to switch empiric therapies in response to increasing resistance levels, whereas patients in developing countries may have to take older drugs. This may lead to the high rate of treatment failure. Consequently, for the same underlying rates of resistance, the burden would be larger in developing countries (Howard *et al*. 2003). Thus the use of empiric therapy based on susceptibility testing of the drugs will be effective against resistant bacterial pathogens.

However, due to lack of laboratory facilities and financial resources, susceptibility testing is not the standard of practices in developing countries and this needs greater

attention. Besides these, developing counties should have a continuous surveillance of the prevalence of the disease and detection of antibiotic sensitivity pattern of the bacterial pathogens in local environment, and these should be the basis for effective therapy (Solomon Gebreselassie, 2002).

2.3.2.3. Development of new drugs and integration of traditional medicines.

In order to reduce morbidity and mortality, caused due to antibiotic resistant bacteria another option is, development of either new and more powerful antibiotics (WMJ, 2000) or use of integrated modern and traditional medicine practices against resistant pathogens (WHO, 1978). This practice needs research work and involvement of the public at large and scientific community, in particular to make it effective.

2.3.2.4 Other measures

To prevent and control infections by emerging antimicrobial resistant strains, measures such as breast feeding, vaccine development, organizing clinical microbiology laboratories locally and at national level, increased emphasis on effective infection controls, emphasis on personal hygiene practice and prudent use of existing antimicrobial agents are recommended (Zeleke Wold Tenssaie, 2001).Besides, focus should be put on standardization and quality control of laboratory procedures to secure comparability of data within and between the different countries (Ringertz *et al.* 1993).

2. 4 Application of Traditional Medicine and Objective of the Study

2.4.1 Traditional Medicine

”An apple a day keeps the doctor away” traditional American rhyme (Cowan, 1999). This traditional saying clearly signifies the potential value of plant products for human walefare. According to Akerele(1984) , traditional medicine is defined as the sum total of knowledge and practices whether they are explained or not , used in preventing diagnosis, and elimination of physical, mental, or social imbalance and relying exclusively on practical experiences and observations, handed down from generation to generation whether orally or in written form. Furthermore, Farnsworth (1985), states that, if traditional medicine in general and medicinal plants in particular are validated:-

1. They will provide locally accessible alternative to imported drugs that will be accepted by the population both because of their cheapness and because they have been used for centuries.
2. They may be used together with western pharmaceutical products, i.e. integrated within the framework of official health services (the Chinese model) in order to take advantage of the positive feature of both traditional and modern medicines.
3. They will in the long run enable pharmaceutical industries based on local resources to evolve the benefit of national economy and provide basis for the discovery of new substances that might be useful against intractable ailments.

Historical background suggests that the use of traditional medicine for the treatment of diseases in all countries whether developed or developing, have been an ancient idea and indeed even the Bible offers description of 30 healing plants (Cowan, 1999). There is also

evidence that the Neanderthals, living 60,000 years ago in present day Iraq had used plants such as holyhock and these plants are still widely used in ethnomedicine practices around the world (Cowan, 1999).

However, the use of traditional medicine for the treatment of diseases has its own advantages and disadvantages. Of the advantages, the major one is that it is a potential source of new drugs. Its major disadvantage is that most preparations expected to have medicinal value are not evaluated scientifically for their efficacy and safety. Besides, the portions of traditional medicines are not standardized nor dispensed to patients in scientific doses or strictly regulated quantities (Sofowora, 1982).

Moreover, integration of traditional medicine with modern medicine has been practiced in countries such as Egypt, Ghana, India, China Sudan and Serilanka, where traditional medicine assumes the major health care (WHO, 1978). It has been estimated that about eighty percent of the world population depend on the traditional medicine for their primary health care needs (Akerele, 1993). In developing countries, the use of medicinal plants does not only have impacts on diseases, but also helps to reduce imports of drugs, thus boosting economic self-reliance. Furthermore, local products tend to be more readily accepted than those obtained from abroad (Akerele, 1993).

The use of herbal/traditional medicine is also popular among African population. For example, eighty percent of the population of Zimbawe depend on traditional medicine, since the modern drugs are beyond their reach. In Ethiopia too; similar proportions of population depend on the use of traditional medicine (Mintesnot Ashebir and Mogessie Ashenafi, 1999). Furthermore, Ethiopia is one of the six countries of the world where about 60% of plants with healing potential are said to be indigenous (Kaba, 1996).

However, only few studies have been made scientifically conducted regarding indigenous medicine (Kaba, 1996).

Due to fast spreading drug and multidrug resistant strains of bacteria in general and *S. pneumoniae* and *S. pyogenes* in particular, the need for new drugs with less toxic effects is a highly demanding public interest of these days. Therefore, intensive research activities are required to find out alternative solutions against drug resistant bacteria of such types. This needs evaluation of crude extracts and active constituents of traditionally used medicinal plants particularly against *S. pneumoniae* and *S. pyogenes*.

In most developing countries like Ethiopia, where the population live in rural and often inaccessible areas, very low health facilities are available, cost of drugs are unaffordable to most of the people and more than 80% of the population rely on traditional medicine, carrying out a drug research is highly demanding. It is therefore; absolutely rational to value medicinal plants for their efficacy and safety use, and integrate them gradually with modern health care system, particularly at primary health care level.

In Ethiopia, some researches on the evaluation of crude extracts of traditionally used medicinal plants have been on progressive trial. A few of the drug researches conducted were ‘’ Evaluation of the antibacterial activity of crude preparation of

Zingiber officinael (‘’ Zinjibl’’), *Echinops* spp. (‘’ Kebericho’’), *Coriandrum sativum* (‘’ Dimbilal’’), and *Cymbopogan citrates* (‘’tej sar’’) on some food born pathogens’’ (Mintesnot Ashebir and Mogessie Ashenafi, 1999), ‘’Investigation of the antibacterial properties of Garlic(*Allium sativum*) on pneumonia causing bacteria’’ (Dawit *et al.* 2002a), and’’ Antibacterial activities of *Plumbago zeylanica* L. roots on some Pneumonia causing pathogens’’ (Hirut et al. 2002). However, drug researches must be encouraged and more researches should be done to avert the threats of diseases-causing pathogens.

In view of these facts, we investigated two Ethiopian traditionally used medicinal plants for this drug research. The following explanation provides brief descriptions of the two plants used for our investigation.

2.4.2. Albizia gummifera (J.F. Grnel) C.A. sm (Fabaceae or (Leguminosae).

It is a deciduous tree preferring forest margins and open forest throughout mountainous region. It is also medium or large tree which occurs mainly in eastern tropical Africa and also in Cameroon, Nigeria, Madagascar and South Africa at altitudes ranging from 1000 to 2300

meters. In Ethiopia, this plant is distributed upland riverine forest, 1700- 2400 m in Gojam, Wollega, Illubabur and Kefa (Thulin, 1989). It grows poorly in higher altitudes colonizing woodlands. Means of propagation is directly sowing of seeds or sowing the pod a tree.

General use and land management: - the wood is medium or large tree fairly strong and does not wrap. It is also used for making beehives, water troughs and boats. Besides these; *A. gummifera* is a nitrogen fixing tree and can be used for soil stabilization. Its leaves form good mulch and are a good shade tree. It has also been reported that the plant is being used as a traditional medicine for Gonorrhoea, Rectal prolepses, Malaria, Skin infection (bacterial or fungal), Pneumonia, Eye disease, Amoebiasis, Syphilis and Wound infection in Ethiopia (Dawit Abebe and Ahadu Ayehu, 1993; Dawit *et al.*, 2003; Aberra *et al.,* In press).

2.4.3 ***Ferula communis* L. (Umbelliferae)**

It is annual or biennial herb up to 3 m high, basal and stem leaves with very conspicuous sheathing. Petals yellow with incurved apex. Propagation is sexual. Fruits vary, strongly dorsally compressed, broadly obviate to broadly elliptic. Upland grassland: 1400-3250 m. Distribution- S. Europe, west Asia North Africa, Sudan, Uganda Kenya and Tanzania. In Ethiopia the plant is distributed in Tigray, Gonder, Gojam, Welo, Shewa, Arsi, Sidamo,Bale and Harerge (Hedberg and Hedberg, 2003).

Furthermore, the plant has also been reported that it is used as a traditional medicine for Skin infection, Schistosomiasis, pneumonia and Wound infection in Ethiopia (Dawit Abebe & Ahadu Ayehu, 1993; Dawit *et al.,*2003;Aberra *et al.* In press)

2.4.4. **OBJECTIVES OF THE STUDY**

This study was thus attempted to evaluate the *in vitro* antibacterial activity of two traditionally used medicinal plants i.e. crude and fractionated extracts of *Albizia gummifera* (Ambabesa oromifa, Sesa- Amh) and *Ferula communis* (Doge- oromifa,, Dog- Amh..) against two major Gram positive bacterial pathogens of the upper respiratory tract infection of children namely *S. pneumoniae* and *S. pyogenes*.

The specific objectives of this study are:-

- To collect and screen clinical isolates of *S. pyogens* and *S.pneumoniae* from the paediatric patients.
- To evaluate MIC (Minimum inhibitory concentration) of crude and fractionated extracts against *S. pyogenes* and *S. pneumoniae*
- To pave the way for the application of the *in vitro* results at *in vivo* (animal model) levels.
- To give insight for researchers to carry out further work on various medicinal plants.
- To promote attention among the Policy makers so that traditional medicines will have their concerns to contribute to the health coverage of the country at Primary Health Care level.

3 MATERIALS AND MEHODS

3.1 Study design

The study was carried out at the Molecular bacteriology Laboratory of Ethiopian health and Nutritional Research Institute (EHNRI) in collaboration with Addis Ababa University, Biology Department. After preliminary assessment of the flow of patients in the selected health institutions, the Tikur Anbessa Specialized Hospital, the Teklehiamanot and the Kirkos health centres, where most of the paediatrics (children aged ≤ 14 years old) (WHO 1992) often get medical treatment were selected for this study. From the two later sites, study subjects were referred to Tikur Anbessa Hospital. In all health centres, assigned nurses were stationed to screen study subjects for the evaluation by Ear, Nose and throat Consultant Surgeon.

All patients who fulfil the clinical diagnosis of AOM (Acute Otitis Media) and acute tonsillitis (AT) when examined at the OPD were enrolled. For the study purpose AOM was defined as an inflammation of the middle ear associated with ear discharge in the past two weeks. AT was defined as a sore throat and disphagia fever in the past few days with inflammed pharynx, coated with exudates. Evaluation by the ENT surgeon was important for the proper assessment of the cases using the appropriate examination kit and collection of specimen from the tympanic membrane or middle ear and surface of the tonsils for the isolation and drug sensitivity characterization of *S. pyogenes* and *S. pneumoniae*

3.2 Questionnaire (data collection)

A preformed questionnaire was used to collect demographic data and physical features of each patient by asking the patients or caretakers. Information was gathered at the time of physical examination and specimen collection on age, sex, address, and

duration of illness, frequency of recurrence, symptoms and signs. It was also inquired whether these patients had been exposed to any traditional practice or use of local or herbal medicine

3.3 Study subjects (patients)

The study involved a total of 191 children who visited and or referred from the HCs to be seen at the paediatrics outpatient clinic of the Tikur Anbessa Specialized Hospital, over a period of 5 months (January –May, 2000). One hundred thirty one of the patients were cases of tonsillopharyngitis whereas the other sixty patients were those suffering from the AOM. All patients were recruited from among those who came to outpatient department (OPD) to get treatment for the specified diseases. Concerned authorities of the Black Lion Hospital, Paediatric and Health Department were officially informed about the study and ethical clearance was obtained from the Departmental Research and Publication Committee (DRPC) of the hospital. All patients of the study subjects were enrolled after getting informed consent from the parents or guardian. The specimens were collected by the otorhinolaryngologist after taking the medical history and physical examinations of the ENT organs.

3.4 Laboratory investigation

3.4.1 Test organisms and standard antibiotics

The organisms used for this study included clinical isolates screened from the out- patient samples collected at the specified Hospital, and the standard reference strain of *S.*

pneumoniae (ATCC= 49619) was obtained from the EHNRI laboratory to be used as control organism. But *S. pyogenes* ATCC 49615 was not available for this investigation.

Standard antibiotics (Tetracycline and Erythromycin) were obtained from Drug Administration and Control Authority, Drug Quality Control Laboratory (DACA) and were used as positive control for the plant extracts. We selected these reference antibiotics based the criteria that:-

1. Some of the species of the test organisms have been reported to be resistant to these antibiotics.

2. Besides, these drugs are relatively cheap and therefore commonly prescribed and available to the community who may easily tend to abuse them heralding the emergence of resistance.

3.4.2 Collection and Transport of specimens

Throat swabs were collected from the tonsillar surface using sterile disposable cotton swabs with wooden applicator sticks, while the middle ear discharges were collected using calcium alginate tips from the patients who were clinically confirmed by the signs and symptoms to have acute otitis media. Specimens were collected from the tympanic membrane or middle ear through the tympanic defect after the initial clearing of the ear canal with a sterile cotton swab. After collection, the swabs were immediately placed in Amies charcoal transport media (Difeco) and brought to the clinical bacteriology laboratory of EHNRI, where the laboratory investigations (Culturing, species identification and susceptibility test) were performed.

3.4.3 Laboratory Identification of Target Bacteria

The specimens collected were primarily inoculated on an appropriate media. i.e. Blood agar base (Oxoid), supplemented with defibrinated 5% sheep blood (local production of blood), was used to allow the growth of both β haemolytic group A *Streptococcus pyogenes* and α haemolytic *Streptococcus pneumoniae*. All plates were incubated at 37^{o}C for 24 hours, after which the typical colonies were isolated and identified. Identification was done, using their classical methods (colonial morphology, haemolysis, catalase test, disk inhibition) and serological test. For optimal results, plates for *S. pneumoniae* identification were incubated in a 5% CO_2 atmosphere using a candle jar (WHO, 2003).

3.4.3.1 Streptococcus *pnuemoniae*

A. Optochin sensitivity test

After 24 hours of incubation, pure colonies of those suspected for α haemolytic *S. pneumoniae* were picked from the blood agar plate using sterile loop and inoculated onto TSY (Trypton soya yeast) broth. After considerable turbidity, the suspects were streaked onto blood agar plate again using a sterile biological loop and an optochin disk of 6 mm Diameter or "p disk" (containing 5µg ethylhydrocupereine hydrochloride) was aseptically placed on the streaked plate surface of the inocula to differentiate between *S. pneumoniae* and viridans streptococci and each plate was incubated at 37 ^{0}C using candle jar for 24 hrs.

After 24 hours, optochin (P disk) reading and interpretation was done according to the recommendation of WHO (2003) as shown in Figure 2

Accordingly:-Alpha (α) haemolytic strains with a zone of growth inhibition by optochin greater or equal to 14 mm diameter were considered to be *S. pneumoniae* (growth inhibition $\geq$ 14 mm diameter).

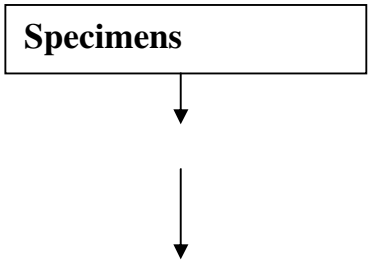
B Slidex Pneumo Kit (Latex Aggultination test)

Slidex pneumo kit (Latex Aggultination test) was also performed for the rapid identification of *S. pneumoniae* isolated from the culture. After 24 hrs incubation, at 37^0C all suspected colonies were tested using slidex pneumo kit. In brief, drops of normal saline were dispensed on 2 clean slides labelled as (slide 1 and 2) and a few of the suspected colonies were mixed with the drop to obtain a slightly opalescent suspension. On the suspension of slide 1 one drop of anti *streptococcus pneumoniae* reagent (R1) was poured while as that of slide 2 one drop of control reagents (R2) was poured and thoroughly mixed using a stirrer. Within 2 minutes, positive results for all isolates of *S. pneumoniae* were indicated by visible agglutination of the latex particle in the R1 reagent (Slide 1) indicating the presence of *S. pneumoniae antigen*

C. Catalse Test

After 24 hrs incubation at 37^0c, all suspected colonies were screened using a rapid slide technique to perform catalase test. This test was used to make further approval and to differentiate *S. pneumoniae* (catalase negative) from catalase positive bacteria such as staphylococci.

.Catalase test showed all the isolates of *S. pneumonias* were *negative* for the test.



Inoculate blood agar plates for 24-48h at 37°C

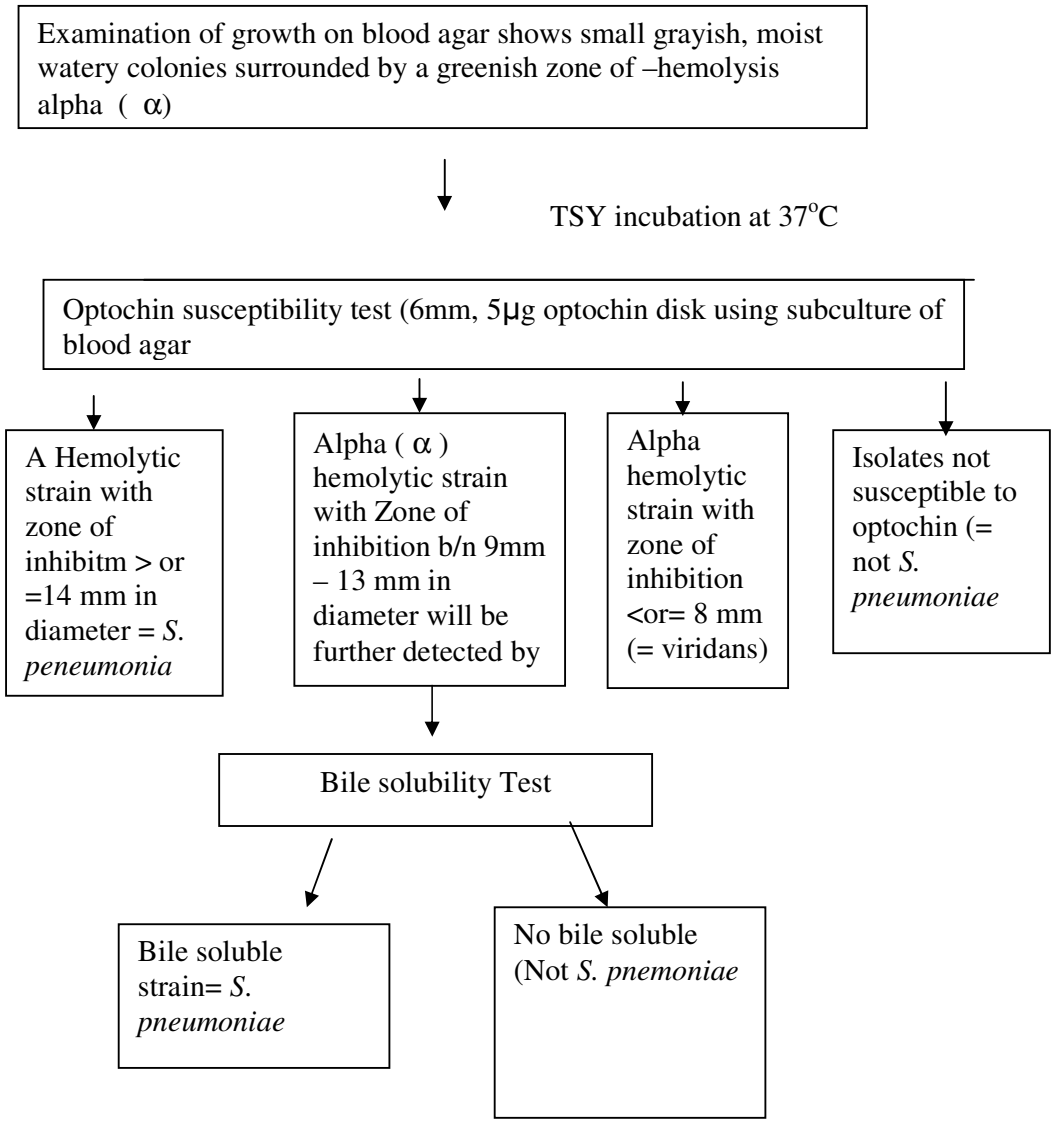


Fig 2 Flowchart for Laboratory identification of *Streptococcus Pneumoniae* for Ear discharges (Adapted from WHO, 2003)

3.4.3.2 *S. pyogenes*

A. Bacitracin Sensitivity test

After 24 hours of incubation, pure colonies of those suspected for *S. pyogenes* (Colonial morphology of pinpoint, with β haemolytic) were picked from the blood agar plates and inoculated on to TSY until, considerable turbidity was observed. Then the turbid suspension was streaked onto blood agar plate again using a sterile biological loop and aseptically a diameter of 6 mm bacitracin disk was placed on the streaked surface of the inocula and each agar plate was incubated at 37 ^{0}C for 24 hrs to screen *S. pyogenes*, since most strains were sensitive to bacitracin (Cheesbrough, 2000).

Bacitracin reading and interpretation was done according to Kilian (1998) shown on Fig 3, with some modification.

- Beta (β) haemolytic strains with the growth inhibition zone by bacitracin (0.04 or 0.05 IU) $\geq$ 11 mm in diameter around the disk were considered as *S. pyogenes*
- Clinical isolates not susceptible to bacitracin were considered not to be *S. pyogenes.*

B. Catalase Test

Furthermore, catalase test was done using a rapid slide techinique to differentiate beta (β) haemolytic *Streptococcus pyogenes* (negative for catalase test) from strains of beta (β) haemolytic *Staphylococcus aureus* (positive for catalase test). The test showed that all clinical isolates of *S. pyogenes* were negative for catalase test. Besides, summary of identificaion of target bacteria is shown in Table 1

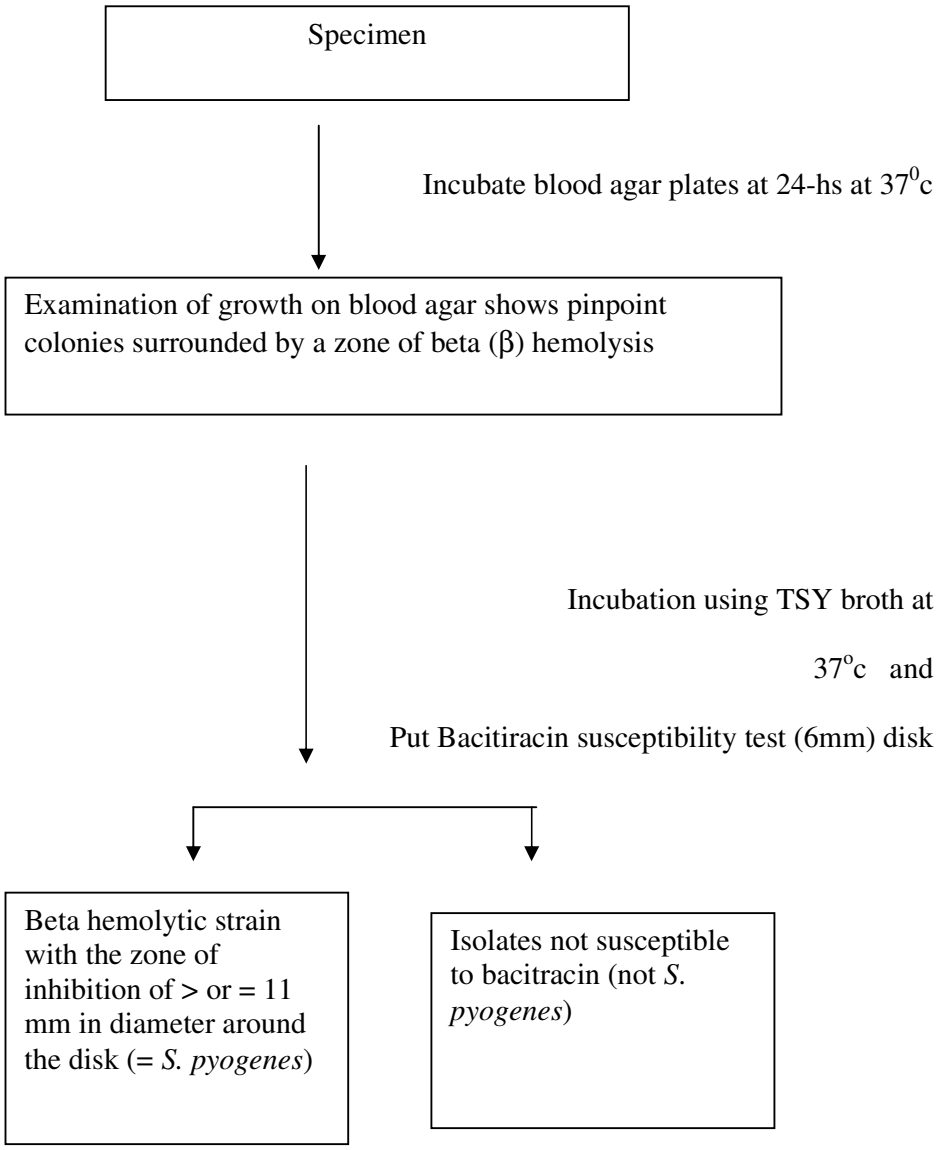


Fig 3. Flowchart for Laboratory identification of *S. pyogenes* for throat Swab

(Adapted from Kilian, 1998) with some modifications)

Table 1 Summary of identification of the target bacteria.

Site of Specimen collection	**Haemolysis**	**Optochin/ Bacitracin (Inhibition)**	**Slidex Pneumo Kit**	**Catalase test**	**Morphology**	**Idetified isolates**
Black Lion Hospital	**α**	**≥14mm**	**Visible agglutination**	**-ve**	**Dew drops (watery) and Ringed appearance .greyish colonies**	***S.pneumoniae***
Same	**β**	**≥11**		**-ve**	**.White to grey . Pinpoint**	***S.pyogenes***

3.4 Plant Materials

3.4.1 Collection and Identification

The plant parts of *Albizia gummifera* (seeds) and *Ferula communis(* root*)* :- used in this study were collected between February, 2000 and May 2002 from the Southern Region of the country approximately 540 km away from Addis Ababa around Bedelle and Bale regions. Identification of plants was done by a taxonomist in the Department of Drug Research of EHNRI (Ethiopian Health and Nutriton Research Institute) and voucher specimens were deposited in the Herbarium of EHNRI, Addis Ababa. The selection criteria of the targeted plants were based on the indigenous Knowledge (Ethno pharmacological screening) and preliminary study based on the effects of the crude 80% methanol extracts of these plants against reference strains of *S. pneumoniae* ATCC 49619 and *S. pyogenes* ATCC 49615 (Aberra *et al.*In press). . The plants collected and identified as *Albizia gummifera* and *Ferula communis* were selected for this study and hydroalcoholic(80% methanol) and their solvent fractionates were used against the clinical isolates identified as *S. pyogenes* and *S. pnenmonia* for their antimicrobial activities.

3.4.2 Plant extract preparation

A. Crude extract preparation

About 300 g powdered seed of *Albizia gummifera* and the root of *Ferula communis*

were dried at room temperature and extracted by maceration / percolation with 80% methanol and water respectively. The extracts were then filtered using Whatman No.1 paper and concentrated under reduced pressure in rotary evaporator to give 50g and 35g of the crude 80% methanol extract and 25 and 20g of the aqueous extracts following freeze drying, respectively to give crude extract. In this investigation we used 80% mthanol extract for antibacterial activity against clinical isolates and for further fractionation

B**.Solvent/Solvent partitioning (fractionation)**

Part of 80% methanol extracts was shaken up in about 200 ml water and partitioned with petroleum-ether (40-60^{0}c). The petroleum fraction was combined, evaporated and labelled as fraction 1 and the water suspension residue was then partitioned in chloroform to give chloroform fraction (labelled fraction 2). The aqueous layer was further partitioned with n-Butanol. The butanol layer was combined and evaporated to dryness (labelled fraction 3) and the aqueous residue that was left following portioning was filtered using coarse texture cloth and lyophilized to give a dried amorphous solid, which was labelled as fraction 4 (Figure 4).

During the preliminary study, petroleum- ether fraction(fraction 1), containing non polar components such as fats, sterols and some non polar compounds , and chloroform fractions fraction 2) containing intermediate polar compounds such as flavonoids, phenols, diterpenes, etc did not exhibit antibacterial activity so we excluded these fractions. Aqueous residue (fraction 4) contained very polar compounds (polar saponins and phenolic glycosides and terpenes), and n- Butanol fractions (fractions 3) contained polar compounds including terpenes, saponins, phenols, etc of which terpenes and saponins were our semi-purified test extracts of *Ferula communis* and *Albizia gummifera* respectively.

80% methanol crude extract of *Albizia gummifera* was also acidified using 2% citric acid and basified using 10% ammonia (PH-9).Then the fraction was extracted by dichloromethane to have aqueous residue and dichloromethane fraction. The later contained alkaloid fractions of *Albizia gummifera.* Identification test for each coded fractions (A2, A3…Tp1, TP2…) (Table 2) were done in the Department of Drug Research of EHNRI during the preliminary investigation of the extracts using the chemical tests and TLC (Aberra *et al.*, In press).

The fractions shown on the Table 2 were used for antimicrobial activities against targeted organisms to determine MIC (Minimum inhibitory concentration) by using agar dilution method

Table 2 Designation of test fractions of plant extracts

Fc-Tp.1	*Ferula communis* Terpene.1
Fc-Tp.2	*Ferula communis* Terpene2
Fc-Tp.3	*Ferula communis* Terpene3
Fc-Tp.4	*Ferula communis* Terpene.4
Ag-A.2	*Albizia gummifera* Alkaloid.2
Ag-A.3	*Albizia gummifera* Alkaloid 3
Ag-S.2	*Albizia gummifera* Saponin 2
Ag-S.3	*Albizia gummifera* Saponin3

Besides, for each species of plant extract and standard drugs, stock solutions were prepared using different solvents. That is, 0.1g of crude 80% methanol and fractionated plant extracts, 0.01g of tetracycline and 0.0005g of erythromycine were measured using analytical balance. Crude 80% methanol plant extracts were dissolved in 90% ethanol containing 10% tween 80 to prepare 5ml solutions while fractionated plant extracts and erythromycin were dissolved in 80% ethanol in different test tubes separately to have 5 ml solutions. But tetracycline was dissolved in distilled water to prepare 5ml stock solution. All of the stock solutions were stored in refrigerator at -20^0c prior to use.

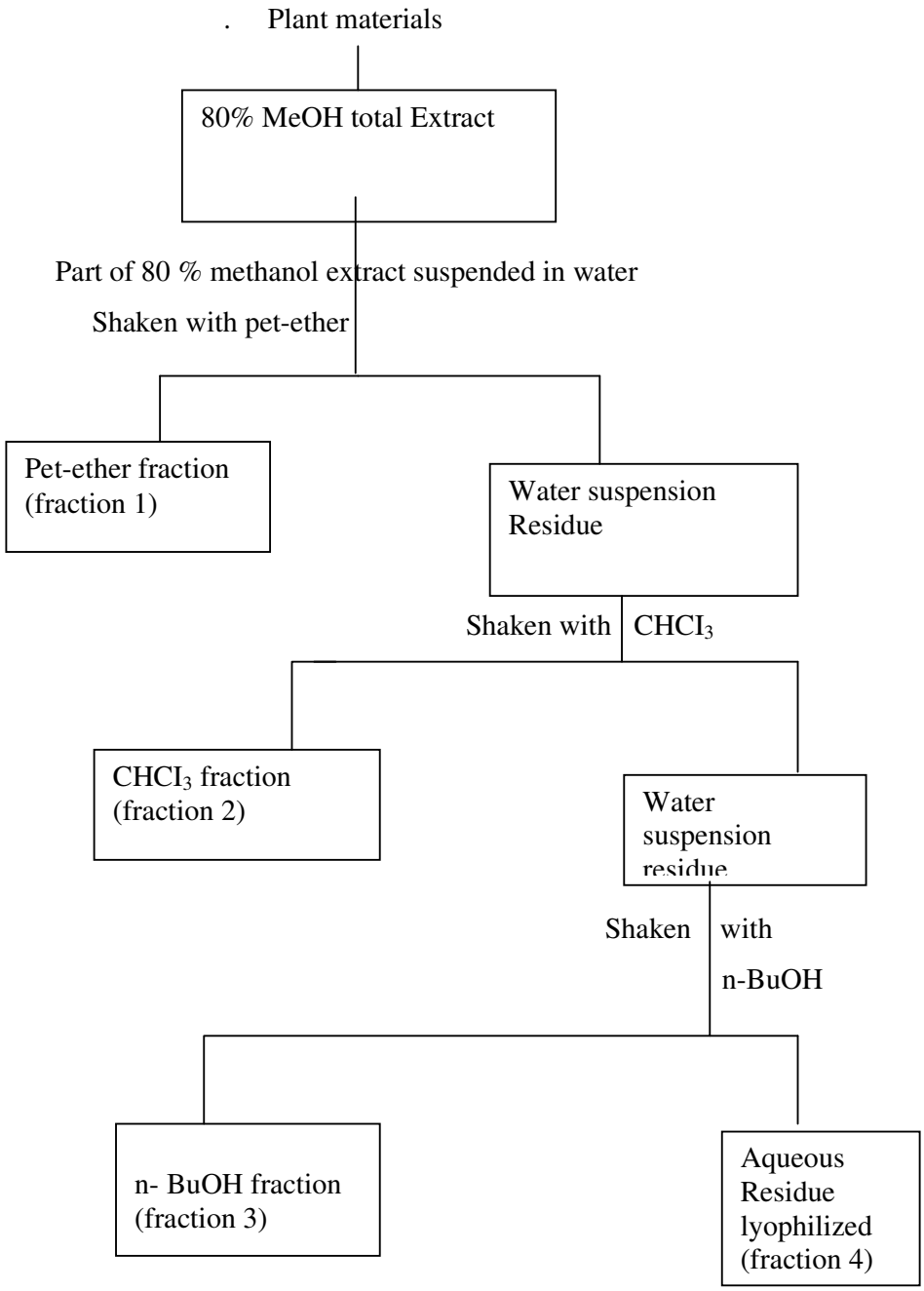


Fig 4: Flow chart for crude extract preparation and fractionation of plant extracts for *in-vitro* Antibacterial Testing with some modification (Adapted from Mahasneh and El-oqlah, 1999) .

3.5 Preparation of Inocula and culture Media for MIC of plant Extracts and standard antibiotics

Each medium was prepared using Muller Hinton agar (oxoid) enriched with 5% defibrinated sheep blood. In each medium, appropriate concentrations of plant extracts and standard drugs were included to determine minimum inhibitory concentrations (MIC). The concentrations that were included in each medium were 2000, 1000, 500, 250, and 125 µg/ml for plant extracts, 100, 50, 25, 12.5, 6.25 etc µg/ml for tetracycline and 5, 2.5, 1.25, 0.63 etc µg/ml for erythromycin. One medium was used only with Muller Hinton agar enriched with 5% sheep blood without the test compounds to provide the appropriate growth control. Moreover, one or more media without the test compounds but only solvent (s) were also used (negative control)

All clinical isolates and reference organisms (*S. pneumoniae*, ATCC 49619) were sub cultured on blood agar supplemented with 5% defibrinated sheep blood prior to inocula preparation. Pure cultures of the freshly grown organisms were prepared using the method described by Andrews (2001) (Table 3) to maintain appropriate growth conditions: Accordingly, the method of choice for fastidious organisms (S. *pyogenes and S. pneumoniae*) was, using direct colony suspension method. In brief, a 24 hrs freshly grown colonies were taken directly from the plate into 4.5 ml of Muller Hinton broth (BBL Microbiology system) and the suspension was made to match the density of 0.5 McFarland standards, by adding Muller Hinton broth to obtain approximately the organism's number in the range of 10^7 and 10^8 cfu/ml. Then the suspension of *S. pyogenes* were further diluted to 1:10 in Muller Hinton broth to give 10^7 cfu/ml which is used as starting inocula for the test.

Then the starting inocula were further standardized using the Steer's replicator i.e. When the inocula, were ready, using Steer's multipoint inoculator, suspension (an organism density of 10^4 cfu/spot) was dispensed onto the surface of the appropriate agar plate containing the plant extracts and reference antibiotics. (Andrews, 2001; Hsueh *et al.*, 2002; Santos *et al.,* 2002). Then the inocula were allowed to be absorbed into the agar before incubation. .After appropriate dispensation of each inoculum, and time for absorption, all the media were incubated at 37^0C for 18-20 hrs. in an aerobic environment but enriched in the candle Jar for *S. pneumoniae*. (Table 3)

Table 3: Showing Appropriate Media and incubation conditions for the clinical isolates and standard reference strain used for determination of MIC (Anderews, 2001)

Organisms	Media used	Incubation condition
1. clinical isolates of *S. pyogenes*	Muller Hinton Agar + 5% defibrinated sheep blood	37^0C for 18- 20h
2. Clinical isolates of *S. pneumoniae*	Muller Hinton Agar + 5% defibrinated sheep blood	37^0C in 4-6 % CO_2 in air for 18-20 h
3. *S. pneumoniae* (ATCC 49619)	Muller Hinton Agar + 5% defibrinated sheep blood	37^0C in 4-6 % CO_2 in air for 18-20 h

3.6 Determination of MIC (Minimum inhibitory concentration) of the plant extracts and standard antibiotics

MIC of the extracts against clinical isolates and test organisms (*S. pneumoniae* ATCC 49619) were determined using conventional serial dilution assay and agar dilution methods. Agar dilution was used to determine the MIC of the samples according to the recommendation of WHO (Rios *et al.,* 1988), hence the agar dilution method is applicable to both polar and none polar samples of extracts.

After 18-20 hrs incubation, the presence or absence of growth at each concentration of the extracts and standard antibiotics were examined by direct visual comparison of the test cultures with the control cultures and the concentration that completely inhibited the visible growth of bacteria was recorded as MIC. i.e. Minimum inhibitory concentration that completely inhibited visible growth of bacteria (Anderews, 2001; Santos *et al.* 2002). All the tests were carried out in triplicates and the modal MIC (Bosio *et al.* 2000) were determined. All the growth formation below the MIC or the growth of one or two colonies or film of growth was discarded or disregarded (Lambert and Pearson, 2000; Adnrews, 2001).

3.7 Data Analysis

The results were interpreted based on the triplicates done for each of the crude extracts and semi purified fraction of the traditionally used medicinal plants, and the modern antibiotics against the clinical isolates and reference strain (*S. pneumoniae* ATCC 49619). The data analysis were made taking the percentage of the modal growth inhibition value obtained for each of the twenty two clinical isolates of *S. pyogenes* and six clinical isolates of *S. pneumonia* and the reference strain.

4 RESULTS

4. 1 Result of clinical data

A total of 191 infants and children aged ≤ 14 years who were clinically symptomatic for acute otitis media (AOM) and acute pharyngitis (tonsillitis) were screened for the study. Among the patients, 31.4% (60 of 191) were symptomatic for Acute Otitis Media (AOM), having clinical symptoms of ear ache; ear discharge etc and 68.6%(131 of 191) were symptomatic for acute tonsillo pharyngitis, having the symptoms of tonsils, pharynx, mostly with exudates, high fever, swallowing problems etc

Age and sex distribution of the patients (subjects) is shown in Table 4. It was also reported that some of the patients used plant products prior to coming to the health centres. Species diagnosis using colonial morphology, Bacitracin, Optochin, and Catalase Tests and Serology Test (only for *S. pneumoniae*) showed that 12% (22 of 191) of the patients were found to be positive for *S. pyogenes* ((3.3 % (2 of 60) from the specimens of ear discharge and 15% (20 of 131) from the throat swab)). Among the 60 specimens collected from the clinically symptomatic patients of AOM, 10 %(6 of 60) of the sample were positive for the *S. pneumoniae.*

Besides, each clinical isolates from each patient was given isolate number in order to differentiate them and observe the effects of plant extracts and reference antibiotics. Accordingly, *S. pneumoniae* was given isolate number 1-5 and 26 (six Isolates) and *S Pyogenes* was given isolate number 6-25, 27 and 28 (twenty two isolates) thus, a total of twenty eight clinical isolates were included in this study and used to test the *in vitro* antibacterial activities of plant extracts.

Table 4 Age and sex distribution of study subjects, suspected for AOM and Acute pharyngotonsillitis

Age Range (years)	Male	Female	Total	%
≤2	29	18	47	24
3-5	28	25	53	28
6-8	19	29	48	25
9-11	11	19	30	16
12-14	5	8	13	7
Total	92	99	191	100

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4.2 Antibacterial Activities of plant extracts, Standard Antibiotics, and Determination of MIC (Minimum Inhibitory Concentration)

4.2.1 MIC for the Crude 80% methanol extracts of *Albizia. gummifera* and *Ferula . communis*

The crude 80% methanol extracts of the seeds of *Albizia gummifera* and roots of *Ferula communis* solubilized by 90% ethanol were tested against clinical isolates of *S pneumoniae* and *S. pyogenes*. Both crude methanol extracts of the plants showed antibacterial activities against all isolates, but extracts of *Albizia gummifera* showed more sensitivity to both clinical isolates as compared to the extracts of *Ferula communis*.The overall results of the *in vitro* susceptibility of the test organisms to the crude 80% methanol extracts of *Albizia. gummifera* and *Ferula communis* are shown in Appendices 2 and 3. The results recorded as MIC for the crude 80% methanol extracts were 500 µg/ml for *Albizia gummifera* and 2000µg/ml for *Ferula communis*, showing different sensitivity against clinical isolates. (Table 5).

Table 5 MIC for 80% crude methanol extracts against ***S. pneumoniae*** and ***S. pyogenes***

Clinical isolate	Crude 80% methanol extracts of	MIC (µg/ml)
S. pneumoniae	*Albizia gummifera*	≤500
	Ferula commins	≤2000
S. pyogenes	*Alizia gummifera*	≤500
	Ferula communis	≤2000

4.2.2 MIC for the Semi purified fractions of ***Ferula communis*** against ***S. pyogenes and S. pneumoniae***.

Simpurified fractions of *Ferula communis*, solubilized by 80% ethanol showed antibacterial activities against all clinical isolates, but, all fractions showed better susceptibility (Table 6) as compared to the crude 80% methanol extract of the same plant (Table 5)). However, aqueous solubilized fractions did not exhibit any antibacterial activity against all isolates. The detailed results are also presented in Appendices 4 and 5. As it was observed that the MIC value for the Semi purified fractions of *Ferula communis* against both isolates ranged from 500-1000 µg/ml and the results are shown in Table 6

Table 6. MIC for the Semi purified fraction of ***Ferula communis*** **against** ***S. pneumoniae*** **and** ***S .pyogenes***

Clinical isolates	Fractions of plants exctracts	MIC (µg/ml)
S. pyogenes	Fc.TP1	≤ 1000
	Fc. Tp2	≤500
	Fc. TP3	≤1000
S. pneumoniae	Fc. Tp1	≤1000
	Fc.Tp2	≤500
	Fc. Tp3	≤1000

4.2.3 MIC for the Semipurified fractions of *Albizia gummifera* against *S pyogenes* and *S. pneumoniae*

The test results of this study also showed that the Semipurified fractions of *Albizia gummifera,,* solubilized by 80% ethanol indicated antibacterial activities against all clinical isolates. But the results indicated that there is variation in susceptibility against clinical isolates (Table 7). However, aqueous solubilized fractions also did not show any antibacterial activity against all organisms. The detailed results are indicated in appendices 6 and 7. Further more, the MIC result for the semipurified fractions of *Albizia gummifera* against *S. pyogenes* and *S. pneumoniae* was also determined, and the values were ranged from 500 µg/ml 1000 µg/ml. (Table 7.)

Table 7 MIC for the Semipurified fractions of *Albizia gummifera against* clinical **isolates.**

Clinical isolates	Fractions of plan extract	MIC (µg/ml)
S. pyogenes	Ag,A2	≤1000
	Ag,A3	≤1000
	Ag,S2	≤500
S. pneumoniae	Ag,A2	≤1000
	Ag A3	≤1000
	Ag S2	≤1000

4.2.4 **MIC for the Semipurefied fraction of both plants against reference strain *(S. pneumoniae* ATCC 49619)**

The results of all simplified fractions of both plants also showed antibacterial activities against reference strain (*S. pneumoniae*. ATCC 49619). It had been observed that, the reference strain was relatively more sensitive than the clinical isolates (See appendix 8,and Table 8). MIC against reference strain was determined and the values were ranged from 500-1000 µg/ml (Table 8)

Table 8 MIC for the semipurified fractions of *Albizia. gummifera and Ferula communis against S. pneumoniae(ATCC 49619)*

Reference strain	Fraction of plant extracts	MIC (µg/ml)
S. pnuemoniae	Fc.Tp1	≤500
	Fc. Tp2	≤500
	Fc Tp3	≤500
	Ag.A2	≤1000
	Ag.A3	≤500
	Ag.s2	≤500

4.2. 5 **MIC for the reference antibiotics against clinical isolates and reference strain (*S. pneumoniae* ATCC 49619).**

Reference antibiotics (Tetracycline and erythromycin) used as a positive control showed antibacterial activity at a very low concentration against all test organisms including the reference strain. The detailed results are shown in appendices 9 and 10. It was also observed that the MIC value of this result was ranged from 0.04-12.5 µg/ml (Table 9)

Table 9. MIC for standard antibiotics against reference strain and all clinical isolates

Clinical isolates and *S. pneumoniae (ATCC 49619)*	Reference antibiotics	MIC (µg/ml)
S. pyogenes	Tetracycline	≤12.5
	Erythromycin	≤0.04
S. pneumoniae	Tetracycline	≤.39
	Erythromycin	≤0.04
S. pneumoniae (ATCC49619	Erythromycin	≤0.04
	Tetracycline	≤0.04

Moreover, all the MIC results of the crude 80% methanol extracts solubilized by 90% ethanol, and Semipurified fractions, solubilized by 80% ethanol and reference antibiotics against all isolates including the reference strain are summarized in table 10 and Figurs, 5-8

Table 10. **Summary of the MIC results.**

Plant and reference Antibiotics	Crude/ Semipurified fractions	Effective against	MIC (µ g/ml)
Albizia gummifera	Crude 80% methanol extract solubilized by 90% ethanol	*S. pyogenes*	≤500
		S. pneumoniae	≤500
Ferula. communis	Crude 80% methanol extract solubilized by 90% ethanol	*S. pyogenes*	≤2000
		S. pneumoniae	≤2000
*Albizia gummifera**	Semipurified 80% methanol extract, solubilized by 80% ethanol	*S. pyogens*	*≤500*
		S. pneumoniae	*≤1000*
		ATCC 49619	≤500
F. communis	Semipurified 80% methanol extract, solubilized by 80% ethanol	*S.pyogenes*	*≤500*
		S. pneumonia	*≤500*
		ATCC 49619	<500
Reference Antibiotics 1. Tetracycline	Solubilized by DH_2O	*S. pyogenes*	*≤12.5*
		S, pneumoniae	*≤.39*
		ATCC 49619	≤.39
2 erythromycin	Solubilized by 80% ethanol	*S. pyogenes*	*<0.04*
		S. pneumoniae	*≤0.04*
		ATCC 49619	*≤0.04*

* - *Semi purified* 80% methanol fraction of *A gummifera*, solubilized by 80% ethanol (Ag.S2) Indicated 41% MIC of 125 µg/ml (appendix 6) against *S. pyognes.*

*All fractions solubilized by DH_2O (Aqueous) did not show any antibacterial activities (Appendix 4-8) against all clinical isolates including reference strain

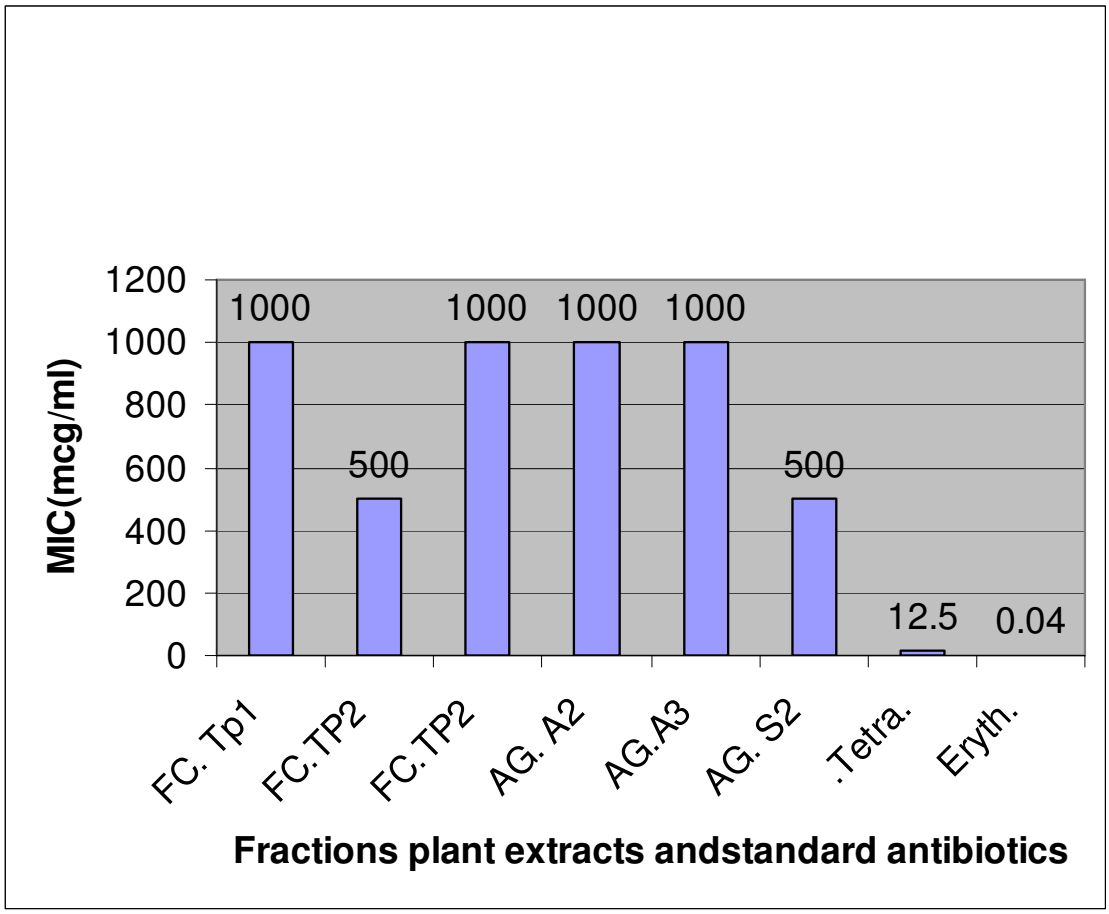

Fig.5. MIC of plant extracts and standard antibiotics against *S. pyogenes*

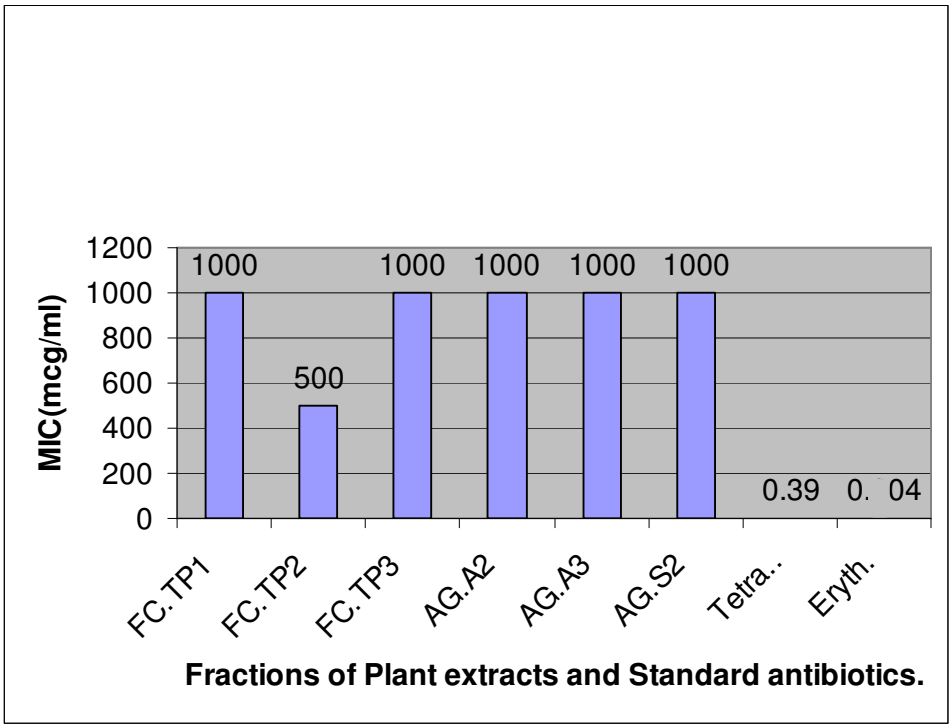

Fig. 6 MIC of plant extracts and standard antibiotics against *S. pneumoniae*

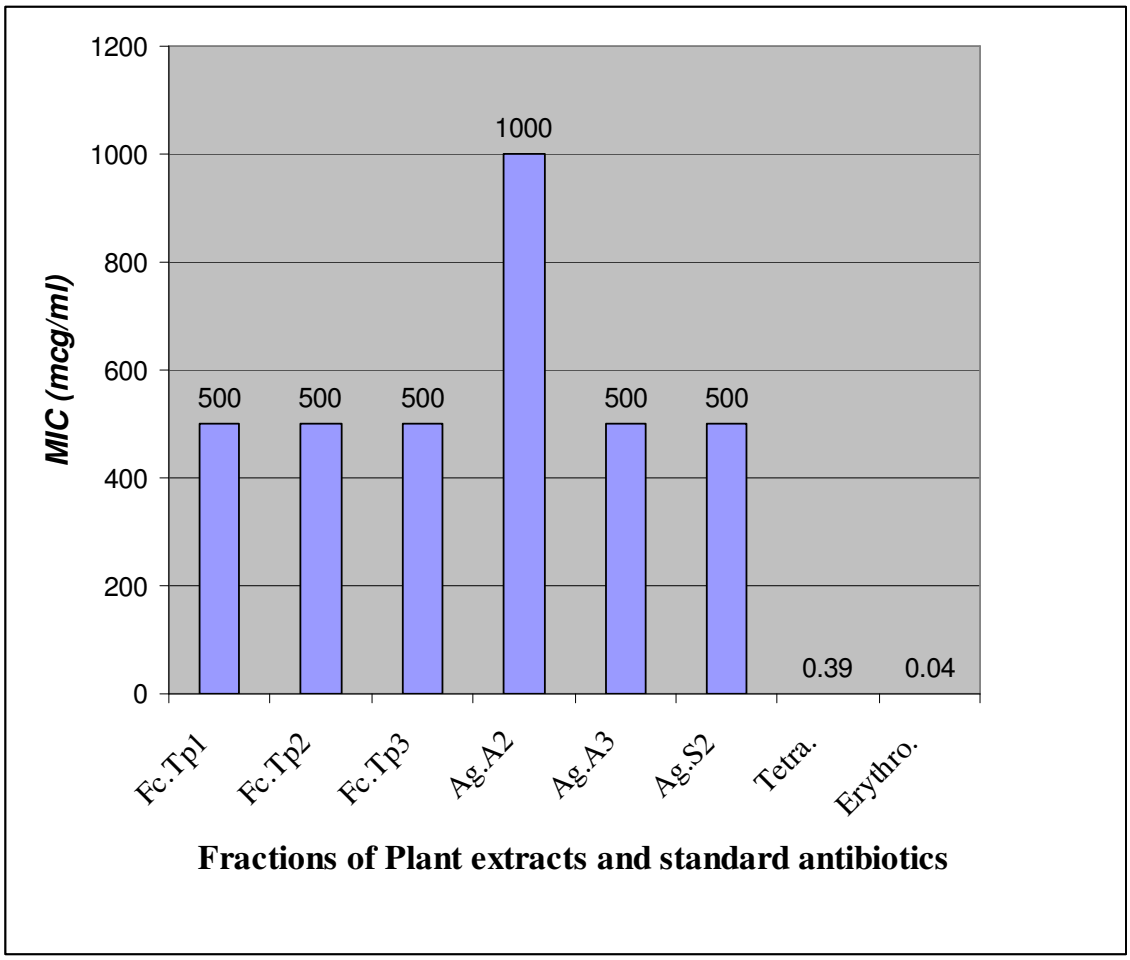

Fig.7 MIC of plat extracts and standard antibiotics against. Reference strain (*S. pneumoniae*, ATCC, 49619)

5. Discussion

When we analysed the data on the basis of age and sex, the result indicated that there were more males 99 (52%) than females 92 (48%) included in this study (Table 4). The higher prevalence of male children in our study may reflect the social attitude where by a male child may receive more attention and is preferentially taken to hospital for treatment. This finding is in agreement with similar other conclusion reported by Samir and his colleagues (1997). When the Age distribution of the patients were again analysed, 148 (77%) of the patients studied were $\leq$ 8 years of age showing these groups are more vulnerable to infection (WHO,1999) and 43(23%) of the patients were 9 and above years.

When the overall data was analysed, on the basis of infection interims of duration, acute infections were determined. We have also observed that patients used traditional medicine before coming to clinic or hospital mainly against tonsillitis / pharyngitis/. Among 131 patients 35 (27%) were found to be users of plant products such as garlic, jingible, feto, kebricho ,chewing leaf of banana, Kundo berbere, Damakese etc showing that there is a tendency for using traditional medicine.

In this study as shown in table 5 and Table 10, crude 80% methanol extracts of *Albizia gummifera,* solubilized by 90% ethanol showed higher antibacterial activity, (MIC 500 µg/ml on both isolates than 80% methanol crude extracts of *Ferula communis* (MIC 2000 µg/ml) solubilized by 90% ethanol. Furthermore in the previous studies (Abera *et al*. In press) on the crude 80% methanol extracts of the seeds of *Albezia gummifera* and the roots of *Ferula*

communis, on *S. pneumoniae*. ATCC 49619 and *S. pyogenes* ATTC 49615 had also showed antibacterial activities against these strains.

However, crude extracts of *Ferula communis* of this study (Aberra et al. In press) also indicated lower antibacterial activities against both strains (1000 µg/ ml) as compared to the crude 80% methanol extracts of *Albizia gummifera* against *S. Pyogenes* (MIC 250 µg/ml) and *S. pneumoniae* (MIC 500µg/ml)

This susceptibility variation was also reflected in our study in that, crude 80% methanol extract of *Albizia gummifera* showed more antibactrial activity (MIC 500 µg/ml) against all clinical isolates whilst 80% crude methanol extract of *Frula commins* showed lower antibacterial activities (MIC 2000 µg/ml) (Table 5 and Table 10). We predict that variation in the antibacterial activities of the crude extracts may be due to the synergistic effects of the bioactive compounds as it was reported by other studies (Santos *et al*. 2002).

The study also showed that Semipurified fractions of *Ferula communis* (Fc.Tp2) solubilized by 80% ethanol showed antibacterial activity against both isolates (table 6, 10 and Figure 5 and 6) at much lower concentration (MIC 500 µg/ml) than the crude extracts of the same plant (MIC 2000 µg/ml). We also predict this susceptibility variation may indicate the simpurified combinations of bioactive compounds of the extract of *Ferula communis* have more antibacterial activity, and this needs further analysis.

As shown in table 7, 10 and Figure 5 and 6, 80% ethanol solubilized Semipurified fractions of *Albizia gummifera* (Ag.S2) also showed antibacterial activity against both isolates. However, less sensitivity (MIC 1000 µg/ml) to *S. pneumoniae* was observed compared to higher sensitivity against *S. pyogenes* (MIC 500 µg/ml). We speculate variations of this result may be due to selectivity of the bioactive compounds of the plant extracts against the clinical isolates. In other studies, this concept has been reported, that gram positive bacteria

were selectively inhibited by compounds from plants extracts and most antibiotics (Basile *et al.* 1999). The reason for this selectivity to either bioactive compounds of plant extracts or antibiotics was suggested probably due to species variation and differences in bioactive compounds in plant extracts and antibiotics (Martin *et al.* 2004).

Aqueous solubilized fractions of both plants did not show any antibacterial activities against all organisms (Appendices 4-8) and this has been observed earlier for other plant extracts (Hirut *et al.* 2002). Besides, this result is also supported by the study carried out by Rios *et al.* (1988), in that some water soluble compounds may have a high diffusion power and lower antibacterial activity showing that water soluble plant extracts may not necessarily contain bioactive compounds.

Absence of antibacterial activity in some crude extracts (preparation) might also be due to a number of factors such as time of collection of plants materials and climate which might in turn affect the amount of active constituents in the plant material (Mintesnot Ashebir and Mogessie Ashenafi, 1999).

80% ethanol solublized fractions of both plants showed lower MIC (500 µg/ml) against standard test organism than clinical isolates(Tables 8 and 10, Fig 7) because, we may speculate that clinical isolates are basically obtained from clinically symptomatic patients, and hence may have high chance of exposure to antibacterial agents that may bring change to the molecular and other factors. Therefore, they are expected to be less sensitive than as compared to standard reference organism with no chance of exposure to the above agents.
Thus in finding new bioactive products which are selectively active against problematic micro organisms, causing certain disease (e.g. resistant pathogen), it is clearly appropriate to

employ; plant extracts against the corresponding clinically isolated pathogenic micro organisms (Vanden Berghe and Vlietinck, 1991).This means that it is necessary to employ new drugs or plant extracts against Standard reference strains along with clinical isolates of the same species.

All MIC values of both fractions of 80% ethanol solublized extracts (500 µm/ml) are significantly at lower concentration (Table 10, Fig.5,6) compared to MIC value of garlic (*Alium sativum*) extracts (MIC 7.80 mg/ml) against clinical isolates of *S. pneumoniae* (Dawit *et al.* 2002a) and MIC value of seed extracts of papaya *(Carica papaya*) (MIC, 18.4mg/ml) against clinical isolates of *S. pneumoniae* (Dawit *et al*. 2000b). However, our results were found to be higher concentration than *Plumbago zeylanica* extracts against clinical isolates of *S. pneumoniae* (MIC 0.2 mg/ml), study carried out by Hirut *et al.* (2002). Moreover, these studies also showed that fractions of *Albizia gummifera* (AG .S-2) indicated 41% MIC at the concentration of 125µg/ml against *S. pyogenes* (Appendix 6) which shows a promise for further purification.

But this finding also showed that MIC of both plant fractions were found to be at higher concentration (500 µg/ml) compared to studies carried out by Bosio *et al.* (2000) *in vitro* activities of propolis against *S. pyogenes* (MIC≤ 234 µg/ml) and this concentration of propolis was reportedly active against *S. pyogenes* and all strains were killed by 234 µg/ ml propolis. However, the fractionated hydroalcoholic extracts of *Albizia gummifera (*Ag-S-2*)* showed 41% MIC at the concentration of 125 µg/ml. against *S. pyogenes* indicating better susceptibility.

Besides this, much lower concentration of plant extracts (MIC 0.0003 µg/ml to 0.7 µg/ ml for *Zingiber officinale* (ginger) and 0.00008 to 1.8 µg/ ml for *Garccinia kola (* bitter kola*)* against *S. aureus, S. pyogenes, S. pneumoniae* and *H. influenzae* was reported by Akoacherf *et al.* (2002) and these MICs are much lower than our findings. See summary of comparative studies in Table 11.

Tetracycline and Erythromycine were used (Table9, 10 and Fig 5-7,) as positive controls showed MIC values ranging from 12.5 to 0.04 µg/ml). In comparison to these values, the minimum inhibitory concentration of ethanol solublized extracts of *Albizia gummifera* and *Ferula communis* appear to be not significant. But studies have shown that it is useful to carry out susceptibility test on both standard (reference) and clinical isolates to establish sensitivity of the test organisms (Venden Verghe and Vlietinck, 1991). According to Venden Verghe and Vlietinck,(1991) , a comparison of the antimicrobial potency of the plant extract and antibiotics cannot be drown from this result because a higher sensitivity may be caused by a highly active compounds present in a quite small amounts or by a substance of comparatively low activity but present in a high concentration of plant extract. However it has to be stressed that semi purified crude fractions of these extracts need, further analysis of bioactive compounds.

Moreover, the over all results of this study indicated that all extracts showed antibacterial activities against clinical isolates of *S. pyogenes and* S. *pneumoniae*. Thus the extracts may contain compounds with potential therapeutic activity and needs further purification and identification, so that it could be further explained clinically using animal models.

Table 11 Summary of comparative studies

Plant/ other	MIC (μg/ml)	Author
Albizia/Ferula	500	Abayneh *et al. 2005*
Alium sativum (garlic)	7800	Dawit *et a*l 2002a
Carica papaya	18400	Dawit *et al* 2002b
Plumbago zeylanica	200	Hirut *et al* 2002
Propolis	234	Bosio *et al* 2000
Garcinia kola(bitter kola)	$8x10^{-5}$- 1.8	Akoacherf *et a*l 2002
Zingiber officinale (ginger)	$3 x10^{-4}$ —0.7	

6. Conclusions and Recommendations

Researches have been done but there is no antimicrobial compound from a higher plant that has yet come into significant clinical use (Rios *et al.* 1988; Cowan, 1999) so, further investigation and intense research should be continued in the hope of finding plant antimicrobials that are effective for the treatment of humans and agricultural infections. Moreover, Ethiopia is rich in medicinal plants and various plants have been used traditionally for the treatment of diseases including respiratory infections. However, the medicinal value of these plants has not yet scientifically proved. Thus, the results of this study.

- Indicate that hydro alcoholic extracts of *Albizia gummifera* and *Ferula communis* may contain antibacterial agents and need further purification.
- Are good indicators that Ethiopian medicinal plants may possess compounds with antimicrobial effects.
- Proves the traditional wisdom of Ethiopian traditional medical practitioners.
- Are good indications that intense screening efforts are required in a number of plants may lead to the identification of highly effective drugs with desirable actions, which would lead to integration of traditional health care with modern health care systems

Our study was a preliminary step in screening Ethiopian medicinal plants for their antibacterial activity against clinical isolates of major respiratory pathogens. Based on our findings we would like to consider the following recommendations.

1. Standard / Reference micro organisms are preferably used as test bacteria to find out new antimicrobially active plant extracts. However, any positive results of screening could indicate that the plant extract may contain active compound(s) against standard test organisms but, this result is not a conclusive proof that active compounds of plant extracts are equally effective against all clinical isolates of the same species existing in the community because clinical isolates may have developed resistance or may not be resistant to antimicrobial agents of that particular types. Therefore, to judge the effectiveness of active compounds in the plant extracts, it is necessary to carry out further, screening tests of plant extracts against both clinical isolates and standard reference strains.

2. It would be advantageous to standardize methods of extraction and *in vitro* testing so that the search could be more systematic and interpretation of the results would be facilitated (Cowan, 1999).
3. Clinical isolates of *S. pyogenes* and *S. pneumoniae* used in this study may contain strains of different susceptibility to conventional drugs and active compounds of plant extracts. Thus, it is essential to confirm the effectiveness of the active compounds in extracts against known resistant strains of clinical isolates.
4. It would be also highly recommendable to carry out susceptibility testing of the drugs before treating the patients. It would also be necessary to perform wise management of bacterial infection.
5. Most traditional medical practitioners prescribe plant parts from two or more species of plants for the treatment of patients; with respiratory infections thus, further studies would be necessary to investigate the possible synergistic effects of the extracts.

6. It would be highly recommendable to evaluate the current status of antimicrobial resistance in clinical isolates of *Streptococcus pneumoniae* and *Streptococcus pyogenes* in nationwide as part of the surveillance study from multicenter antimicrobial Resistance programme in Ethiopia (SMARPE).

7. Since bacteria do not respect national borders, there should be a common susceptibility testing methods (Wise and phillips, 2000) and worldwide standardization of the methodologies for determining MIC and break point criterion used for interpretation

7 .References

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8. Appendices

Appendix 1. Questionnaire

The objective of this questionnaire is only to assess. Clinical background of the children to study bacterial isolates. Only mothers or parents who are willing (Volunteers) will be questioned and I offer great thanks for your cooperation.

Date _____

Card No_____

Name of Hospital_____

1. Child's

   1.1 Name _____

   1.2 Age_____ years

   1.3 Sex _-_____Male_____ Female_____

   1.4 Kebele_____

   1.5 Weight _____kg

2. Family Educational background

2.1 Illiterate_____2.3 Read & write _____

2.2 Elementary_____ 2.4 College or above_____

3 Status of a patient

2.3 Inpatient_____

2.4 Out patient Yes____ No_____

2.5 Antibiotics taken for the last week or so if Yes, which antibiotics_____?

2.6 Longevity of sickness_____-

3. Does the child have recurrent attack of sore throat

Yes___ No _____ earache (ear discharge) yes____ No_____

4. Do you use traditional medicine before taken to health instruction

Yes____ No____

If yes, which one?_____

5. Do you use traditional practice?

Yes____ No ____ if yes, which one?_____

Appendix 2. **: In*vitro* Antibacterial Activities of Crude Extracts of *Albizia gummifera* and *Ferula communis* against Six clinical** Isolates of *S . Pneumoniae*

Crude Extracts	Solvents	Isolate Number	Concentration of serial dilution of Plant Extracts and percentage Growth Inhibition or percentage Growth															Controls
			$2x10^3$ µcg/ml				$10x10^2$ µg/ml				$5x10^2$ µg/ml				25x10 µg/ml				Media &Solvent
			G(+)	%	I(-)	%	G(+)	%	I(-)	%	G(+)	%	I (-)	%	G(+)	%	I (-)	%	
Albizia gummifera	*9% ethanol*	1-5,26			-	100%			-	100%			-	100%	1.,2,3,4 ,26	83%	5	17 %	Growth 100%
Ferula communis	>>	>>			-	100%	3	17%	1,2,4 ,5,26	83%	1,3,26	50%	2,4,5,	50%	1,2,3,4, 5,26	100%			Growth 100%

Key: G= percentage Growth. I. = percentage growth inhibition

* Good Growth = + * NO growth — * Isolate No of *S. pneumoniae*: 1-5, 26 Isolate .Isolate No of *S. pyogenes* : 6-25, 27,28

Appendix 3: ***In vitro*** **Antibacterial Activities of Crude Extracts of *Albizia gummifera* and *Ferula communis* against twenty two clinical Isolates.** of *S . pyogenes*

Crude Extracts	Solvents	Isolate Number	Concentration of serial dilution of Plant Extracts and percentage Growth Inhibition or percentage Growth															Controls
			$2x10^3$ µg/ml				$10x10^2$ µg/ml				$5x10^2$ µg/ml				25x10 µg/ml				Media &Solvent
			G(+)	%	I(-)	%	G(+)	%	I(-)	%	G(+)	%	I (-)	%	G(+)	%	I (-)	%	
Albizia gummifera	*9% ethanol*	6-25 27-28			-	100%			-	100%			-	100%	6 9 10 11-16 19,24 27	55%	7,8, 17,18 20,21 22,23 25,28	45%	Growth 100%
Ferula communis	>>	>>			-	100%	18 19 21	14%	6-17 20, 22 23 24 25 27 28	86%	9,18, 19,20, 22,28, 24	32%	6,7,8, 10,11, 12,13, 14,15, 16,17, 21,23, 27,25	68%	6,15, 9,12, 18, 19,20, 21,28, 22,16, 17,23, 25,27	68%	7,8, 10, 11, 13, 14, 24	32%	Growth 100%

Appendix 4: ***In vitro*** **Antibacterial Activities of Fractionated Extracts of *Ferula communis* against twenty two clinical Isolates of *S. Pyogenes***

Extracts	Solvents	Isolate Number	Concentration of serial dilution of Plant Extracts and percentage Growth Inhibition or percentage Growth															Controls
			$2x10^3$ µg/ml				$10x10^2$ µg/ml				$5x10^2$ µg/ml				25x10 µg/ml				Media &Solvent
			G(+)	%	I(-)	%	G(+)	%	I(-)	%	G(+)	%	I (-)	%	G(+)	%	I (-)	%	
FC, TP.1	*8%% ethanol*	6-25 27-28			-	100%			-	100%	24,27 ,6,11, 12,13, 14,16, 18,20, 21,17, 22	59%	7-10 15,19 ,23, 25,28	41%	ALL	100%			Growth 100%
FC,TP.2	*8% ethanol*	.>>			-	100%			-	100%	-		-	100%	,9,6-18, 20-22 24,25,27	86%	19, 23, 28	14 %	Growth 100%
FC,TP.3	*8% ethanol*	.>>			-	100%			-	100%	6, 11-13, 15-18 24	41 %	7,8,9, 10,14, 19,20, 21,22, 23,25, 27,28	59 %	ALL	100%			Growth 100%
FC,TP 4	DH_{20}	.>>	ALL	100 %			ALL	100%			ALL	100 %			ALL	100%			Growth 100%

Appendix 5**: *In vitro* Antibacterial Activities of Fractionated Extracts of *Ferula communis* against six clinical Isolates of *S. Pneumoniae***

Crude Extracts	Solvents	Isolate Number	Concentration of serial dilution of Plant Extracts and percentage Growth Inhibition or percentage Growth																Controls
			$2x10^3$ µg/ml				$10x10^2$ µg/ml				$5x10^2$ µg/ml				25x10 µg/ml				Media &Solvent
			G(+)	%	I(-)	%	G(+)	%	I(-)	%	G(+)	%	I (-)	%	G(+)	%	I (-)	%	
FC, TP.1	*8% ethanol 2% tween80*	1-5 26			-	100%			-	100%	26	17%	1,2,3,4, 5	83%	ALL	100%			Growth 100%
FC,TP. 2	8% ethanol	.>>			-	100%			-	100%	-		-	100%	3,26	33%	1,2, 4,5	67%	Growth 100%
FC,TP. 3	*8% ethanol*	.>>			-	100%			-	100%	3	17%	1,2,4,5, 26	83%	ALL	100%			Growth 100%

FC,TP 4	DH_{2O}	.>>	ALL	100%			ALL	100%			ALL	100%			ALL	100%			Growth 100%

Appendix 6: ***In vitro*** **Antibacterial Activities of Fractionated Extracts of *Albizia* gummifera against twenty two clinical Isolates of *S. Pyogenes***

Crude Extracts	Solvents	Isolate Number	Concentration of serial dilution of Plant Extracts and percentage Growth Inhibition or percentage Growth																		Controls Media &Solvent
			$2x10^3$ µg/ml				$10x10^3$ Mcg/ml				$5x10^2$ µg/ml				25x10 µg/ml				125 µg/ml				
			G(+)	%	I(-)	%	G(+)	%	I(-)	%	G(+)	%	I (-)	%	G(+)	%	I (-)	%	G(+)	%	I (-)	%	
A.GA.2	*8% ethanol*	6-25 27-28			-	100%			-	100%	15,1621,2224,25	27%	6-14 17-20 23,27,28	73%	9,10, 11-22 ,24,25,27,28	82%	6,7,8, 23	18%					Growth 100%
A.GA.3	*8% ethanol*	.>>			-	100%			-	100%	17,18,24,25,	18%	6-16, 19-23 27,28	82%	11,12,13-17 19,20,24,27,28	55%	6-10, 18,21,22,23,25	45%					Growth 100%

A.G. **S.2**	*8% ethanol*	.>>			-	100%			-	100%			-	100%	6,15, 16,24,25,	23%	7-14 17-23 27,28	77%	6-10 13-15 16,20 24,23, 25	59%	11,12, 17-19 21,22,27,28	41%	Growth 100%
A.G S.3	DH_{20}	.>>	All	100 %			All	100 %			ALL	100%			All	100 %							Growth 100%

Appendix 7**: *In*** vitro Antibacterial Activities of Fractionated Extracts of Albizia gummifera against six clinical Isolates of S. pneumoine

Extracts	Solvents	Isolate Number	Concentration of serial dilution of Plant Extracts and percentage Growth Inhibition or percentage Growth																		Controls
			$2x10^3$ µg/ml				$1x10^3$ µg/ml				$5x10^2$ µg/ml				2.5x10 µg/ml				125 µg/ml				Media &Solvent
			G(+)	%	I(-)	%	G(+)	%	I(-)	%	G(+)	%	I (-)	%	G(+)	%	I (-)	%	G(+)	%	I (-)	%	
A.G A.2	*8% ethanol*	1-5,26			-	100%			-	100%	3,1, 26	50%	2,4,5	50%	1,2, 3,4, 5,26	100%							Growth 100%
A.G A.3	*8% ethanol*	.>>			-	100%			-	100%	3,26	33%	1,2,4,5,	67%	1,2, 3,4, 5,26	100%							Growth 100%
A.G. S.2	*8% ethanol*	.>>			-	100%			-	100%	3,26	33%	1,2,4,5	67 %	3,26	33%	1,2,4, 5,	67%					Growth 100%
A.G S.3	DH_2O	.>>	All	100 %			All	100 %			ALL	100%			All	100 %							Growth 100%

Appendix 8: ***In vitro*** **Antibacterial Activities of Fractionated Extracts of *Ferula communis* and *Albizia gummifera* against Standard test organism (*Streptococcus pneumoniea*, ATCC 49619)**

Crude Extracts	Solvents	Isolate Number	Concentration of serial dilution of Plant Extracts and percentage Growth Inhibition or percentage Growth																		Controls
			$2x10^3$ µg/ml				$1x10^3$ µg/ml				$5x10^2$ µg/ml				25x10 µg/ml				125 µg/ml				Media &Solvent
			G(+)	%	I(-)	%	G(+)	%	I(-)	%	G(+)	%	I (-)	%	G(+)	%	I (-)	%	G(+)	%	I (-)	%	
A.G A.2	*8% ethanol*	29			-	100%			-	100%	29	100%			29	100%							Growth 100%
A.G A3	*8% ethanol*	29			-	100%			-	100%			-	100%	29	100%							
A.G S.2	*8% ethanol*	29			-	100%			-	100%			-	100%	29	100%							Growth 100%
A.G S.3	DH_2O	29	29	100%			29	100%			29	100 %			19	100%							
F.C T.P.1	*8% ethanol*	29			-	100%			-	100%			-	100%	29	100%							
F.C T.P2	*8% ethanol*	29			-	100%			-	100%			-	100%	29	100%							
F.C T.P3	*8% ethanol*	29			-	100%			-	100%			-	100%	29	100%							
F.C T.P.4	DH_2O	29	29	100%			29	100%			29	100%			29	100%							Growth 100%

Appendix 9: *In vitro* **susceptibility of *S. pneumoniae s. pyogenes* and Reference strain (*S. pneumoniae* ATCC, 49619) to standard drug (Tetracycline)**

	Percentage of organisms inhibited by drugs (tetracycline) at a concentrations (µg/ml) of:-										
organism	100	50	25	12.5	6.25	3.125	1.56	.78	.39	.195	.098
S. pnumoniae (6 isolates)	100	100	100	100	100	100	100	100	100	17	-
S. pyogenes (22 isolates)	100	100	100	100	87	87	87	87	60	28	-
S. neumoniae (ATCC.49619)	100	100	100	100	100	100	100	100	100	-	-

Appendix10: *In vitro* Susceptibility of *S.pneumoniae*, *S. pyogenes* and Reference strain (*S. pneumoniae*,ATCC 49619) to standard drug (Erythromycin)

	Percentage of organisms inhibited by drugs(Erythromycin)at a concentration(µg/ml) of:-										
Organism	5	2.5	1.25	0.63	0.31	0.16	0.08	0.04	0.02	0.0098	0.004
a. *S. penumoniae* (6 isolates)	100	100	100	100	100	100	100	100	17	-	-
. *S. pyogenes* (22 isolates)	100	100	100	100	100	100	100	100	87	23	-
. .*pneumoniaen* (ATCC. 49619)	100	100	100	100	100	100	100	100	-	-	-