2. REVIEW OF LITERATURE

2.1. Streptococcus pneumoniae

*S. pneumoniae* are Gram-positive, encapsulated cocci, facultative anaerobic and α-hemolytic bacteria usually arranged in short chains. *S. pneumoniae* is a human pathogen which inhabits the upper respiratory tract of healthy individuals. Infections caused by *S. pneumoniae* can be divided into two categories. In invasive infections (e.g. meningitis, pneumonia, and bacteremia), pneumococcus can be isolated from blood or other normally sterile body fluids. In mucosal infections such as sinusitis, otitis media, and conjunctivitis, pneumococcus can be isolated from mucosal excretions only (Musher 1992, Feldman & Klugman 1997, Bogaert et al., 2004).

The spectrum of pneumococcal diseases differs in different age groups and different populations (Musher 1992, O’Brien & Santosham 2004, Bogaert et al., 2004, Hausdorff et al., 2005). Several risk factors for pneumococcal infection, such as age, race, immunodeficiency, other illness, socio-economic status, previous antibiotic therapy and day-care attendance have been reported (O’Brien & Santosham 2004). Pneumococcus is a leading pathogen, causing infections with high mortality and morbidity (Austrian 1977, Scott et al. 1996, Hausdorff et al. 2000a, Hausdorff et al. 2000b, O’Brien & Santosham 2004). At least one million children die annually from pneumococcal diseases, and most of them are young children in developing countries (WHO 1999, Williams et al., 2002). Invasive pneumococcal infections also occur in industrialized countries, especially among children and elderly people (Eskola et al. 1992, Scott et al. 1996, Sankilampi et al., 1997, Hausdorff et al., 2005).

2.1.1. Biology of Streptococcus pneumoniae

Over 130 years since the first discovery of pneumococcus, the traditional phenotypic definition of *S. pneumoniae* has not changed. In a Gram-stain, pneumococcus appears as an oval-shaped, gram-positive coccus, 1-2 μm in diameter, typically in pairs, sometimes singly or in short chains. The gram positive reaction of young cells may be lost when the culture is aged. Pneumococcus grows as α-hemolytic, centrally depressed colonies on blood agar, and generally upon primary isolation it is heavily encapsulated. Streptococci, and thus also pneumococcus, belong to the heterotrophic bacterial species that uses organic compounds as
a source of carbon, and their energy-yielding metabolism is fermentative, yielding low levels of lactic acid. Glucose and other carbohydrates are fermented. Production of the leucine amino peptidase (LAP) enzyme is a typical characteristic of all streptococci, whereas production of pyrrolidonylarylamidase (PYR) is rare among streptococci, occurring only in S. pyogenes isolates and in some pneumococcal isolates (Ruoff et al., 2003). Published genome analysis suggests that S. pneumoniae has pathways for catabolism of pentitols as well as for cellobiose, fructose, fructose, galactose, galactitol, glucose, glycerol, lactose, mannitol, mannose, raffinose, sucrose, trehalose, and maltose saccharides. In addition, ten amino acids and N-acetylglucosamine can be used as nitrogen and carbon sources (Tettelin et al., 2001).

2.1.2. Virulence factor

Pneumococcal extra cellular virulence factors can be divided into two groups: glycome based and proteome-based factors (Jedrzejas 2004). The first group uses sugars as building blocks and consists mainly of capsule polysaccharides and teichoic and lipoteichoic acids. The second group includes numerous surface-attached proteins and enzymes. This group can be divided further into three categories: peptidoglycan-bound proteins (e.g., hyaluronate lyase and neuraminidase), choline-binding proteins (pneumococcal surface protein A, choline-binding protein A, autolysin) and cytoplasmic lipid bilayer attached macromolecules (pneumococcal surface antigen A, formerly called pneumococcal surface adhesion A) (Paton et al., 1993, Jedrzejas 2004). The pathogenicity of S. pneumoniae has been attributed to various structures, most of which are situated on its surface. One group of factors, such as the capsule and a recently identified protein, provides resistance to phagocytosis and thus promotes the escape of S. pneumoniae from the host immune defense. Other factors, including cell wall components and the intracellular toxin pneumolysin, are involved mainly in the inflammation caused by infection.

2.1.2.1 Capsule

The capsule has long been recognized as the major virulence factor of S. pneumoniae. Experimental proof for this was provided by the difference in 50% lethal dose between encapsulated and unencapsulated strains. Encapsulated strains were found to be at least 105 times more virulent than strains lacking the capsule (Watson, D. A., et al., 1990).
2.1.2.2. Cell Wall and Cell Wall Polysaccharide

In contrast to the capsular polysaccharide, purified peptidoglycan and especially cell wall polysaccharide have been found to induce inflammation similar to that seen after infection with whole *S. pneumoniae*. Typical pneumococcal diseases such as otitis media, meningitis, and pneumonia can be mimicked in animals that have received injection of purified cell wall or its degradation products (Carlsen, B. D. *et al.*, 1992).

2.1.2.3. Neuraminidase

Several virulence determinants have been identified in *S. pneumoniae* including the neuraminidase A (NanA), which can cleave terminal sialic acid residues that are α (2-3) and α (2-6) linked to galactose or α (2-6) linked to N-acetyl-galactosamine. The *S. pneumoniae* contains several loci with sequence similarity to neuraminidases; nanA and nanB are expressed and possess activity (Berry, A *et al.*, 1996; Camara, M *et al.*, 1994). A third putative neuraminidase, nanC, remains to be characterized (Shakhnovich, E *et al.*, 2002; Tettelin, H *et al.*, 2001).

The role of *S. pneumoniae* neuraminidase activity is not fully elucidated; however, evidence indicates that NanA is involved in colonization and virulence. One suggested contribution of NanA to colonization is to increase the availability of ganglioside receptors that are important for pneumococcal adherence (Tong, H *et al.*, 1999). A nanA mutant has significantly reduced ability to colonize tracheal epithelium (Tong, H *et al.*, 2002) and persist in a chinchilla model of nasopharyngeal colonization (Tong, H *et al.*, 2000). Furthermore, this mutant was cleared more efficiently in a chinchilla middle ear infection model (Tong, H *et al.*, 2000). In contrast, little difference was identified in an intraperitoneal infection model.

Evidence suggests that NanA may contribute to long-term colonization by modifying other nasopharyngeal organisms and host proteins. NanA has been demonstrated to desialylate the cell surfaces of *Neisseria meningitidis* and *Haemophilus influenzae* (Shakhnovich, E *et al.*, 2002). Both these species reside in and possibly compete for the same host niche as pneumococci, and desialylation of lipopolysaccharides of competitors may well provide an advantage. In addition, NanA may contribute to a protease independent mechanism to modify the function of host glycoproteins that bind to the pneumococcus and protect the airway (King, S. J *et al.*, 2004). In patients with pneumococcal infection, a direct correlation exists between levels of N-acetyl neuraminic acid in cerebrospinal fluid and
development of coma and adverse outcome (O'Toole, R et al., 1971). Recently, immunization with NanA has also been demonstrated to afford protection against nasopharyngeal colonization and experimental otitis media (Long, J et al., 2004).

2.1.2.4. Pneumolysin:

Pneumolysin (Ply) is an efficient intracellular toxin that can lyse any eukaryotic cell which has cholesterol in its membrane. Ply is a significant factor in pneumococcal virulence. Ply consists of a single 53 kDa polypeptide chain and is a pore-forming toxin, expressed during the late log phase of growth and produced by virtually all clinical isolates. The toxin has several functions, particularly in the early phase of pneumococcal infection (Paton et al. 1993, Jedrzejas 2001). Its presence was earlier considered to be characteristic only to S. pneumoniae, but later on it has been shown that S. mitis and other α-hemolytic streptococci can also harbor the ply gene (Whatmore et al. 2000).

2.1.2.5. Pneumococcal surface protein A (PspA):

Pneumococcal surface protein A (PspA) is the foremost studied surface protein among the numerous choline-binding proteins of S. pneumoniae (Novak & Tuomanen 1999). This highly variable protein (from 60 to 200 kDa) is produced by all pneumococci examined to date (Crain et al. 1990). PspA has an important role in protecting pneumococcus from the host immune system. It has been shown to be significant in the pathogenesis of disease. Its ability to bind lactoferrin may help it in the colonization of host mucosae. Recently it has been found that human lactoferrin has similar receptor-like properties as PspA, but the significance of the finding is still under research (Jedrzejas 2004).

2.1.2.6. Autolysin:

Autolysins are a group of enzymes that degrade bacterial peptidoglycan. Their action leads to cell lyses. The major pneumococcal autolysin, S. pneumoniae N-acetylmuramoyl-L-alanine amidase, also known as LytA amidase, belongs to extra-cellular choline-binding proteins, and its size is 36 kDa. At the moment, the exact role of LytA in the virulence of pneumococci is unclear. Mutations of the lytA gene reduce virulence. Moreover, LytA induces protective antibodies in mice (Jedrzejas 2001). The lytA gene is the first bacterial autolytic gene that was cloned (Garcia et al. 1985). Obregon and coworkers (Obregon et al. 2002) presented that atypical pneumococcal isolates had mutations in the lyt
A gene, and the gene has also been found in closely related streptococci (Lull et al. 2006). Bile solubility, which tests the ability of deoxycholate to dissolve the cell wall of streptococci, depends on the presence of the autolysin gene lytA (Obregon et al. 2002).

2.1.2.7. Pneumococcal surface antigen A (PsaA):

Pneumococcal surface antigen A (PsaA) protein (37 kDa) is also an important virulence factor. This extra cellular, lipid-linked protein is part of a bacterial transport system and provides for the transport of Mn2+ and Zn2+ into the cell (Jedrzejas 2004). Morrison and Coworkers (Morrison et al. 2000) have detected by using a PCR that all 90 serotypes of S. pneumoniae contain the psaA gene. However, this gene has also been detected in other Streptococcal species such as S. mitis, S. oralis and S. anginosus. Pneumococcal proteins are under abundant research as new vaccine candidates (Jedrzejas 2001, Bogaert et al. 2004).

2.2. MULTIDRUG RESISTANCE

Frighteningly, the number of reported antibiotic resistant clinical isolates of this bacterium has increased markedly over the last 20 years, and multi-drug resistant strains have already emerged (Mera et al., 2005). The increasing global emergence and rapid spread of multidrug-resistant Streptococcus pneumoniae is a serious concern (Vilhelmsen S.E et al., 2000). Clonal dissemination of problematic pneumococcal strains has created clinically important treatment problems (Odenbalt I, et al., 2003; Bean DC et al., 2005). Pneumococcal resistance to antimicrobial drugs was first reported in the mid-1960s (Kislak J,W et al., 1965).

Before the antibiotic era, the population of pneumococci isolated from humans was mainly dominated by strains with capsular polysaccharides of serotypes 1, 2, and 3 (Griffith, 1928; Finland & Barnes, 1977). Within the last 25 years, escalating emergence of penicillin- and multidrug-resistant S. pneumoniae clones have been witnessed, a number of which have spread worldwide (McGee et al., 2001). In global surveys, serotypes 6, 9, 14, 19 and 23 account for over 80% of resistant S. pneumoniae out of 90 serotypes, and have also dominated the population of S. pneumoniae, and many of the worldwide clones have appeared with different capsular types (Pantosti et al., 2003).
Specifically related to respiratory tract infections, there is a rapid increase in resistant pathogens that cause both community and hospital acquired infections. In South Africa, resistance to the respiratory pathogen, *Streptococcus pneumoniae*, is recorded as having first emerged in 1978. Resistance patterns to the usual antimicrobial agents are very alarming. It is estimated that eighty percent of the worldwide antibiotic usage takes place in the community instead of in hospitals, and surprisingly enough, 80% of this usage is for respiratory tract infections and also recorded relatively high rates of antimicrobial resistance to antibiotics such as penicillins, macrolides, and cotrimoxazole for respiratory pathogens viz. *Streptococcus pneumoniae*, *Haemophilus influenza*, *Moraxella catarrhalis*, *Klebsiella pneumoniae* and *Streptococcus pyogenes*.

In global surveys, serotypes 6, 9, 14, 19 and 23 account for over 80% of resistant *S. pneumoniae* out of 90 serotypes, and have also dominated the population of pneumococci and many of the worldwide clones have appeared with different capsular types (Trzeciński et al., 2004). Such variants are thought to arise through natural transformation involving recombinational replacements, within and around the capsular biosynthesis (cps) locus (Coffey et al., 1998). Epidemic multidrug-resistant strains dominate clinical *S. pneumoniae* isolates in many regions of the world (Trzeciński et al., 2004) as there have been numerous reports globally regarding the failure of antibiotics to treat a range of pneumococcal infections due to drug resistance (Hart & Kariuki, 1998). Between 3-35% of pneumococcal illness is due to drug resistant *S. pneumoniae* (Centers for Disease Control, 1999).

Globally, the prevalence of antibiotic-resistant *S. pneumoniae* continues to increase (Jones et al., 2002). Though the level of penicillin resistance varies by country, it usually ranges between 5% and 50% (Jacobs, 2004). Some regions such as South Africa have reported resistance as high as 79% (Beckmann et al., 2005). In Uganda, the pneumococcal antibiotic resistance rates in isolates colonizing the nasopharynx of children were on the upper end of the global spectrum, with 83.5% showing resistance to penicillin (Joloba et al., 2001). Other countries with high prevalence rates of antibiotic-resistant pneumococci have identified pneumococcal clones that are responsible for the dispersion of resistance throughout the population locally and internationally (Blossom et al., 2006).
Antibiotic-resistant isolates, including isolates resistant to penicillin, usually belong to serogroups 6, 9, 14, 19, or 23 and to defined clonal groups. The strains which are responsible for most disease and drug-resistant S. pneumoniae are 6B, 14, 19 and 23F (Centers for Disease Control, 1999). The most successful clone in terms of geographical dispersion and prevalence is the multidrug-resistant serotype 23F pandemic clone (Spain23F-1) (Trzcinski et al., 2004), which originated in Spain, was also found in Mexico, South Africa, South Korea, Portugal, Croatia, France and the United States (Hart et al., 1998). Many penicillin resistant strains are also resistant to other drugs such as chloramphenicol, erythromycin, tetracycline and trimethoprim-sulfamethoxazole (Tomasz 1994). A crucial study done in 2007 in Kampala at Mulago hospital showed that all S.pneumoniae bacteremia isolates were resistant to cotrimoxazole (Namayanja, 2007).

Carol A et al., (1996) analyze five isolates of S.pneumoniae resistant to tetracycline but lacking tet (M) were studied. The tetracycline resistance gene, tet(O), was detected for the first time in the pneumococcus. The gene was amplified and sequenced and found to share 99% nucleotide sequence identity and 99, 99, and 98% deduced amino acid sequence identity with the tet(O) resistance genes of Streptococcus mutans, Campylobacter coli, and Campylobacter jejuni, respectively.

Stephen G Jenkins et al., (2008) states that Macrolide resistance among S. pneumoniae is mediated by two major mechanisms: methylation of ribosomal macrolide target sites, encoded by the erm(B) gene, and drug efflux, encoded by the mef(A) gene. While erm(B)-mediated resistance predominates across much of the world, the dominant genotype in the USA is mef(A). S. pneumoniae isolates with both erm(B) and mef(A) genes have also been documented in the USA and are typically multidrug resistant and clonal in nature. These findings have raised concerns over the continued clinical utility of antibacterial agents, such as the β-lactams and macrolides, for the empiric treatment of many community-acquired RTIs.

Resistance to erythromycin in pneumococci has been observed since 1967 and was first reported in Africa in 1978 in South African multiresistant pneumococcal strains (Widdowson & Klugman, 1998). The mechanisms that involve the formation of erythromycin drug resistance include; the alteration of the ribosomal target site, the production and utilization of active efflux mechanisms and the production of inactivating enzymes (Amsden, 1999). The first mechanism that results into resistance to erythromycin in
the pneumococcus include an alteration of the rRNA receptor by methylation, the N 6 -methylation of a specific adenine residue in 23S rRNA, which results in reduced affinity between the antibiotic and the ribosome (Widdowson & Klugman, 1998). This is achieved by the production of a ribosomal methylase which alters the ribosomal target site of the macrolides. This mechanism of resistance is coded for by the gene ermB, erythromycin ribosomal RNA methylation (also known as gene ermAM); this gene was first described in Streptococcus sanguis. The ermB gene confers co-resistance to most macrolides, lincosamides, and streptogramin B antibiotics (resulting in the so-called MLSB phenotype).

The second mechanism of erythromycin resistance in the pneumococcus is due to the production of a proton dependent efflux mechanism of the antibiotic from the cell, which is encoded by the gene mefE, (macrolide efflux). Unlike the ermB-induced ribosomal modification, this efflux mechanism is macrolide specific and does not affect the lincosamides or streptogramins. This mefE gene confers resistance only to the 14- and 15-membered macrolides, resulting in the M phenotype (Widdowson & Klugman, 1998; Amsden, 1999). Other, less commonly described mechanisms are mutations in the 23S rRNA gene and/or alterations in riboproteins L4 and L22 (Farrell et al., 2005).

In pneumococci and related streptococci, the frequent association of erythromycin and tetracycline resistance is related to transposons such as Tn1545, Tn3872, and Tn9002, resulting from the insertion of the ermB gene into the Tn916 family of conjugative transposons that harbor the tetM gene (Calatayud, et al., 2007). Tn916 is a well-known transposon that carries the tetM gene and has the int gene (integrate) and the exs gene (excisionase), which encode transposition functions. The efflux pump mechanism in pneumococci is codified by three subclasses of mefA genes, including mefE, mefA, and the recently described subclass mefI (Mingoia, et al., 2007). The mefE gene is the most frequently found and is carried by the 5.5-kb macrolide efflux genetic assembly (mga) element, an orphan and nonconjugative element containing five open reading frames (ORFs), of which mefE is the first, mefE gene is part of a genetic insertion element that lacks the genes necessary for transposition and it is inserted at various chromosomal sites, including the composite transposons (Gay and Steaphan et al., 2001). Since this genetic element has no enzymes that favor its transposition or conjugation, for it to move it has to be incorporated into a genomic sequence, an insertion sequence or into a composite transposon. Once it is incorporated, transformation is the favorable means of propagation (Zollezi et al., 2004). The mefA is carried by a defective transposon (Tn1207.1). (Calatayud, et al., 2007).
Recently, two new composite elements of the Tn916 family, containing the tetM gene plus mega (Tn2009) and the tetM and ermB genes plus mega (Tn2010), have been described.

Mohammad Kangar et al. (2012) described some penicillin resistant and macrolide resistant Streptococcus pneumoniae strains has carried out on 70 samples suspected to be S. pneumoniae isolated from patients who were admitted in Intensive Care Unit (ICU) detection of the resistance gene including  
\textit{erm(B)},  
\textit{mef(A)},  
\textit{php1a},  
\textit{php2b} and  
\textit{php2x} genes. The \textit{lytA} gene was detected in 50 samples. There was prevalence of resistant strains to erythromycin (56%), penicillin (40%), ampicillin (56%), cefotaxime (50%), tetracycline (10%), trimethoprim-sulfamethoxazole (48%), nalidixic acid (16%), clarithromycin (48%), azithromycin (44%) and levofoxacin (4%). All strains were susceptible to chloramphenicol, amikacin, streptomycin and gentamicin. Gene analysis showed that 29 strains (58%) had \textit{mef(A)} gene, and 24 strains (48%) had the \textit{erm(B)} gene. Out of all the penicillin resistance and intermediate strains, 6 (20%) and 1 (3.33%) strains harbor mutations in \textit{php1a} and \textit{php2x} genes, respectively, but \textit{php2b} was not identified in any sample. Resistance to penicillin, trimethoprim-sulfamethoxazole, clarithromycin and azithromycin in S. pneumoniae is a serious problem in this area and the local pattern of resistance/susceptibility must be considered for therapeutic regimens. The \textit{mef(A)} gene was a predominant mechanism of macrolide resistance in this area. With regards to low frequency of \textit{php} resistance genes, monitoring of other kinds of mechanisms is recommended.

2.3. Treatment

The first vaccination attempts were made among South African gold miners by using whole pneumococcal cells and later in young men by using capsular polysaccharides of types 1, 2, 5 and 7. In 1977, 14-valent and in 1983, 23-valent polysaccharide vaccines were introduced (Watson et al., 1993,WHO 1999, O'Brien & Santosham 2004). Unfortunately, polysaccharide vaccines were not immunogenic in young children, the elderly and immunocompromised patients, i.e. in people who are considered to be at a high risk for life threatening pneumococcal infection. Later, new second-generation vaccines, 7-, 9- and 11-valent polysaccharide protein conjugate vaccines, have been developed (Klein & Eskola 1999, O'Brien & Santosham 2004, Peltola et al., 2004). These conjugate vaccines seem to work best in infants and high-risk groups (Klein & Eskola 1999, Whitney et al.2003, O'Brien & Santosham 2004).
2.4. Medicinal plants

Man and plant relationships are strongly persisting. Since earliest days of existence, interaction with plants as shelter, source of nutrients and as medicinal agents was evident. Right from the beginning man has struggled for the alleviation of diseases and through the ages has accumulated substantial amount of knowledge of drugs derived from various plants. These efforts have led to the emergence of various disciplines of medicine (e.g. Allopathic, Homeopathic, Ayurvedic, and Chinese etc). One of the most challenging pursuits in the realm of pharmaceutical and medical sciences is the search for newer and more potent drugs with little toxic effects, self-administrable, less expensive and completely reversible. Much of these properties are observed in the drugs of plant origin. Medicinal plants, which form the backbone of traditional medicine, have in the last few decades been the subject of very intense pharmacological studies (Unny et al., 2003). This has been brought about by the acknowledgement of the value of medicinal plants as potential sources of new compounds of therapeutic value and as sources of leading compounds in drug developments (Mati and Staden, 2003).

In developing countries, it is estimated that about 80% of the population rely on traditional medicine for their primary healthcare (WHO, 1999). Medicinal plants might contain one or many different compounds having medicinal activities; their pure compounds could be used or mixed together to make very effective medicines (Steiner, 1986). Therefore, the need arises to screen medicinal plants for bioactive compounds as a basis for further phytochemical studies. Herbal remedies have been applied for treatment of many ailments since ancient times all over the world and about 25% of current drugs are derived from plants. Farnsworth et al., (1985)

2.5. Phytochemical compounds

With improved isolation and spectroscopic techniques, a growing number of active constituents are being isolated structurally characterized, and, in due course, many are synthesized in the laboratory. Some times, more active, better-tolerated drugs were produced either by chemical modifications (semi-synthesis), or by total synthesis of analogues of the active natural principles. In this study three medicinal plants were conducted for phytochemical investigation of natural products (secondary metabolites) which are: *Allium sativum, Allium cepa* and *Ficus bengalensis*. 

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At the present time, the genus *Allium* (Alliaceae) has over 500 members, each differing in taste, form and color, but close in biochemical, phytochemical and neutral actical content. L.D.Lawson *et al.* (1998) *Allium* were revered to contain numerous phenolic compounds which arouse great interests. R.S. Rivlin (2002) A. Bilyk *et al.*, (1984). The major part of pholinos is constituted by flavonoids. CrozierA *et al.*, (1997), Rhodes M.J, C. *et al.*, (1996) such as quercetin, isorhamnetin, kaempferol and their conjugate phenolic acids. HydroxyBenzoic acids, *Protocatechuic acid*, *Phloroglucinol acid*, *Pyrocatechol*. Flavonoids such as *Quercetin*, *Quercetin-glycosides*, *isorhamnetin glycosides*, *Kampferol glycosides*. Anthocyanins such as *Cyanidin glycosides*, *Peonidin glycosides*, *Pelargonidin glycosides*, Noreddine.

*Allium* species contain a wide variety of organosulfur compounds, particularly alk(en) yleysteine sulfoxides. Trauma to the plants, such as chewing, converts the organosulfur oxides to a complex mixture of sulfur-containing organic compounds. Many of these compounds or their metabolites are responsible for the odors, flavors, and pharmacologic effects of these plants. Many *Allium* species' organosulfur compounds appear to be readily absorbed through the gastrointestinal tract and are metabolized to highly reactive oxidants. Amagase H *et al.*, (2001)

*Allium* species, especially *Allium* vegetables, are characterized by their rich content of thiosulfimates and other organosulfur compounds, such as the well known lachrymatory factor. The thiosulfimates or alkane(ene) thial-S oxide are formed by the action of the enzyme allinase (E.C. 4.4.4,1,4) from their respective S-alk(en)yl cysteine sulfoxides which are the main responsible of onion flavor and produce the eye-irritating compounds that induce lachrmation. However, depending on the *Allium* species, and under differing conditions, thiosulfimates can decompose to form additional sulfur constituents, including diallyl, methyl allyl, and diethyl mono-, di-, tri-, tetra-, penta-, and hexasulfides, vinylthiins, and (E)- and (Z)-ajoene (Noreddine, B and Virginia, L. 2003)

A comprehensive analysis of the phytochemicals in *Allium porrum* contains carotenoids, chlorophyll, and saponins were identified by Fattorusso *et al.* (2000) and five kaempferol glycosides by Fattorusso *et al.* (2001). The USDA Flavonoid Database (2003) also lists kaempferol as the major *A. porrum* flavonoid, although additionally lists a very small amount of quercetin. Moderate levels of phenolics were measured by Turkmen *et al.*
(2005) Analysis of the phytochemicals in *Allium ascalonicum* saponins and high levels of quercetin, isorhamnetin and their glycosides in shallots were identified by Fattorusso et al. (2002)

A typical sensorial property of allium species is the strong aroma. Several aroma volatiles contribute to their flavor. In this study, a manual headspace solid phase micro extraction (HSSPME) coupled to gas chromatography-mass spectrometry (GC-MS) method was developed to study the aroma volatiles from four kinds of Allium species, namely chive, onion, scallion and shallot, with the comparative simultaneous distillation extraction. The GC-MS analysis identified 30 aroma volatiles. The chromatographic data were treated with the chemometric method of principal component analysis (PCA) in order to group different varieties of the aroma characteristics of Allium species Zhuo-Min Zhang et al., (2006).

Three saponins, named minutoside, minutoside, minutoside, and two known sapogenins, alliogenin and neoagigenin, were isolated from the bulbs of *Allium minutiflorum* Regel. Elucidation of their structure was carried out by comprehensive spectroscopic analyses, including 2D NMR spectroscopy and mass spectrometry. The structures of the new compounds were identified as (25R)-furost-2a,3b,6b,22a,26-pentaol 3-O-[b-D-xylopyranosyl-(1 4)-O-b-D-galactopyranosyl(1 4)-O-b-D-galactopyranoside (1), (25S)-spirostan-2a,3b,6b-triol 3-O-b-D-xylopyranosyl(1 3)-O-b-D-galactopyranosyl(1 4)-O-b-D-galactopyranoside (2), and (25R)-furost-2a,3b,5a,6b,22a,26-pentaol 3-O-[b-D-xylopyranosyl(1 3)-O-b-D-galactopyranosyl(1 4)-O-b-D-galactopyranosyl(1 4)-O-b-D-galactopyranoside (3), Elisa Barile et al., (2007).

Phytochemical investigation of *Allium leucanthum* Phytochemical investigation allowed the isolation of seven saponins from the flowers. Their structures were elucidated on the basis of NMR and HRESIMS spectrometry data. Identified a new compound, such as leucospirenone A (5), has been identified as (25R)-5a-spirostane-2a, 3b, 6b-triol, 3-O-β-glucopyranosyl(1 3)-β-glucopyranosyl(1 4)-β-glucopyranosyl(1 3)-β-glucopyranosyl(1 4)-β-galactopyranoside, Lasha Mskhiladze et al., (2008). The phytochemical screening of the methanolic extract of *Allium wallichii* Kunth revealed the presence of terpenoids, flavonoids and reducing sugars as main chemical groups. Kishor Acharya et al., (2011).
The phytochemicals in *Allium roseum* contains Sulphurous compounds such as Methional, 2,4-Dimethylthiophene, 2,4-Dimethylthiophene, Dimethyl trisulphide, 2-Propenyl methyl distilfide, 1-Propenyl methyl distilfide. The actives volatiles compound, or rosy garlic’s odor (flavor) detected in *A. roseum* also by gas chromatography mass spectrometry with static headspace sampling are essentially: DMDS (dimethyl disulfide), M2AIS2 (distilfide allyl dimethyl), M2S3 (trisulphide dimethyl), M2S2 (distilfide dimethyl), A1S2 (distilfide diallyl), M2AIS3 (trisulphide allyl dimethyl), MAIS4 (trisulphide allyl methyl), A1S3 P (trisulphide diallyl 2-propenyl) and MAIS4 (tetra sulfide allyle dimethyl), two γ-glutamyl peptides γ-L-glutamylS- (allyl)-L-cysteine (GluAISC), γ-L-glutamylS-(trans-1-propenyl)-L-cysteine (GluPeCS), their corresponding sulfoxide derivatives, (+)-S-(2-propenyl)-L-cysteine sulfoxide (allii), (+)-S-(trans-1-propenyl)-L-cysteine sulfoxide (iscallii)and (+)-S-methyl-L-cysteine sulfoxide (methiin) Najiaa Hanen et al., (2011)

To investigate the content of phenolic compounds (PhC) in flowers of *Allium schoenoprasum* (chive), *Tragopogon pratensis* (meadow salsify) and *Rumex acetosa* (common sorrel) and their effect on proliferation of HaCaT cells. Antiproliferative effects were evaluated in vitro using the following concentrations of phenolic compounds in cultivation medium: 100, 75, 50 and 25 μg/mL. Phenolic composition was also determined by HPLC. The results indicate that even low concentrations of these flowers’ phenolic compounds inhibited cell proliferation significantly and the possible use of the studied herb’s flowers as sources of active phenolic compounds for human nutrition. Zdenka Kucekova et al., (2011)

The qualitative phytochemical screening of the *Allium ursinum* revealed the presence of flavonoids, steroids, phenols, glycosides, lipids, and saponins with the absence of anthraquinones. In preliminary NMR analysis of BuE, there were characteristic signals of glycosirodoid nature of the mixture containing single methyls (0.8-1.1). Zolfaghari et al., (2012). *Allium ursinum* phytochemical compounds analysed through GCMS studies. The most abundant of the volatile compounds from *A. ursinum* were diallyl disulfide (19.96), diallyl trisulphide (38.74), 3-vinyl-(4H)-ditiin-1,2 (42.90) and 2-vinyl-(3H)-1,3-ditiin (57.87). Radu Vasile Bagiu et al., (2012)

Ficus species contain flavonoid glycosides, alkaloids, phenolic acids, steroids, saponins, coumarins, tannins, triterpinoidsoleanolic acid, rosolic acid, α-hydroxy urosolic
acid, protocatechuic acid, maslinic acid. The nonenzymatic constituents include phenolic compounds, flavonoids, vitamin C. The enzymatic constituents present are ascorbate oxidase, ascorbate peroxidase, catalase, peroxidase. The phenolic compounds present are gallic acid and ellagic acid. Furano coumarins that are reported are psoralen, bergapten Ram P et al 1979, ß-sitosterol and a new tetracyclic triterpene — glaumol acetate are reported from the leaves, bark and heartwood of F. palmata, Subramanian, P.M et al 1978.

Triterpinoid constituents rhoiptelenol, 3ß-hydroxyisohop-22(29)-en-24-oic acid were isolated from the methanolic extracts of fresh leaves and stems of Ficus thunbergii. This species also contains lupenyl acetate, ß-amyrry acetate, ß-amyrin acetate, lupeol, ß- amyrin, ß-amyrry, glutinol, ursolic acid, betulinic acid in its leaves and stems Juniichi Kitajima et al 1994. Besides the leaves, bark and fruits of F. benjamina contains cinnamic acid, lactose, naringenin, quercetin, caffeic acid, stigmasterol Hassan Abdalla Almatry et al 2002. Two new pentaecyclic triterpenes 8ß,26-cyclo-urs-21- en- 13ß,20ß-diol and 3ß-acetoxy-8,26-cyclo-urs-21-ene-20ß-ol and also 3ß-friedelinone, oleanolic acid, betulinic acid, lupeol acetate, ß and ß amyrine, 3ß,5,7,4ß-tetra hydroxyl flavones, 3ß,5,7,3ß,4ß-pentahydroxy flavonate are reported from the stem bark of Ficus cordata Herve M et al 2008.

4, 4, 24-trimethylcholest-8-en-3ß-ol, mixture of campesterol, stigmasterol and sitosterol, stigmasterol 3ß-B-glucoside and 4, 5, 7-trihydroxy flavan-3-ol. In addition to xanthoholoxin, ß-amyrry and ß-amyrry from n-hexane and ethyl acetate fractions of ethanol extract of Ficus carica (Thunb) leaves Hassan Abdalla Almahy and Almatry 2005. Phytochemicals are the chemicals produced by Ficus carica Linn. Literature survey indicated the presence of coumarins, flavonoids, sterols, triterpenoids, anthocyanins etc. in various parts of the plant. Dried seeds contain fixed oil containing the fatty acids viz oleic acid, linoleic acid, linolenic acid, palmitic acid, stearic acid, arachidic acid. Leaves contain bergapten, 4ß,5ß-dihydropсорalen, rutin, 24-methylencecolartanol umbelliferone, marmesin, stigmasterol, ß-sitosterol, ficusogenin, lupeol, psoralen ß-taraxasterol ester and tyrosine moisture, protein, fat, crude fiber, ash, N-free extract, pentosans, carotene on a dry weight basis 22, 23, 24, 25, 26, 27, 28. The latex contains 6-O-linoyle-ß-D-glucosyl-ß-sitosterol, 6-O-linoyle-ß-D-glucosyl-ß-sitosterol, 6-O-oleyl-ß-D-glucosyl-ß-sitosterol, 6-O-palmitoyl-ß-D-glucosyl-ß-sitosterol, caoutchouc, resin, albumin, cerin, sugar and malic acid, rennin, proteolytic enzymes, diastase, esterase, lipase, catalase, and peroxidase 29. Fruits contain cyanidin-3-O-glucoside, cyanidin-3-O-rhamnoglucoside, saturated fat, cholesterol,
sodium, insoluble sugars, protein, vitamin A, vitamin C, calcium, iron. Roots contain psoralen, bergapten22, 23, 26, 28. Fig. 3 shows the structures of phytochemical constituents present in Ficus carica Linn. Anshul Chawla et al., (2012)

The phytochemical analysis exhibited that F. lyrata latex extract contains alkaloids, flavonoids, coumarins, saponins, and terpenes. Some phenolic compounds, with reported pharmacological properties have already been isolated from fig leaves, namely furanocoumarins like psoralen and bergapten, flavonoids like rutin, quercetin, and luteolin, phenolic acids like ferric acid, and also phytosterols like taraxasterol Bidarigh S et al., (2011). The phytochemical analysis of Ficus sur J. Two pentacyclic triterpenoids of oleanane and ursene structures have been isolated from the latex of the F. sur. The compounds isolated from the latex are naturally acetylated in the 3-position and their structures have been elucidated on the basis of spectroscopic studies, Sisay Feleke and Abeba Brehan, (2005).

Phytochemical constituents of purified sample of Ficus platypylla gum was investigated using GC-MS technique. The results obtained indicated the presence of methoxy-phenyl-oxime (0.79 %), 1-methoxyethyl benzoate (4 %), palmitic acid (7.58 %), oelic acid (10.15 %), octadecanoic acid (6.2 %), 1-phenanthrene-carboxylic acid (podeca) (17.78 %), albiatic acid (19.28 %), (6,8,9-trimethyl-4-(1-phenylethyl)-3-oxabicyclo[3.3.1]non-6-en-1-yl)methanol(11.16%)andtetraicosamethyl-cyclo dodecasiloxane (31.07 %). Ficus platypylla gum extract has been found to be a good adsorption inhibitor for the corrosion of mild steel in solutions of HCl, Nnabuk O Eddy et al., (2012).

The Ficus microcarpa bark Twelve phenolic compounds were identified in BE fraction by GC-MS and HPLC analyses. The compounds areCatecho Coumaran p-Vinylguaiaicol Syringol 1-p-Propylphenol ,Vanillin,p-Propylguaiaicol ,Isovanilllic acid ,4-n-Propylresorcinol, Syringaldehyde, Protocatechutic acid, Oleanolic acid. The strong antioxidant and antibacterial activities of F. microcarpa bark extract may be attributed to its high level of phenolic compounds. Changwei A, et al., (2008)

The phytochemical compounds of Ficus racemosa Linn leaf contains sterols, triterpenoids (Lanosterol) and alkaloids, tannins and flavonoids. Stem-bark gives glucon acetate, β-sitosterol, leucocyanidin-3-O-β-D-glucopyranoside, leucopelargonidin-3-O-β-
D-glucoopyranoside, luteopelargonidin-3-O-α-L-rhamnopyranoside, lupeol, ceryl behenate, lupeol acetate and α-amyrin acetate. From trunk bark, lupeol, β-sitosterol and stigmastanol were isolated. Fruit contains glucomacetate, glucose, tiglic acid, esters of taraxasterol, lupeol acetate, friedelin (7), higher hydrocarbons (Hentriacontane) and other phytosterols. A new tetacyclic triterpene glucomacetate which is characterized as 13α, 14β, 17β-H, 20α-H-lanosta-8, 22-diene-3β-acetate and racemosic acid were isolated from the leaves. An unusual thermobable aspartic protease was isolated from latex of the plant. The stem bark and fruit showed presence of glucom Anita Rani shiksharhi and Stuti mittal (2011)

The essential oil from the root bark of Ficus exasperata cultivated in Nigeria were examined by GC and GC-MS. The essential oil was characterized by the presence of α-terpinol (33.7%), α-pinene (10.8%), sabinene (5.6%), β-patchoulene (4.7%), 1,8-cineole (3.1%), and α-thujopsene (2.1%) as the major compounds. The volatile oil was assessed against clinical strain of Candida albicans. The low minimum inhibition concentration (MIC) of this compound (1.1 μg/mL) is among the basis of this report. IA. Oladosu et al., 2009

Bark and leaves of Ficus mollis (Moraceae) are reported as folk medicinal plant parts. The samples of bark and leaves of F. mollis (vahl) are subjected to HPTLC analysis. Heavy metal contents such as mercury, lead, cadmium, arsenic and minerals like iron, copper, manganese, zinc, cobalt analysis using atomic absorption spectroscopy and pesticide residues by GC-MS were carried out. The study revealed that heavy metals are within the permissible limit. The data evolved in the present work will aid in identifying these drugs in dry form and in standardization of the drug. Sreenivasulu Munna et al., (2010).

GC-MS analysis was carried out to quantify the different compounds present in the root extract of Ficus religiosa. The results shows 25 compounds are present in the root extract of F. religiosa. Among the 25 compounds following compounds shows high peak value trans-o-Dithiane-4,5-diol, 1,3,4,5-Tetrahydroxy-Cyclohexane Carboxylic acid, Stimaet-5-en-3-ol, Lupe-20(29)-en-3yl acetate, Methyl Commate C, Acetic acid, 17-(1,5-dimethylhex-4-enyl)-4,4,8,14-pentamethyl-2,3,4,5,6,7,8,9, Eswaralakshmi R, and Arifa Khatoon (2012)
The oil composition of three Ficus species (Moraceae): *Ficus lutea* Vahl., *Ficus polita* Val., and *Ficus thonningii* Blume., were studied by GC and GC/MS. The main compounds in *F. lutea* were acorenone B (20.7%) and phytol (16.2%), with significant quantities of demethoxyageratechromene (6.0%), 6, 10, 14-trimethyl-2-pentadecanone (5.1%) and zingiberene (5.2%). However, *F. polita* had phytol (23.3%) and 6, 10, 14-trimethyl-2-pentadecanone (15.0%) in abundance, in addition to sizeable proportions of (E)-6, 10-dimethyl-5, 9-undecadien-2-one (7.3%) and drimenol (5.8%), while *F. thonningii* comprised 6, 10, 14-trimethyl-2-pentadecanone (18.8%) and phytol (14.7%). Acorenone B (7.6%) and β-gurjunene (6.3%) were also observed in higher amounts. Phytol and 6, 10, 14-trimethyl-2-pentadecanone seem to be the marker components of Nigerian grown Ficus species as it is evident in this report and previous studies, Ogunwande LA et al., (2008)

The methanol extract of stems of *Ficus tikoua* Bur., a new benzofuran glucoside, named 6-carboxyethyl-5-hydroxybenzofuran 5-O-β-D-glucopyranoside (1), together with one known benzofuran glucoside (2) were isolated. Their structures were elucidated by 1D and 2D (1H-1H COSY, HMQC, and HMBC) NMR spectroscopy and HRMS techniques. The antioxidant activities of the isolated compounds were assayed based on the scavenging activities of DPPH free radical. Compounds 1 and 2 exhibited moderate antioxidant activities, and the IC50 values were 242.8 μg·mL⁻¹ and 324.9 μg·mL⁻¹, respectively. Shao-Peng W, et al (2011)

2.6. Antibacterial activity

Almost in all countries of the world, the plants which are believed to be important for health are being used for centuries. According to the research of World Health Organization (WHO) based on the literature of herbs and codexes of 91 different countries, the total amount of herbs is nearly 20,000 (Mahindru ,1992). In different laboratories of most countries, the antimicrobial effects of herbs have been searched since 1926 (Vorderbank et al., 1949; Holopainen et al., 1988) Despite the improvement on medicine and technology at the present day, the widespread consumption of natural products and the economical crisis made herbs more effective and more purposive. Hence, the present systematic review focused on their active phytoconstituents of plants that have been found to possess antibacterial action against *Streptococcus pneumoniae* and their various strains
Mamidou Koné W., et al., (2007) studied antibacterial activities of some herbal plants used in Northern Côte d’Ivoire. The crude extracts were from *Erythrina senegalensis* (Fabaceae), *Piptostigma thomsonii* (Caesalpinioideae), *Waltheria indica* (Sterculiaceae), *Andira inermis* (Fabaceae), *Uapaca togoensis* (Euphorbiaceae), *Koctia hispida* (Rubiaceae) and *Combretum molle* (Combretaceae) showed a promising in vitro bactericidal activity against *Streptococcus pneumoniae*.

Doughari, J., et al., (2007) studied the Antibacterial activity of *Carica papaya L* against *Staphylococcus aureus*, *Streptococcus pyogenes*, *Streptococcus pneumoniae*, *Bacillus cereus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Salmonella typhi* and *Shigella flexneri*. The Antibacterial activities were found to be using the cup plate agar diffusion method.

Bagyalakshmi D, et al (2009) studied the antimicrobial activity of crude extracts of five medicinal plants *Glycyrriza glabra*, *Datura metali*, *Coccinia grandis*, *Sida spinosa* and *Lablab purpureus* used in traditional Indian medicine was tested against *Streptococcus pyogenes*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Streptococcus pneumoniae*. Among the three solvents used the most effective extract was found to be methanol extraction. The most effective antimicrobial plant was identified as *Glycyrriza glabra* followed by *Datura metali*, *Coccinia grandis*. Least activity was observed in *Sida spinosa* and *Lablab purpureus*.

Ahmed, T., et al., (2009) tested the chemical composition and antimicrobial activity of *Sesame radiatum* and *Sesame indicum*. The essential oil for *S. radiatum* and *S. indicum* was analyzed by gas chromatography-mass spectrometry (GC/MS) technique. Phenolic and carboxylic acids were the main constituents. This oil exhibited bacteriostatic and fungistatic activities against *Streptococcus pneumoniae* and *Candida albicans*. Finally, *S. radiatum* and *S. indicum* strongly effective against *Streptococcus pneumoniae*. Hakan Uslu et al., (2009) studied *Pelargonium sidoides* extracts have antibacterial effect against *Neisseria spp, Haemophilus influenzae*, *S. pneumoniae*, *Staphylococcus epidermidis* and *Moraxella catharralis*. Antibacterial activity was evaluated by using microdilution broth method.
Warda K et al., (2009) studied antibacterial activities of some herbal plants used in south of Morocco. The methanol extracts of Marrubium vulgare, Thymus paliatus, Eryngium ilicifolium and Lavandula stoechas are tested against Streptococcus pneumoniae responsible for pharyngitis, rhinitis, and otitis and sinusitis infections. Significant activity has been observed with methanol extracts of three plants; M. vulgare, T. paliatus and L. stoechas showed a promising in vitro bactericidal activity against Streptococcus pneumoniae.

Mustafa Oskay et al., (2009) studied antibacterial activities of ethanolic extracts of 19 plant species were studied against multi-drug resistant clinical isolates using agar well diffusion method. Extracts of Liquidambar orientalis, Vitis vinifera, Rosmarinus officinalis, Panica granatum, Cornus sanguinea, Euphorbia peplus, Echallium elatum, Inula viscosa and Liquidambar orientalis showed broad-spectrum antibacterial activity with inhibition zones ranging from 8 to 26 mm. The most resistant organisms were Escherichia coli (E. coli), Ampicillin-, amoxicillin- and sulfamethoxazole-resistant, Stenotrophomonas maltophilia (Amoxicillin- and nalidixic acid-resistant) and Klebsiella pneumoniae (Ampicillin-, amoxicillin- and aztreonam-resistant), and the most susceptible species were Staphylococcus aureus (Penicillin G- and oxacillin-resistant), Streptococcus pyogenes (Penicillin G-, erythromycin- and clindamycin-resistant) and Pseudomonas aeruginosa (Sulfamethoxazole- and novobiocin-resistant), Streptococcus pneumoniae (Sulfamethoxazole- and penicillin G-resistant, and oxacillin- and lincomecinsensitive) respectively. Minimum Inhibitory Concentrations (MIC) of crude extracts were determined for the seven highly active plants showing activity against methicillin resistant S. aureus (MRSA), E. coli, P. aeruginosa, S. Pneumoniae and the reference bacteria (E. coli ATCC 11229 and Kocuria rhizophila ATCC 9341 NA), MICs of active extracts ranged from 8 to 142 mg/mL against one or other test bacteria.

Lalitha S, et al., (2010) studied antibacterial evaluation of the methanolic extract and aqueous fractions of the whole plant of the Acacia mellifera was carried out using the agar-well diffusion method. The extracts and their fractions were tested against two gram positive organisms S. pneumoniae and Staphylococcus aureus and two gram negative organisms- Klebsiella pneumoniae and Escherichia coli. The result of the plant extracts of A. mellifera showed that methanol extracts contain a greater proportion of terpenoids. Ethyl acetate extract of A. mellifera contain flavonoids. Methanol extracts showed antimicrobial
activity against *S. pneumoniae* and *K. pneumoniae* with zones of inhibition ranging from 10-20 mm. Ethyl acetate extracts was found to have antimicrobial activity on *S. aureus* and *E coli* with zones of inhibition ranging from 10-26 mm. The inhibitory activity of these extracts confirmed the potential use of the plant in the treatment of microbial induced ailments.

Bii C, et al., (2010) tested the *Prunus africana* stem bark decoctions are used for antibacterial and antifungal activity Disc diffusion assay was used to evaluate antimicrobial activity against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *S. pneumoniae*, methicillin resistant *Staphylococcus* (MRSA), *Candida albicans*, *Cryptococcus neoformans*, *Microsporum gypseum* and *Trichophyton mentagrophyte*. The methanol extracts were active against *T. mentagrophyte*, *S aureus*, Methicillin resistant *S aureus* and *S pneumoniae* at concentrations of 0.039, 0.073, 0.156 and 2.5 mg/ml, respectively. The antifungal and antibacterial activity of *P. africana* demonstrated supports the claimed antimicrobial uses of the plant in the traditional medicine and provides scientific prove for their medicinal uses.

Ravi Kant Upadhyay et al., (2010) studied the antimicrobial activity of six different plant essential oils i.e., citrus (Citrus lemon), olive (Olea europaea), ajwain (Trachyspermum ammi), almond (Amygdalus communis), Bavchi (Psoralea corylifolia) and neem (Azadirachta indica) oils have been evaluated against Gram-positive bacteria Lactobacillus acidophilus, Streptococcus pneumoniae, Staphylococcus aureus, Micrococcus luteus, Bacillus cereus and Gram-negative bacteria Klebsiella pneumoniae, Escherichia coli. Among all essential oils almond and neem oils were found to be highly bactericidal, as it has shown lowest MIC and MBC values and high growth inhibition zone diameter in comparison to antibiotics. Present study reveals significantly higher broad-spectrum antibacterial activity in essential oils than antibiotics.

Mustofa H.A.M, et al., (2011) studied the different organs of Rumex vesicarius L were screened for their antibacterial activity against six human pathogenic bacterial isolates by disk diffusion assay. Ether extract of root was found to be the most effective against Pseudomonas aeruginosa, Klebsiella pneumoniae, Staphylococcus aureus and Streptococcus pyogenes. Methanol extract of roots was found to be the most most effective against Streptococcus pneumoniae and ethanol extract of flowers most effective one against Escherichia coli. Finally Rumex vesicarius L showed a promising in vitro bactericidal activity against various pathogenic bacteria.

Preethi D, et al., (2011) tested the Stevia rebaudiana, different solvent extracts from leaf, and flower were assayed for in vitro antibacterial activity against pathogenic bacteria such as Bacillus subtilis, Klebsiella pneumonia, Proteus vulgaris, Streptococcus pneumoniae, Staphylococcus aureus and Pseudomonas fluorescense, the zone of inhibition were compared with different standard antibiotics. Phytochemical screening of the crude extracts revealed the presence of secondary compounds such as alkaloids, flavonoids, steroids, tannins and phenols. The organic solvent extracts of flowers are very active against the tested bacterial stains when compared with leaf extracts Elamparithi D. and Boominathan M.,(2011) This study investigated the antibacterial effect of Terminalia chebula crude extract and fraction on two pathogenic bacteria. Generally, crude extracts and fractions were effective against both bacterial strain by disc diffusion method, respectively. However, ethanol mixes methanol fraction at 8:12 and 10:10 combinations were exhibited 5 mm and 7 mm zone of inhibition against Streptococcus pneumoniae and Staphylococcus aureus, which was greater anti-bacterial activity than
the all individual crude extract and fractions. Compared to crude extract, the fraction elicited higher antibacterial properties.

Elampariithi D. and Boominathan M. (2011) state that antibacterial activity of individual crude extract and fraction and combination active fraction of *Camellia sinensis*. For antibacterial test, disc diffusion technique was used against human pathogenic bacterial strains. The significant inhibition of ethanol mixed methanol active fraction was inhibit 7 mm zone against *Streptococcus pneumoniae*, and 8 mm zone against *Staphylococcus aureus*, it was higher activity than all individual fractions and crude extract.

Yushauu M. et al. (2011) studied antibacterial activities of ethanolic extracts of *Ammna squamosa* (L.) leaves were studied against clinical respiratory tract isolates of *Klebsiella pneumoniae*, *Proteus* species, *Pseudomonas* species, *Staphylococcus aureus*, *Streptococcus pneumoniae* and α-haemolytic *Streptococci* using disc diffusion and microbroth dilution techniques. Sensitivity test results showed that water fraction of the plant was active on *Staphylococcus aureus* and *Streptococcus pneumoniae* (10 mm) at 50μg/disc concentration while ethanolic extract of the plant was active against *Klebsiella pneumoniae*, *Streptococcus pneumoniae* and *Proteus* species at 200μg/disc concentration with zone diameter formed by *Klebsiella pneumoniae* (11 mm) being wider than that formed in response to standard Augmentin disc (06 mm).

Shiv Shanker Gautam et al. (2012) studied the antibacterial potential of various extracts (petroleum ether, acetone, methanol and aqueous) of *Nepeta ciliaris* against three gram-positive (*Staphylococcus aureus*, *Streptococcus pneumoniae* and *Streptococcus pyogenes*) and one gram-negative (*Pseudomonas aeruginosa*) bacterial pathogens. The agar well diffusion method was adopted to examine antibacterial and minimum inhibitory concentration (MIC) values of most effective extracts against the susceptible bacteria. Erythromycin was used as positive control to determine the sensitivity of the strains. Out of the four bacterial species tested, *S. pneumoniae* was the most susceptible. The *N. ciliaris* is potentially a good source of antimicrobial agents.
Souad Akroum and Korichi Lakaoui (2012) tested the antimicrobial activity of their ethanolic and methanolic extracts of *Vicia faba L.*, *Vaccinium macrocarpon*, *Punica granatum*, *Lavandula officinalis*, *Artemisia absinthium*, *Linum capitanum* and *Camellia sinensis* on some pathogen bacteria, then their ability to in vivo inhibit the growth of *Streptococcus pneumoniae*. The phytochemical screening has given the composition of the most active extracts. According to the obtained results, the ethanolic extract of *Lavandula officinalis* and *A. Absinthium* has shown an inhibition of all the tested bacteria. The ethanolic extract of *L. Officinalis* has given the highest activity against *S. Pneumoniae* followed by the methanolic extracts of *C. Sinensis* and *P. granatum*. The phytochemical screening showed that the most active extracts contained mainly phenolic compounds.

Siraj ahmed kakar et al., (2012) studied the Crude Methanol Extracts of four plants of Balochistan (*Berberis baluchistanica*, *Sericidium quattense*, *Iphiona acheri*, *Ferula costata*) have been tested for a wide array of antimicrobial activity against three gram positive bacteria *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Streptococcus pyogene* and four gram negative bacteria *Escherichia coli*, *Salmonella typhimurium*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*. All the plant extracts were found to be effective against all the tested bacteria.

Inderjit Kaur et al., (2012) studied the antimicrobial activity of aqueous and methanolic extracts of *Adhatoda vasica* were evaluated against the bacteria isolated from the sputum samples of asthmatic patients. *A. vasica* showed a broad spectrum of antibacterial activities against gram-positive *Staphylococcus aureus* and *Streptococcus pneumoniae* bacterial species in comparison to the gram-negative *E.coli* and *Klebsiella pneumoniae* bacterial species. On the basis of the results obtained in the present study, concluded that the aqueous and methanolic extract of *Adhatoda vasica* has significant amounts of antioxidant and antimicrobial agents.

2.7. Molecular docking

In the area of molecular modeling, docking is a method which predicts the preferred orientation of one molecule to a second (Lengauer et al., 1996). Knowledge of the preferred orientation in turn may be used to predict the strength of association or binding affinity between two molecules using scoring functions. Docking is frequently used to predict the binding orientation of small molecule drug candidates to their protein targets in order to in
turn predict the affinity and activity of the small molecule. Hence docking plays an important role in the rational design of drugs. Given the biological and pharmaceutical significance of molecular docking, considerable efforts have been directed toward improving the methods.

Hex is an interactive molecular graphics program for calculating and displaying feasible docking modes of pairs of protein and DNA molecules. Hex calculates protein-ligand docking, assuming the ligand is rigid, and it can superpose pairs of molecules using only knowledge of their 3D shapes. It is still the only docking and superposition program to use spherical polar Fourier correlations to accelerate the calculations, and it is one of the few docking programs which has built-in graphics to view the results (Ritchie et al., 2000).

The structure-based virtual screening (SBVS), an approach used widely in drug design and discovery, possesses many advantages, such as rapidness, economization, efficiency and high-throughput. In the recent years, SBVS has attracted great attention in developing innovative antimicrobial agents. In SBVS method, Protein-Ligand interaction plays a significant role in structural-based drug designing. The docking results are ordered by energy values and the lowest energy docking solution is the seed member for drug design. Lowest energy orientation is the prediction for target (Ritchie et al., 2003).

*S. pneumoniae*, an important human pathogen, contains at least two genes, *nanA* and *nanB*, that express sialidase activity. NanA is a virulence determinant of pneumococci which is important in animal models of colonization and middle ear infections. The gene encoding NanA was detected in all 106 pneumococcal strains screened that represented 59 restriction profiles. Sequencing confirmed a high level of diversity, up to 17.2% at the nucleotide level and 14.8% at the amino acid level. Result suggests that *nanA* is an important target of the immune system in the interaction between the pneumococcus and host.

One of the important biological and clinical properties of *Streptococcus pneumoniae* is the antibiotic/multi-drug resistance (Pagliero et al., 2004). Generally β-lactam antibiotics are the drug of choice and penicillin-binding proteins (PBPs) are its target; but excessive use of β-lactam antibiotics leads to antibiotic resistance. In order to study the mechanism of antibiotic resistance, the recently reported structures of PBP2B, 2WAF (wild type and sensitive strain, R6) and 2WAE (mutant and resistant strain, 5204) and the predicted structures of other mutant and resistant strains such as G54, Hungary19A-6 and SP195 were
considered. Generally, mutations within the transpeptidase domain of PBP2B of S. pneumoniae lead to decreased affinity of β-lactam antibiotics and thus resistance emerges. The study of interaction with β-lactam antibiotics such as amoxicillin, penicillin G and cefprozil with the above PBP2B through induced-fit docking (IFD) of Schrödinger software revealed the mechanism of resistance. The Glide and IFD scores and interaction of active site and mutated residues revealed that the wild type R6 as sensitive, mutant strains 5204 and G54 as highly resistant and the other mutant strains Hungary19A-6 and SP195 as intermediate resistant. It was observed that the mutant strains Hungary19A-6 and SP195 exhibit intermediate resistant due to the existence of mutations only upto 538th and 516th positions, respectively, and not till the end of the C-terminus. The study also showed that if the mutations are extended till the end of the C-terminus, then the antibiotic resistance of induced mutated Hungary19A-6 and SP195 strains increase from intermediate to high resistance, as in the case of mutant 5204 and G54 strains (Jothi et al., 2010).

Simrika Thapa and Abdullah Zubaer (2011) stated that Streptococcus pneumoniae is the major pathogen that causes pneumonia in the pediatric population in Bangladesh and other developing countries. The capsule of this pathogen is the main virulent factor, the synthesis being governed by the capsular polysaccharide gene cluster that encodes for regulatory proteins such as CpsB. Studies have suggested CpsB to be a potential drug target in case of a high rate of vaccine failure as vaccines are intended to target only a few serotypes among 90 available ones. Thus the protein-targeted drug against CpsB is a good alternative to protect against pneumonia. In this study, homology modeling and validation of this protein was performed with certain bioinformatic tools which facilitate the execution of molecular docking against the ligands from the ZINC database. The two potential ligands bind substantially in the active site of this regulatory protein with considerable binding energy in the docking study, which means they could be considered good inhibitors. This study aims to understand the functional aspects of this and also to examine the development of a novel drug against pneumonia.