Chapter 2

Review of Literature

*Klebsiella* is among the most common pathogens isolated in intensive care units (ICUs), (Sanchez *et al*., 2013). *K. pneumoniae* is a member of the Enterobacteriaceae family, which along with *E. coli* accounts for the vast majority of urinary tract infections (UTIs). It occupies diverse ecological niches ranging from soil to water, it also represents one of the most important human pathogens (Podschun *et al*., 2001). It is also a frequent cause of nosocomial bloodstream infections and community-acquired pneumonia among alcoholics. Respiratory infections caused by *K. pneumoniae* are responsible for high rates of mortality and morbidity. *Klebsiella* spp are often resistant to multiple antibiotics (Podschun & Ullman, 1998). The most frequent resistance include resistance to Aminoglycosides, Fluoroquinolones, Tetracyclines, Chloramphenicol and Trimethoprim/Sulfamethoxazole (Nathisuwan *et al*., 2001).

Spread of the multidrug resistance in bacteria is an alarming feature. The rapid spread of bacterial strains expressing multidrug resistance (MDR) has necessitated the discovery of new antibacterials and resistance-modifying agents. There are several mechanisms to develop resistance against antibiotics in bacteria; one of them is the development of efflux pump mediated drug resistance. Drug resistant micropathogens, efflux out the maximum concentration of drugs with the help of efflux pump. Efflux pumps play a major role as a mechanism of antimicrobial resistance in Gram-negative pathogens (Padilla *et al*., 2010). The presence of AcrAB efflux pump (EP) in *K. pneumoniae* is a major mechanism of the development of antibiotic resistance (Zhang & Buckling, 2012) and also contributes to antimicrobial resistance and virulence in the Gram negative bacteria (Padilla *et al*., 2010).

Since the initial discovery of bacterial efflux pumps in the 1980s, several Gram-positive and Gram-negative MDR strains have been characterized in which efflux pump mediated drug resistance was developed (Stavri *et al*., 2007). Efflux mechanisms have become largely recognized as key components of resistance to many classes of antibiotics because they are able to extrude structurally different compounds, including antibiotics; the latter are rendered therapeutically ineffective (Seasotiya & Dalal, 2014). Antibiotic resistance might develop quickly through changes in the expression of efflux pumps, including changes to some antibiotics considered to be drugs of last preference. It is therefore very important that new antibiotics, resistance-modifying agents and, more specifically efflux pump inhibitors (EPIs) to be identified. The use of bacterial resistance modifiers such as EPIs could facilitate the re-introduction of therapeutically ineffective
antibiotics back into clinical use such as Ciprofloxacin, Tetracycline and Chloromphenicol etc., and might even suppress the emergence of MDR strains (Stavri et al., 2007).

*K. pneumoniae* is a Gram Negative, rod shaped, non motile, encapsulated bacteria belongs to family Enterobacteriaceae. It causes infection to the human beings and transmitted through air, soil and water. It is inhabited in respiratory tract and causes several clinical manifestations.

### 2.1 Clinical Importance

*K. pneumoniae* is a Gram negative bacterium found in the normal flora of the mouth, skin and intestine (Ryan et al., 2004). It is facultative anaerobic, non-motile, rod shaped and is encapsulated bacteria. Ishida and co-workers (1998) reported that the *K. pneumoniae* is one of the major bacteria causing pneumonia with symptoms like sudden onset of high fever & currant jelly sputum. *K. pneumoniae* also often causes urinary tract infections (Hoban et al., 2003, Jones et al., 2004). In addition to being the primary cause of respiratory tract infections, *K. pneumoniae* is also commonly involved in acute pyelonephritis in pregnant women with urinary tract abnormalities such as urolithiases, hydronephrosis or congenital deformities. *Klebsiella* species are also often involved in neonatal sepsis and have a marked tendency to exist as mixed infections or as secondary infections with other pathogenic bacteria (Sikarwar & Batra, 2011). *K. pneumoniae* also emerges as an important cause of neonatal nosocomial (Gupta et al., 1993) and community acquired (food borne) infections (Podschun & Ullman, 1998). *Klebsiella* accounts for 6 to 17% of all nosocomial urinary tract infections (UTI) and even higher incidence is seen in patients at risk such as patients with neuropathic bladders or with diabetes mellitus (Podschun & Ullman, 1998).

### 2.2 Epidemiology

*K. pneumoniae* causes serious epidemic and endemic nosocomial infections. *Klebsiella* spp. accounts for 8% endemic hospital infections and 3% epidemic outbreaks (Podschun & Ullman, 1998). It is responsible for 6-17% UTI’s, 7-14% pneumonia, 4-15% septicaemia, 2-4% wound infections and 4-17 % nosocomial infections in ICUs (Podschun & Ullman, 1998). Carriers in hospitalized patients carry 19% pathogens in the pharynx, 77% in the stool and 42% on the hands (Podschun & Ullman, 1998). The principal reservoirs for spread of *Klebsiella* in the hospital setting are the gastrointestinal tract of patients and the hands of hospital workers. The ability of this pathogen to spread
rapidly often leads to nosocomial outbreaks, especially in neonatal wards (Hart, 1993). World Health Organization (2004) reported that approximately, 3.9 millions of people died by pneumonia in the year 2004. Pneumonia is the fourth cause of human death following cancer, heart diseases and brain diseases in Japan, (Watanabe et al., 2009). K. pneumoniae should be considered as a first cause of liver abscess (Lim et al., 2003). Pyogenic liver abscess is a potentially life-threatening disease, with a reported mortality of up to 31% (Chan et al., 2007). K. pneumoniae is emerging as the leading cause of liver abscess although in past the most common pathogen was E. coli (Sang et al., 2003), hence, it is important to treat patients infected with K. pneumoniae by using antimicrobial agents at early stage of infection (Pedersen et al., 1997). In an analysis of K. pneumoniae liver abscess from two hospitals in New York, 78.3% of patients were of Asian origin. These findings raise the possibility that geographic distribution patterns of virulent K. pneumoniae subtypes may play important roles in its pathogenesis (Lin et al., 2012). Lin et al., (2012) also reported that the infections of K. pneumoniae are more prominent in Asian countries. However, bacterial strains can only persist inside the gastrointestinal tract if they are firmly established on the intestinal mucous surface and hence are able to resist the strong waves of peristalsis.

2.3: Drug Resistance

Since the introduction of penicillin during the Second World War antibiotics have become miracle drugs. However, today the miracle may be over due to increasing antibiotic resistance in various bacteria, including multi-resistance bacteria (Borjesson et al., 2009). Infectious diseases caused by microbes like bacteria and fungi affect millions of people worldwide. In these pathogens now drug resistance is rising rapidly. Drug resistance is the reduction in the effectiveness of a drug in curing a disease. MDR is defined as resistance to three or more different classes of antibiotics (Tenover; 2006, Karthy et al., 2009). Pathogenic species of bacteria which have become resistant to the drugs causes infections to the human beings which cannot be treated with the available antibiotics (Usha et al., 2010). The emergence and rapid spread of drug resistant K. pneumoniae is becoming a serious problem worldwide (Li et al., 2012). Recently, it has been reported that multidrug resistant K. pneumoniae has often been isolated in hospitals, and this has become a big clinical problem (Pagani et al., 2000, Sekowska et al., 2002, De Champs et al., 2004 & Hasdemir et al., 2004). Respiratory infections caused by K. pneumoniae show high rates of mortality and morbidity, but their management is often
difficult due to multidrug resistance found in different strains of bacteria (Padilla et al., 2010). As with many bacteria the recommended treatment has changed as the organism has developed resistance. Klebsiella organisms are often resistant to multiple antibiotics. The most frequent resistances include resistance to Aminoglycosides, Fluoroquinolones, Tetracyclines, Chloramphenicol and Sulfamethoxazole-trimethoprim (Jacoby, 1994). Fluoroquinolones have been considered an adequate therapeutic option, however several studies indicate that an increasing percentage of Klebsiella strains are resistant to these antimicrobials (Deguchi et al., 1997, Martinez et al., 1998 & Wang et al., 2004). It is important to understand the mechanism of resistance for the development of proper strategies to solve the problem (Sibanda et al., 2007). Resistance can be described in two ways: (a) intrinsic or natural whereby microorganisms naturally do not possess target sites for the drugs and therefore the drug does not affect them or they naturally have low permeability to those agents because of the differences in the chemical nature of the drug and the microbial membrane structures especially for those that require entry into the microbial cell in order to affect their action (b) acquired resistance whereby a naturally susceptible microorganism acquires ways of not being affected by the drug (Byarugaba, 2004). Mechanisms for acquired resistance are: (a) the presence of an enzyme that inactivates the antimicrobial agent (b) the presence of an alternative enzyme for the enzyme that is inhibited by the antimicrobial agent (c) a mutation in the antimicrobial agent’s target, which reduces the binding of the antimicrobial agent (d) post-transcriptional or post-translational modification of the antimicrobial agent’s target, which reduces binding of the antimicrobial agent (e) reduced uptake of the antimicrobial agent (f) active efflux of the antimicrobial agent (g) overproduction of the target of the antimicrobial agent (h) expression or suppression of a gene in vivo in contrast to the situation in vitro (i) previously unrecognized mechanisms (Fluit et al., 2001).

Multidrug resistance increasingly being reported in bacteria and is often mediated by genetic mobile elements such as plasmids, transposons, and integrons (Dessen et al., 2001). A plasmid is a small DNA molecule within a cell that is physically separated from a chromosomal DNA and can replicate independently of it. A transposon is a DNA sequence that can change its position within the genome. Integrons are mobile DNA elements with the ability to capture genes, notably those encoding antibiotic resistance, by site specific recombination, and they have an intergrase gene (int), a nearby recombination site (attI), and a promoter, Pant (Hall, 1997).
Besides these, bacterial drug resistance can be summarized into five major mechanisms (Campos *et al*., 2004)

1. Inactivation of drugs due to degradation
2. Modification by enzyme
3. Alteration in target of drugs
4. Emergence of bypass which is not inhibited by drugs reduced membrane permeability for drugs.
5. Active efflux of drugs from cells by drug efflux pumps, especially by multidrug efflux pumps (Fig 2.1).

Efflux pump mediated antimicrobial resistance involved one mechanism out of two mechanisms: either (i) Expression of the efflux pump protein is increased or (ii) The protein contains an amino acid substitution, which makes the protein more efficient to efflux out the drug. In either case, the intracellular concentration of the substrate antimicrobial is lowered due to the efflux out of the antimicrobial agent and the organism becomes less susceptible to that agent (Pedersen *et al*., 1997, Ishida *et al*., 1998, Hoban *et al*., 2003 & Jones *et al*., 2004).

![Fig 2.1: Mechanisms representing antibacterial resistance. A generic bacterium is depicted in which various mechanisms for resistance to antimicrobial agents are indicated. (a) Drug target modification, (b) Drug inactivation by enzymes, (c) Reduced drug permeability by membrane modification (d) Active efflux of drugs from the bacterial cell. Yellow circles indicate antimicrobial agent molecules; red arrows indicate movement of molecules, and black arrows are pointing to intra- and extracellular structures (Kumar *et al*., 2013).](image-url)
2.4: Resistance due to Efflux pump

Bacterial efflux transporters pump, all antibiotic classes out of the cell before they can reach their prospective targets (Piddock, 2006, Davin et al., 2008; Li & Nikaido, 2009). Antibiotic efflux transporters found in both eukaryotes and prokaryotes may be specific, facilitating the efflux of only one compound or a class of compounds, exhibiting broad specificity for chemical compounds that are structurally unrelated (Nelson et al., 1994). Phylogenetically, bacterial antibiotic efflux pumps belong to five super families (Paulsen, 2003, Saier 2003, Li & Nikaido, 2004) namely:

1) ATP-binding cassette (ABC), which are primary active transporters energized by ATP hydrolysis
2) Small multidrug resistance (SMR) subfamily of the drug/metabolite transporters (DMT) Super family
3) Multi-antimicrobial extrusion (MATE) subfamily of the multidrug/Oligosaccharidyl-lipid/Polysaccharide flippases (MOP) super family
4) Major facilitator super family (MFS)
5) Resistance/Nodulation/Division super family ((RND) (Fig 2.2)).

Drug resistance in K. pneumoniae due to efflux pumps is an alarming problem worldwide. As shown in Table 2.1, efflux pumps found in K. pneumoniae belongs to MFS and RND families and creates drug resistance towards Chloramphenicol, Fluoroquinolones, Tetracyclines, Glycylcyclines and Florfenicol (Piddock, 2006).
Table 2.1: Bacterial Efflux Systems Involved in Antibiotic Resistance in *K. pneumoniae*

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>Efflux System</th>
<th>Pump Family</th>
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<tbody>
<tr>
<td>Chloramphenicol</td>
<td>Cml,CmlA,CmlB</td>
<td>MFS</td>
</tr>
<tr>
<td>Chloramphenicol and Florfenicol</td>
<td>Flo, FloR, pp-Flo</td>
<td>MFS</td>
</tr>
<tr>
<td>Glycylcyclines</td>
<td>AcrAB-TolC,Mex XY,OprM,MexAB-OpM,MexCD-OprJ</td>
<td>RND</td>
</tr>
<tr>
<td>Fluorquinolones</td>
<td>AcrAB-TolC</td>
<td>RND</td>
</tr>
<tr>
<td>Quinolones</td>
<td>AcrAB</td>
<td>RND</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>AcrAB</td>
<td>RND</td>
</tr>
</tbody>
</table>

The RND pumps are found mainly in Gram-negative bacteria and display a wide spectrum of lipophilic and amphiphilic substrates, including several classes of antibiotics, antiseptic compounds, dyes, and detergents (Tseng *et al.*, 1999). Over expression of RND efflux systems associated with MDR has been also demonstrated in *Klebsiella* spp (Mazzario *et al.*, 2002, Kumar *et al.*, 2005, Van Amsterdam *et al.*, 2005). Recent studies suggest that RND efflux pumps have a role in invasion, adherence and colonization of the host cell. Therefore, the inhibition of efflux pumps in some cases might also reduce the bacterial virulence *in vivo* (a & b Piddock, 2006).

AcrAB- TolC is an efflux pump found in *K. pneumoniae* belonging to RND family is responsible for most common drug resistance in this bacterium. In AcrAB–TolC system, the transporter protein AcrB (Aires & Nikaido, 2005), captures its substrates either from within the phospholipid bilayer of the inner membrane or from the cytoplasm, and then transports them to the extracellular medium through TolC, which forms a channel in the outer membrane (Eswaran *et al.*, 2004). The cooperation between AcrB and TolC is mediated by the periplasmic accessor protein AcrA. Efflux through RND-family pumps is driven by the proton motive force (Eswaran *et al.*, 2004), an electrochemical gradient in which the movement of hydrogen ions drives transport of the substrate. In Enterobacteriaceae, Tol C can function as the protein channel for different RND-family efflux pumps. These efflux pumps can extrude different compounds form the bacterial cytoplasm and the periplasm (Fig2.3).
Fig 2.3: Structure of an RND efflux pump. The figure shows a scheme of the structure of the AcrAB-TolC system. As shown, the system is a tripartite complex formed by the inner membrane AcrB protein, the outer membrane protein TolC and the membrane fusion protein AcrA. The activity of the AcrB RND protein is coupled to the proton gradient (Blair and Piddock, 2009).

2.5. Efflux Pump Inhibitors

Efflux pump inhibitors are resistance modifying agents; they may be synthetic or derived from plants sources. They approach in combating drug resistance. Efflux pump inhibitors are expected to (1) decrease intrinsic resistance and consequently expand the spectrum of activity of resistant antibiotics to previously non-susceptible species; (2) overturn acquired resistance; and very importantly (3) decrease the frequency of emergence of resistant strains.

2.5.1: Synthetic Inhibitors of efflux pumps in *K. pneumoniae*

(a) Alkoxyquinolones

Alkoxyquinolones increase the efficacy of Chloramphenicol, Norfloxacin, Tetracycline and Cefepime up to 8-folds. e.g. 2, 8-dimethyl-4-(2’-pyrrolidinoethyl)-
oxyquinoline, restores drug susceptibility in resistant clinical strains of *K. pneumoniae* against Chloramphenicol and in some other structurally unrelated antibiotics (Chevalier *et al.*, 2004).

**(b) Carbonyl cyanide-m-chlorophenylhydrazone (CCCP)**

CCCP is a protonophore. It considerably affects the energy level of the membrane and cell viability by causing a dissipation of the proton motive force of the membrane, affecting the transporters that depend on this mechanism. Besides its high toxicity for the cell, it is described as a substrate of bacterial efflux pumps (Mahamoud *et al.*, 2007). It has shown inhibitory activity in *Mycobacterium smegmatis* and in *Mycobacterium fortuitum* by inhibition of the MFS efflux pump (Rana *et al.*, 2014). This is a strong uncoupler that disturbs electrochemistry gradient to inhibit RND, MATE, MFS and SMR efflux pumps. CCCP increase the intracellular concentrations of antibiotics and become a necessary tool for studying efflux systems MICs (Poole, 2002).

**(c) PAβN- MC-207,110 or Phenylalalnine arginyl β-naphthylamide**

It is a dipeptide amide and had potentiated the activity of levofloxacin by 8 fold at 10 μg/mL, against *P. aeruginosa* (Barrett; 2001). It also increases the susceptibility of erythromycin 8-32 fold and & Rifampicin 8-64 folds against *Campylobacter jejuni* & *Campylobacter coli* (Hannula & Hanninen 2008). It also inhibited the efflux pump in *E.coli* and reduced the susceptibility of Rifaximin (Gomes *et al.*, 2013). PAβN converts Ciprofloxacin resistant strains of *P. aeruginosa, Acinetobacter baumannii* & *E.coli* to susceptible ones (Cetinkaya *et al.*, 2008). In combination with Fluoroquinolones, it seems to have inhibitory activity against the MexCD-OprJ and MexEFOprN pumps of *P. aeruginosa*, and against the AcrAB-TolC efflux pump of Gram-negative bacteria, including *K. pneumoniae, E. coli, S. typhimurium* and *E. aerogenes* (Rana *et al.*, 2014). It is also toxic in nature. Its toxicity prevents its use in clinical applications. However, it is widely used to experimentally determine & evaluate the efflux mechanisms of bacterial pathogens. In addition, it is used to measure the efflux activity and determine the level of inhibitor-sensitive efflux for specific antibiotics in various bacteria (Mahamoud *et al.*, 2007).

**(d) Arylpiperazines**

Arylpiperazines inhibits the efflux pumps activity against Fluoroquinolones, Tetracyclines, Chloramphenicol etc as substrates. Arylpiperazines have a potency to reverse multidrug resistance in cells overexpressing RND-type efflux pumps (Bohnert &
Kern, 2005). Some of the members of Arylpiperazines family are capable of reversing multidrug resistance in *E. coli* over-expressing RND Efflux Pumps, e.g. 1-(1-Naphthylmethyl)-piperazine enhanced the susceptibility of *E. coli* to Fluoroquinolones and Levofloxacin (Rana *et al.*, 2014).

(e) **Quinoline derivatives**

They inhibit the efflux pumps which use Chloramphenicol, Norfloxacain & Tetracycline as substrates, e.g.: 4-sustituted thioalkyl, alkylamino, alkoxy-quinolines & MC-02, 595. Various Quinoline derivatives significantly increase the intracellular concentration of Chloramphenicol as reported with other inhibitors, thereby suggesting the inhibition of the drug transport by AcrAB-TolC pump, which is fully active in the clinically resistant isolates investigated (Mahamoud *et al.*, 2006). They have been proved as promising inhibitors of antibiotic efflux pump in multidrug resistant *Enterobacter aerogenes* isolates (Rana *et al.*, 2014).

(f) **Pro-D-hPhe-3-aminoquinoline (MC-04,124)**

They enhance the antibacterial activity of Azithromycin, Clarithromycin and Erythromycin. They also enhance the activity of Levofloxacin against *P. aeruginosa* (Renau *et al.*, 2001).

2.5.2: **Plant derived EPIs against Gram positive bacteria**

(a) **Carnosic acid**

Carnosic acid is a natural compound obtained from *Rosmarinus officinalis*; it is structurally unrelated to known antibiotics, which can function as an efflux pump modulator by dissipation of the membrane potential. Therefore, carnosic acid would be a good candidate to be employed as a novel therapeutic agent to be used in combination therapies against drug-resistant *S. aureus* infections (Fernandez *et al.*, 2013).

(b) **Carnosol**

Carnosol isolated from herb Rosemary (*Rosmarinus officinalis*) have potentiated tetracycline and erythromycin against *S. aureus* strains possessing the Msr (A) efflux pumps (Rana *et al.*, 2014).

(C) **Baicalein**

Baicalein had synergistically restored the antibacterial actions of ciprofloxacin against the NorA efflux pump overexpressed SA-1199B strain of *S. aureus* (Chan *et al.*, 2011). Baicalein is obtained from *Thymus vulgaris*. 
(d) 2, 6-Dimethyl-1, 4-phenyl-pyridine-3, 5-dicarboxylic acid diethyl ester

It is obtained from *Jatropha elliptica* and acts as an EPI against *S. aureus*. This compound demolishes the efflux pump NorA and restores the level of intracellular drug concentration (Rana et al., 2014).

(e) Piperine

Piperine is obtained from *Piper nigrum* and acts as an EPI against *S. aureus* (Rana et al., 2014). Piperine is involved in the inhibition of clinically overexpressed mycobacterial putative efflux protein (Rv1258c). Piperine may also be useful in augmenting the antimycobacterial activity of Rifampicin in the clinical settings (Sharma et al., 2010).

(f) Reserpine

Reserpine is the most common EPI discovered against *S. aureus* and *Bacillus subtilis*. It is an indole alkaloid which inhibits gram positive efflux pumps, mainly involved in Fluoroquinolone efflux but it is neurotoxic at the concentration required to inhibit pumps in vivo (Klyachko et al., 1997). Resepine is obtained from *Rauwolfia vomitoria* and acts as an EPI against *Bacillus subtilis* (Rana et al., 2014). It is a broad spectrum natural EPI. It is active against many different efflux pumps viz. NorA, TetK, Bmr in *Streptococcus pneumoniae, Staphylococcus aureus, Bacillus subtilis* respectively. A major limitation of combining this EPI with drugs is that this needs to be used at higher concentrations which may be proved toxic at clinical levels (Rana et al., 2014).

Although several EPIs have been discovered from plants but most of them are against Gram positive bacteria mostly against *S. aureus*.

2.5.3: Plant derived EPIs against Gram negative bacteria

(a) Baicalein

Baicalein is obtained from *Thymus vulgaris* and acts as an EPI against Tet K efflux pump of *E. coli* and enhances the activity of tetracycline (Rana et al., 2014).

(b) Pheophorbide a

Pheophorbide-a is obtained from *Berberis aetnensis* and shows EPI activity against *E. coli* and *P. aeruginosa* by enhancing the activity of ciprofloxacin (Rana et al., 2014).

(c) Theobromine

Theobromine is obtained from *Theobroma cacao* and acts as an EPI by enhancing the activity of ciprofloxacin against MexAB-oPrM and AcrAB–ToIC efflux pumps of *P.*
aeruginosa and K. pneumoniae respectively. Its EPI activity has also been proved against Salmonella typhimurium (Piddock et al., 2010).

(d) Cathinone

Cathinone enhances the activity of ciprofloxacin against Salmonella typhimurium by reducing the function of AcrAB-TolC efflux pump and is obtained from Catha edulis (Piddock et al., 2010).

2.5.4: Microbial derived EPIs

EA-371α & EA-371δ, these compounds are isolated from Streptomyces. They inhibit the MDR pump MexAB-OprM of P. aeruginosa (Stavri et al., 2007).

To date, no efflux pumps inhibitors has been licensed for use in the treatment of bacterial infections in human or veterinary settings, although research continues. In the treatment of bacterial diseases, one drug development program involving co-administration of an EPI with an antibiotic agent has reached human clinical trials. In this trial, an aerosolized formulation of the EPI compound MC-601, 205 is being combined with ciprofloxacin for the treatment of pulmonary exacerbations in cystic fibrosis patients in a phase II trial being conducted by Mpex Pharmaceuticals (Barbara & Versace, 2009).

2.6: Plants as a Source of EPIs

Plants used in this study were selected on the basis of synergism with antibiotics used commonly against K. pneumoniae. The description of the plants used in the study is given below:

2.6.1: Syzygium aromaticum

Scientific name: Syzygium aromaticum
Common name: Clove
Family: Myrtaceae
Parts used: Dried buds

History

This plant is used globally as a common spice. It has been used medicinally for bad breath and as insect repellent (Tayel et al., 2009).
Herbal Properties and Uses

Clove oil has been widely used for preventing tooth decay and as a cure for athlete’s foot (Pinto et al., 2009).

Cultivation & Growing Methods

The tree is a perennial. This plant grows best in tropical conditions, such as those regions surrounding the Indian Ocean (Cloyd et al., 2009).

Synergistic activity

As stated in shodhganga, synergistic interactions were shown by the combination of Syzygium aromaticum with antibiotics against different bacteria. Clove has also shown synergistic effect with various antibiotics against *P. aeruginosa*, *K. pneumoniae* & *Proteus* spp (Nascimento et al., 2000).

2.6.2: Syzygium joabolanum

<table>
<thead>
<tr>
<th>Scientific name: Syzygium joabolanum</th>
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<tbody>
<tr>
<td>Common name: Jamun</td>
</tr>
<tr>
<td>Family: Myrtaceae</td>
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<tr>
<td>Parts used: Leaves</td>
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History

Many species of *Jambolan* have been used in the folk medicine to treat infectious diseases (Maciel et al., 2008).

Herbal Properties and Uses

*Syzygium joabolanum* is widely used for treating patients with diabetes mellitus (Maiti et al., 2013).

Cultivation & Growing Methods

*Syzygium joabolanum* is common herb found in India, Pakistan, Southern Asia and Brazil. This tree can reach height of up to 40 feet. It produces creamy or greenish white flowers (Maiti et al., 2013).

Synergistic effect

*Syzygium joabolanum* has shown synergism with standard antibiotics against *P. aeruginosa* (Nascimento et al., 2000).
2.6.3: *Thymus vulgaris*

Scientific name: *Thymus vulgaris*

Common name: Thyme

Family: Labiatae

Parts used: Leaves

History

Thyme has a long history of folk use for a wide range of ailments. This plant has been used since ancient times for its aromatic & medicinal properties (Kulevanova et al., 2000).

Herbal Properties and Uses

Thyme is rich in essential oil which contains active ingredients responsible for most of the medicinal properties. *S. aureus, E.coli* and *Shigella sonnei* are a few species of bacteria against which thyme has been shown to have antimicrobial activity (Bagamboula et al., 2003).

Cultivation & Growing Methods

*Thymus vulgaris* is an evergreen shrub growing up to 0.2m. This is grown in dry to medium well dried soil in full sun. Sandy or rocky soils are best. It is grown in Asia, Southern Europe and the Mediterranean region (Kulevanova et al., 2000).

Synergistic activity

Baicalein a trihydroxy flavones isolated from the leaves of the thyme (*Thymus vulgaris*), was identified as possessing a strong synergistic activity with Tetracycline, Oxacillin, Cefmetazole and Ampicillin against MRSA (Rana et al., 2014). *Thymus vulgaris* has shown synergism with standard antibiotics against *P. aeruginosa* (Nascimento et al., 2000).
2.6.4: *Punica granatum*

Scientific name: *Punica granatum*
Common name: Pomegranate
Family: Lythraceae
Parts used: Seeds

**History**

*Punica granatum* has been used for many years to cure a wide range of diseases across different cultures and civilization (Bhowmik *et al.*, 2013).

**Herbal Properties and Uses**

*Punica granatum* has immune stimulatory, anti-oxidant, anti-inflammatory and diuretic properties (Bhowmik *et al.*, 2013).

**Cultivation & Growing Methods**

There are several ways of growing pomegranate: from a seedling, a cutting or from seed. *Punica granatum* is a shrub or a small tree, growing up to 10m height. It is native to central Asia (Bhowmik *et al.*, 2013).

**Synergistic activity**

The methanolic extract of *Punica granatum* caused an increase in Ethidium bromide uptake in *S. aureus* strain RN-7044, having an Ethidium bromide efflux mechanism (Rana *et al.*, 2014). *Punica granatum* has shown synergism with standard antibiotics against *P. aeruginosa* (Nascimento *et al.*, 2000).
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2.6.5: *Tectona grandis*

**Scientific name:** *Tectona grandis*  
**Common name:** Teak  
**Family:** Lamiaceae  
**Parts used:** Leaves

**History**

*Tectona grandis* is a major constituent in many of the traditional medicines. Traditionally, it is used against bronchitis, hyperacidity, diabetes, leprosy and helmintiasis (Nidavani et al., 2014).

**Herbal Properties and Uses**

Extracts of various parts of teak showed expectorant, anti-inflammatory, anti helmintic properties (Nidavani et al., 2014).

**Cultivation & Growing Methods**

Teak is a large, deciduous tree reaching over 30 m in height. *Tectona grandis* is indigenous to the Indian peninsula & continental Southeast Asia (Verinumbe & Okali, 1985).

**Synergistic activity**

Synergistic activity was observed between methanolic leaf extract of *Tectona grandis* and tetracycline against *K. pneumoniae* & *S.enterica* serovar Typhimurium (Purushotham et al., 2010).
2.6.6: *Hemidesmus indicus*

Scientific name: *Hemidesmus indicus*

Common name: Indian Sarsaparilla

Family: Apocynaceae

Parts used: Roots

**History**

*Hemidesmus indicus* is used in Ayurveda system, widely for the treatment of oligospermia, gastritis, anorexia, menorrhagia etc (Sreekumar et al., 2000).

**Herbal Properties and Uses**

Indian Sarsaparilla is a very commonly used for the treatment of gastrointestinal and cardiovascular disorders (Ganesan et al., 2012). It is also used as blood purifier and anti-diarrhoeal and anti-viper venom (Sreekumar et al., 2000).

**Cultivation & Growing Methods**

Indian Sarsaparilla is an aromatic medicinal twining shrub distributed in moist localities of India & Sri Lanka (Sreekumar et al., 2000).

**Synergistic activity**

Synergistic activity was seen between *Hemidesmus indicus* and various antibiotics against many bacteria (Gayathri & Kannabiran, 2009).
2.6.7: *Ficus bengalensis*

**Scientific name:** *Ficus bengalensis*  
**Common name:** Banyan  
**Family:** Moraceae  
**Parts used:** Bark

**History**

The *Ficus* trees have been produced for food, religious and practical uses (Mathew et al., 2011).

**Herbal Properties and Uses**

*Ficus* species possess immense medicinal values. The bark of *Ficus* species is used to make a formulation to cure various uterine problems in women (Mathew et al., 2011).

**Cultivation & Growing Methods**

The propagation of *Ficus* species is mainly through seed and vegetative means. *Ficus* species are distributed all over India and requires deep alluvial sandy soil with good drainage (Mathew et al., 2011).

**Synergistic activity**

Synergistic activity was seen between *Ficus bengalensis* and various antibiotics against many bacteria (Gayathri & Kannabiran, 2009).
2.6.8: *Pterocarpus marsupium* Roxb

Scientific name: *Pterocarpus marsupium* Roxb  
Common name: Indian Kino tree  
Family: Fabaceae  
Parts used: Bark

**History**

Indian kinotree is an important medicinal plant widely distributed in India. It is used for various diseases like diabetes, angina and cancer (Prathap *et al.*, 2012).

**Herbal Properties and Uses**

Indian kinotree has a number of therapeutic uses. Indian kinotree has anti-hyperlipidemic properties and helps in reducing total cholesterol and serum triglyceride levels in the body (Prathap *et al.*, 2012).

**Cultivation & Growing Methods**

Indian kinotree is a medium to large, deciduous tree that can grow up to 30 metres. It is native to India & Nepal. It is known as vijyasar in India (Prathap *et al.*, 2012).

**Synergistic activity**

Synergistic activity was seen between *Pterocarpus marsupium* Roxb and various antibiotics against many bacteria (Gayathri & Kannabiran, 2009).
2.6.9: *Alium sativum*

Scientific name: *Alium sativum*
Common name: Garlic
Family: Liliaceae
Parts used: Bulb

**History**
Garlic has been used for more than 5000 years to treat numerous diseases and was even used as a remedy against the plague in the middle ages (Capasso, 2013).

**Herbal Properties and Uses**
*Alium sativum* is rich in antioxidants and help in destroying free radicals. This herb has diverse biological activities, including anti-carcinogenic, antiatherosclerotic, antithrombotic, antimicrobial, anti-inflammatory and antioxidant effects (Capasso, 2013).

**Cultivation & Growing Methods**
Garlic prefers a well-drained soil with at least 4% organic matter and a pH of 6.0-7.0. Organic matter helps to hold moisture and is especially important in lighter soil types (Capasso, 2013).

**Synergistic activity**
Synergistic activity was observed between *Alium sativum* synthesized nanoparticles with β-lactam penem antibiotics (Ampicillin & Amoxyclav), (Hari *et al.*, 2013). Aqueous extract of garlic has shown synergism with ciprofloxacin in some bacterial isolates (Salah *et al.*, 2013). According to the study done by Betoni *et al.*, 2006; synergism was observed between extract of garlic and tetracycline against *S. aureus*. As studied by shahnaz *et al.*, 2009; Allicin a compound isolated from garlic has shown synergism with Ciprofloxacin & Enoxacin against *P. aeruginosa*. 
2.6.10: *Alium cepa*

Scientific name: *Alium cepa*
Common name: onion
Family: Liliaceae
Parts used: Bulb

**History**

Onion has been cultivated for 5000 years or more. They were first grown in Iran and West Pakistan. India is the second largest producer of onions in the world (Kumar *et al.*, 2010).

**Herbal Properties and Uses**

Onion prevents cancer and cardiovascular diseases. Onions may guard against many chronic diseases (Kumar *et al.*, 2010).

**Cultivation & Growing Methods**

Onion is an herbaceous biennial plant and its bulb is edible. Onion bulbs are white, yellow or red. The green stems & leaves can reach 3 ft in height (Kumar *et al.*, 2010).

**Synergistic activity**

According to the study done by Tsao *et al.*, 2001; Diallyl sulphides present in onion showed synergism with Ceftazidime, Gentamicin, Imipenem & Meropenem against *P. aeruginosa* & *K. pneumoniae*. 
2.6.11: *Melissa officinalis*

**Scientific name:** *Melissa officinalis*

**Common name:** Lemon balm

**Family:** Lamiaceae

**Parts used:** Leaves

**History**
Lemon balm is one of the important medicinal plants (Bahtiyarca, 2006).

**Herbal Properties and Uses**
Lemon balm oil is used as a mild sedative, spasmolytic and antibacterial agent. Essential oil of lemon balm is useful in the treatment of Alzheimer’s disease. Lemon balm also has an ability to prevent production of thyroid – stimulating hormone (Bahtiyarca, 2006).

**Cultivation & Growing Methods**
Lemon balm is a perennial herb that lives at least three years. It reaches a height of about 1m (Bahtiyarca, 2006).

**Synergistic activity**
According to the study done by Stefanovic & Comic, 2012; synergism was observed between ethanol & ethyl acetate extract of lemon balm with Chloramphenicol, Tetracycline and Amoxicillin against different bacteria.
2.6.12: *Thespesia populnea*

**Scientific name:** *Thespesia populnea*

**Common name:** Tulip tree

**Family:** Malvaceae

**Parts used:** Leaves/ Flowers

**History**

Tulip tree was originated in India. *Thespesia populnea* is widely used by the traditional medicinal practitioners for the treatment of infectious diseases (Moon *et al*., 2010).

**Herbal Properties and Uses**

Tulip tree has many medicinal properties, such as antifertility, antibacterial, anti-inflammatory, antioxidant, purgative and hepatoprotective activity (Vasudevan & Parle, 2006).

**Cultivation & Growing Methods**

Tulip tree is cold sensitive, but can stand mild frosts. It is a mangrove associate. It has a bush like appearance and grows up to height of 40 ft (Vasudevan & Parle, 2006).

**Synergistic activity**

Synergistic activity was observed between methanolic extract of *Thespesia populnea* and oxytetracycline against many different bacteria including *Shigella boydii* (Kumar *et al*., 2009).
2.6.13: *Pelargonium graveolens*

**Scientific name:** *Pelargonium graveolens*

**Common name:** Geranium

**Family:** Geraneaceae

**Parts used:** Leaves

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**History**

*Pelargonium* species have originated in South Africa. It is introduced to India in the beginning of 20th century (Saxena *et al.*, 2008).

**Herbal Properties and Uses**

*P. graveolens* is an aromatic & hairy herbaceous shrub, up to 1m height (Dzamic *et al.*, 2014). Oil of *Pelargonium graveolens* is useful for problems like eczema and athlete’s foot and for mite control (Verma *et al.*, 2010).

**Cultivation & Growing Methods**

*Pelargonium graveolens* is grown in China, Egypt, Algeria, Morocco and Reunion Island. It requires a light well drained neutral to alkaline soil (Verma *et al.*, 2010).

**Synergistic activity**

Synergism was observed between *P. graveolens* essential oil and ciprofloxacin against uropathogens (Malik *et al.*, 2011). Essential oil of *Pelargonium graveolens* was found to reduce the effective dose of norfloxacin against *E.coli, B. cereus, B. subtilis & S.aereus* in combination (Charde *et al.*, 2014).
2.6.14: *Centella asiatica*

![Centella asiatica leaves](image)

- **Scientific name:** *Centella asiatica*
- **Common name:** Centella
- **Family:** Umbelliferae
- **Parts used:** Leaves

**History**

*Centella asiatica* is used in Ayurvedic medicine for the treatment of anxiety (Del Guercio & Piovella, 1995).

**Herbal Properties and Uses**

Today *Centella asiatica* leaves are used in various pharmaceutical and cosmetic products for the treatment of venous and skin disorders (Kartnig, 1998).

**Cultivation & Growing Methods**

*Centella asiatica* is a slender plant, rooting at the nodes, growing in damp areas in different tropical countries. It is native to India, China, Indonesia, Australia, the South Pacific, Madagascar, and southern and middle Africa (Del Guercio & Piovella, 1995).

**Synergistic activity**

Synergistic activity was observed between *Centella asiatica* extract with nalidixic acid, chloramphenicol & tetracycline against AcrAB-ToIC efflux pump containing strains of *S. enterica* serovar Typhimurium (Stavri *et al.*, 2007).
2.6.15: *Citrus aurantium*

![Image of Citrus aurantium](image)

Scientific name: *Citrus aurantium*
Common name: Bitter orange
Family: Rutaceae
Parts used: Leaves

**History**

The fruit of *Citrus aurantium* has been used as a medicinal herb in China for many years. It is known as “bitter orange” (Stohs et al., 2011).

**Herbal Properties and Uses**

*Citrus aurantium* has many uses such as a food product, fragrance, home remedies and of course, weight loss. It is topically used to treat inflammation of the eyes and as an ointment for skin diseases. Its juice is used as mouthwash for thrush and as a diuretic (Stohs et al., 2011).

**Cultivation & Growing Methods**

*Citrus aurantium* is cultivated between 40°N and 40°S, up to 1800 m altitude in the tropics and up to 750 m altitude in the subtropics. The optimum mean daily temperature for growth is 23 to 30°C. Growth is markedly decreased above 38°C and below 13°C. Active root growth occurs when soil temperatures are high than 12° C (Stohs et al., 2011).

**Synergistic activity**

*Citrus aurantium* extract showed synergistic activity against strains of *S. enterica* serovar Typhimurium over expressing AcrAB-TolC efflux pump (Stavri et al., 2007).
2.6.16: *Glycyrrhiza glabra*

Scientific name: *Glycyrrhiza glabra*
Common name: Licorice
Family: Leguminaceae
Parts used: wood

**History**

The genus *Glycyrrhiza* includes about 20 species. *Glycyrrhiza glabra* has an effect on the adrenals, helping to stimulate glucocorticoid production (Somjen *et al.*, 2004).

**Herbal Properties and Uses**

*Glycyrrhiza glabra* is used as medicine in Ayurveda for rejuvenation. Licorice is used to treat a wide array of illnesses including lowering cholesterol levels, healing respiratory tract disorders and boosting immunity levels. It also lowers stomach acid levels, relieves heartburn and indigestion and prevents ulcer formation. It can be used to fight dermatitis, eczema and psoriasis (Somjen *et al.*, 2004).

**Cultivation & Growing Methods**

Licorice is a legume that is native to southern Europe, India and parts of Asia. Licorice is an herbaceous perennial, growing to 1 m in height. It grows best in well-drained soils in deep valleys with full sun (Somjen *et al.*, 2004).

**Synergistic activity**

Synergistic activity was observed between *Centella asiatica* extract with nalidixic acid, chloramphenicol & tetracycline against AcrAB-TolC efflux pump containing strains of *S. enterica* serovar Typhimurium (Stavri *et al.*, 2007).
2.6.17: *Rosemarinus officinalis*

Scientific name: *Rosemarinus officinalis*
Common name: Rosemary
Family: Lamiaceae
Parts used: Leaves

**History**

From the early time, Rosemary is used to enhance the memory (Aruoma et al., 1996).

**Herbal Properties and Uses**

It is used as a tonic, astringent, diaphoretic and stimulant. Oil of Rosemary is useful in curing many cases of headache (Aruoma et al., 1996).

**Cultivation & Growing Methods**

*Rosemarinus officinalis* is a woody, perennial herb with fragrant evergreen leaves. It is grown as an ornamental plant. It grows on friable loam soil with good drainage in an open, sunny position (Aruoma et al., 1996).

**Synergistic activity**

As reported by Toroglu, 2011, essential oils of *Rosemarinus officinalis* have shown synergism with standard antibiotics like Gentamicin, Erythromycin, Norfloxacin and Amoxicillin/clavulanic.
2.6.18: *Myristica fragrans*

Scientific name: *Myristica fragrans*
Common name: Nutmeg
Family: Myristicaceae
Parts used: seeds

**History**
Nutmeg is used as a traditional medicine in the Middle East & Asia. It is used as an ant diarrhoeal drug (Packiyasothy et al., 1991).

**Herbal Properties and Uses**
Nutmeg is used to stimulate appetites, as an emmenagogue and abortifacient (Packiyasothy et al., 1991).

**Cultivation & Growing Methods**
Nutmeg is cultivated in many parts of Asia and in India. It is a perennial evergreen tree. The trees are slow growing. It requires well drained loam and sandy clay soil (Packiyasothy et al., 1991).

**Synergistic activity**
*Myristica fragrans* has shown synergism with different antibiotics against *A. baumannii* (Kongcharoensuntorn et al., 2007).
2.6.19: *Brassica nigra*

Scientific name: *Brassica nigra*
Common name: Mustard
Family: Brassicaceae
Parts used: seeds

**History**

*Brassica nigra* is a native to the Mediterranean region. It is originated in the Middle East (Duke, 1979).

**Herbal Properties and Uses**

Mustard is used as an appetizer, digestive, diuretic, emetic, irritant and stimulant. It is useful for pulmonary congestion, arthritis and rheumatism and also used as diuretic, emetic, rubefacient and stimulant. Its seed flour is used as an antiseptic (Anand *et al.*, 2007).

**Cultivation & Growing Methods**

Land should be prepared to fine tilth. Mustard is an annual plant, growing up to 2 metres in height. In it blooming occurs during the summer and lasts about 1-2 months (Duke, 1979).

**Synergistic activity**

Silver nanoparticles synthesized from *Brassica nigra* have shown synergism with vancomycin against all bacteria (Pandit, 2014).
2.6.20: *Trigonella foenumgraceum*

Scientific name: *Trigonella foenumgraceum*
Common name: Fenugreek
Family: Leguminaceae
Parts used: seeds

**History**
In the early literature this plant is considered as a hypoglycaemic and anti-inflammatory (Ekta & Gupta, 2014).

**Herbal Properties and Uses**
This plant is used for digestive problems such as loss of appetite, upset stomach, constipation and inflammation of the stomach. It is used for kidney ailments, a vitamin deficiency disease called beriberi, boils, tuberculosis, and cancer & in lowering blood sugar in people with diabetes. Seeds of fenugreek are useful in piles (Vats *et al.*, 2002).

**Cultivation & Growing Methods**
Fenugreek is a legume crop. It is a slender annual herb. It is native to India and Southern Europe (Ekta & Gupta, 2014).

**Synergistic activity**
As stated in shodhganga, synergistic interactions were shown by the combination of *Trigonella foenumgraceum* with antibiotics against different bacteria.
2.6.21: *Sesamum indicum*

**Scientific name:** *Sesamum indicum*  
**Common name:** Sesame  
**Family:** Pedaliaceae  
**Parts used:** seeds

**History**  
Sesame was one of the first crops processed for oil. Largest commercial producers of sesame include India, China & Mexico (Carvalho *et al.*, 2001).

**Herbal Properties and Use**  
It is anti-cancerous and possesses laxative properties. It is very efficient in lowering the cholesterol levels. Sesame oil is used as emollient & demulcent. Seeds and fresh leaves of sesame are also used as poultice (Carvalho *et al.*, 2001).

**Cultivation & Growing Methods**  
This is an annual plant growing up to 100cm height. It is cultivated mainly for its seeds. Sesame is a member of Pedaliaceae family & contains white bell-shaped flowers (Kumar *et al.*, 2010).

**Synergistic activity**  
The effect of linoleic acid-gentamicin combination was found synergistic against *S. aureus* (Sirot *et al.*, 1983).
2.6.22: *Cuminum cyminum*

Scientific name: *Cuminum cyminum*
Common name: Cumin
Family: Apiaceae
Parts used: seeds

History

*Cuminum cyminum* belongs to family Apiaceae & is one of the earliest cultivated herbs in Asia, Africa & Europe (Deepak, 2013).

Herbal Properties and Uses

Cumin seeds are prominently used as carminative, eupeptic, antispasmodic and astringent. It is also used in the treatment of mild digestive disorders, diarrhoea & morning sickness. It is also useful in gastrointestinal, gynaecological & respiratory disorders (Battaib *et al*., 2010).

Cultivation & Growing Methods

Cumin is an annual and delicate plant and is a member of Apiaceae family. Seeds of cumin are used for induction of appetite, increase of milk in cattle industries. Cumin is also used for inflammation and oedema of paunch (Sardooyi *et al*., 2011).

Synergistic activity

As reported by Toroglu, 2011; combination of *Cuminum cyminum* essential oil & cephalothin antibiotics showed a synergistic effect on *S. aureus, P. phycocyanus & A. hydrophila*. In combined application of *C. cyminum* essential oil and Gentamicin antibiotics discs synergistic effect was seen in many microorganisms. When a combination of *C. cyminum* essential oil and Erythromycin antibiotics discs were applied, a synergistic effect was observed in *E. coli, Micrococcus luteus, Lactobacillus acidophilus* and *Candida albicans* (Shaaban *et al*., 2013).
2.6.23: *Rhytididelphus squarrosus*

Scientific name: *Rhytididelphus squarrosus*
Common name: Springy Turf-moss
Family: Hylocomiaceae
Parts used: Whole aerial vegetative part

**History**

*Rhytididelphus squarrosus* is a species of moss found in America (Vizma *et al*., 2012).

**Herbal Properties and Uses**

Bio-sorbent prepared from moss *Rhytididelphus squarrosus* biomass was used for biosorption of cationic dyes was reported by Remenarova *et al.*, (2009). *Rhytididelphus squarrosus* was found to have antibacterial property of against *S. aureus* (Vizma *et al*., 2012).

**Cultivation & Growing Methods**

*Rhytididelphus squarrosus* is a moss with main stems often growing erect. Plants are generally yellow-green. *Rhytididelphus squarrosus* is found in open and shady areas. *Rhytididelphus squarrosus* often grows in areas where moisture persists such as lawns and along streams (Vizma *et al*., 2012).

**Synergistic activity**

Synergism was observed between Arachidonic acid a compound isolated from *Rhytididelphus squarrosus* with different antibiotics against MDR *P. aeruginosa* (Bourboulis *et al*., 2000).
2.6.24: Murraya koenigii

Scientific name: *Murraya koenigii*
Common name: Curry tree
Family: Rutaceae
Parts used: Leaves

**History**

*M. koenigii* is used in Indian cookery for centuries and have a significance role to play in traditional medicine. The plant has tonic and stomachic properties (Jain *et al.*, 2012).

**Herbal Properties and Uses**

The leaves, the bark and the roots of *Murraya koenigii* can be used as a tonic and a stomachic. The bark and the roots are used as a stimulant. They are also used to cure eruptions and the bites of poisonous animals. The green leaves are eaten raw for curing dysentery, and the infusion of the washed leaves is used to stop vomiting (Parmar & Kaushal, 1982).

**Cultivation & Growing Methods**

A small shrub, about 2.5 metres in height; the main stem is dark green to brownish, the width of the main stem is 16 cm. *Murraya koenigii* is commonly found in the outer Himalayas, from the Ravi eastwards, in Assam, Chittagong, Upper and Lower Burma. The shrub is of common occurrence in Himachal Pradesh. Every part of this plant has a strong characteristic odour. The people of southern India use the leaves of this plant as a spice in different curry preparations (Parmar & Kaushal, 1982).

**Synergistic activity**

Synergism was observed between aqueous extract of leaves of the *Murraya koenigii* and Norfloxacin against *Klebsiella pneumoniae* (Deshwal *et al.*, 2014).
2.6.25: *Trachyspermum ammi*

Scientific name: *Trachyspermum ammi*
Common name: Carom seeds
Family: Apiaceae
Parts used: Seeds

**History**

*Trachyspermum ammi* is traditional potential herb and is widely used for curing various diseases (Jeet *et al.*, 2012).

**Herbal Properties and Uses**

The roots of *Trachyspermum ammi* are diuretic in nature and the seeds possess excellent aphrodisiac properties. Its seed oil is used in case of gastrointestinal ailments, lack of appetite and bronchial problems. The oil exhibits antimicrobial & fungicidal effects (Jeet *et al.*, 2012).

**Cultivation & Growing Methods**

*Trachyspermum ammi* is native to Egypt and is cultivated in Iran, Iraq, Pakistan Afghanistan and India. This plant is widely grown in arid and semi arid regions. It grows well in soil containing salt (Jeet *et al.*, 2012).

**Synergistic activity**

Synergistic Activity was observed between antibiotics and *Trachyspermum ammi* (Ajwain) extract on MDR *Staphylococcus aureus* (Charde *et al.*, 2014).
2.6.26: *Foeniculum vulgare*

Scientific name: *Foeniculum vulgare*
Common name: Fennel
Family: Apiaceae
Parts used: Seeds

**History**

*Foeniculum vulgare* fruits have been used as traditional herbal medicine in Europe and China (Rahimi & Ardekani, 2013).

**Herbal Properties and Uses**

Fennel is a commonly used household remedy, being useful in the treatment of disorders of digestive system. The seeds are most active medicinally and are the part normally used. An essential oil is often extracted from the seeds for medicinal use. The plant is analgesic, anti-inflammatory, antispasmodic, aromatic, carminative and diuretic (Rahimi & Ardekani, 2013).

**Cultivation & Growing Methods**

Fennel is an evergreen Perennial tree growing to 1.5 m. It grows in sandy, loamy and clay soils and prefers well-drained soil (Rahimi & Ardekani, 2013).

**Synergistic activity**

Synergism was observed between extract of Fennel and standard antibiotics against MDR *Pseudomonas*.
2.6.27: *Nigella sativa*

Scientific name: *Nigella sativa*  
Common name: Black cumin seeds  
Family: Ranunculaceae  
Parts used: Seeds

**History**

This plant is originated in Western Asia. It is grown in India, particularly in Punjab and Bengal (Toma *et al*., 2005).

**Herbal Properties and Uses**

Nigellone, oil obtained from *Nigella sativa* offers both anti-spasmodic and bronchodilating properties which contributes to its potency against respiratory ailments. This plant acts as an antihistamine which helps to reduce the negative symptoms of allergy sufferers. Thymoquinone another oil obtained form *Nigella sativa* contains excellent anti-inflammatory and analgesic properties. It is also a strong anti-oxidant (Toma *et al*., 2005).

**Cultivation & Growing Methods**

This is an annual herb of height 12-18 inches. Its sowing is done in spring through summer and in autumn (Toma *et al*., 2005).

**Synergistic activity**

Combination of compounds isolated from oil of *Nigella sativa* with antibiotics (Ampicillin, Cephalexin, Chloramphenicol, Tetracycline, Gentamicin, and Ciprofloxacin) exerted synergism in *S. aureus* (Halawani, 2009).
2.6.28: *Curcuma longa*

Scientific name: *Curcuma longa*
Common name: Turmeric
Family: Zingiberaceae
Parts used: Roots

**History**

Turmeric plant has a long use in the Chinese and Ayurvedic systems of medicine (Dorai *et al.*, 2001).

**Herbal Properties and Uses**

Curcumin is the main component of turmeric. Curcumin has antioxidant, antiviral, antifungal and anti-inflammatory properties. It is useful in reducing postsurgical inflammation. Curcumin also inhibits the growth of *Helicobacter pylori* which is the main cause of gastric ulcers (Akram *et al.*, 2010).

**Cultivation & Growing Methods**

Turmeric is a rhizomatous perennial herb of the Zingiberaceae family. It is native to southeast India and needs a temperature range of 20°C and 30°C (Dorai *et al.*, 2001).

**Synergistic activity**

*Curcuma longa* has shown synergistic activity with isoniazid against *Staphylococcus epidermidis* & *Mycobacterium* spp. (Rana *et al.*, 2014).
2.6.29: Zingiber officinale

Scientific name: *Zingiber officinale*
Common name: Ginger
Family: Zingiberaceae
Parts used: Rhizome

History
Ginger is an important herb described in Ayurveda. Ginger is used as an appetizer and is also beneficial for heart (Malhotra & Singh, 2003).

Herbal Properties and Uses
Ginger shows cholesterol lowering activity. It also shows antiviral activity against *Rhinoviruses*. It also shows anti-inflammatory and anti platelet aggregation activities. Ginger can be used against ulcer. It has been reported by Mustafa and Srivastava in 1990 that ginger may exert prophylactic effects in migraine headache without side effects (Malhotra & Singh, 2003).

Cultivation & Growing Methods
Ginger is a perennial rhizome, which spreads underground. The plant is native to Southeast Asia and is also cultivated in India (Malhotra & Singh, 2003).

Synergistic activity
*Zingiber officinale* (ginger) extracts have tetracycline-resistance modifying effects against clinical extensively drug-resistant *Acinetobacter baumannii* (Chen et al., 2010).
2.6.30: Terminalia chebula

Scientific name: Terminalia chebula
Common name: Black Myrobalan
Family: Combretaceae
Parts used: Seeds

History

Terminalia chebula is an important medicinal plant used in Ayurveda, in traditional Chinese medicine and also in Tibetan medicine. In Tibetan medicine, it is called as the King of medicines. Almost all Tibetan herbal formulas include T. chebula. From the ancient time, this plant is used in the treatment of asthma, sore throat, vomiting, hiccup, diarrhea, dysentery, bleeding piles, ulcers, gout, heart and bladder diseases (Rathinamoorthy & Thilagavathi, 2014).

Herbal Properties and Uses

Terminalia chebula is used to cure several ailments such as fever, cough, diarrhoea, gastroenteritis, skin diseases and candidiasis. It contains tannins, which has astringent and antidiarrheal effects. Its 100% decoction works against a variety of bacteria including Shigella, P. aeruginosa, Diphtheria bacilli, S. aureus, E. coli, Pneumococcus, hemolytic Streptococcus, Proteus, and S. Typhimurium (Rathinamoorthy & Thilagavathi, 2014).

Synergistic activity

As stated in shodhganga, synergistic interactions were shown by the combination of Terminalia chebula with antibiotics against different bacteria.

1. Antibacterial properties
Chapter 2

Review of Literature

*T. chebula* exhibits antibacterial activity against a number of Gram-positive and Gram-negative bacteria (Bag *et al*., 2013). Its activity has been demonstrated against *S. aureus, S. typhi, C. perfringens* and *E. coli* (Prakash *et al*., 2012). *T. chebula* is effective in inhibiting the urease activity of *Helicobacter pylori*, a ubiquitous bacterium implicated in the development of gastritis, ulcers and stomach cancer (Bag *et al*., 2013).

2. **Antifungal properties**

Aqueous extract of *T. chebula* shows antifungal activity against a number of dermatophytes and yeasts (Dutta *et al*., 1998; Barazani *et al*., 2003). It is effective against the pathogenic yeasts like *Candida albicans* and dermatophytes *Epidermophyton, Floccosum, Microsporum gypseum* and *Trichophyton rubrum* (Barazani *et al*., 2003). Its inhibitory effect has also proved on three dermatophytes (*Trichophyton* spp.) and three yeasts (*Candida* spp.) have also been documented (Mehmood *et al*., 1999). Its aqueous extract of galls showed inhibitory effects on three dermatophytes (*Trichophyton* spp.) and three yeasts (*Candida* spp.) (Barazani *et al*., 2003). *In vitro* anticandidal activity of methanol extract of *T. chebula* was observed against *Candida albicans* (Bonjar, 2004). Its seed extract exhibited antifungal activity against *Trichophyton glabrata* (Barazani *et al*., 2003).

3. **Immunomodulatory activity**

Aqueous extract of *T. chebula* produces an increase in humoral antibody titer and delayed type hypersensitivity. Crude extract of *T. chebula* can stimulate cell-mediated immune response (Bag *et al*., 2013).

4. **Wound healing**

Ethanolic extract of *T. chebula* shows significance response in wound healing. Tannin extracts from dried immature fruits can also promote wound healing (Rathinamoorthy & Thilagavathi, 2014).

5. **Antioxidant activity**

*T. chebula* has excellent anti-oxidant activity. Its leaves, bark and fruit possesses high antioxidant activity and phenolics were found to be responsible for this activity (Bag *et al*., 2013). The aqueous extract of *T. chebula* has protected the antioxidant enzymes from reactive oxygen species (ROS) produced by gamma radiation in the rat liver microsomes and mitochondria (Prakash *et al*., 2012). Its
methanolic extract was also found to inhibit lipid peroxide formation and to scavenge hydroxyl and superoxide radicals in vitro (Bag et al., 2013).

6. **Antidiabetic and Retinoprotective Activities**

According to the study done by Murali et al., in 2007, aqueous extract of *T. chebula* reduces the elevated blood glucose and increase in glycosylated haemoglobin. The in vitro studies showed that the insulin release was nearly two times more than that in untreated diabetic animals.

7. **Anti-arthritic activity**

The hydro alcoholic extract of *T. chebula* inhibited the joint swelling as compared to control in both formaldehyde-induced and CFA-induced arthritis. *T. chebula* treatment has also reduced serum TNF-α level and synovial expression of TNF-R1, IL-6 and IL-1β. *T. chebula* could be used as a disease-modifying agent in treatment of rheumatoid arthritis (Nair et al., 2010).

8. **Antidepressant activity of *T. chebula***

Depression is a common symptom of the present day world. The water extract of *T. chebula* contains total phenolic & tannin content. The water extract has good antioxidant activity & tannins have neuroprotective functions. Hence, *T. chebula* can be used as an adjuvant in the treatment of depression (Manohar et al., 2012).

9. **Anticancer activity of *T. chebula***

Cancer is a class of diseases in which a group of cells display uncontrolled growth. According to the study done by Ahuja et al., 2013, *T. chebula* might be a great choice for the treatment of cancer & its ethanolic extract can be used as a potential agent of anticancer chemotherapy. Another study had reported the inhibitory action on cancer cell growth by the phenolics of *T. chebula* Retz fruit and found that chebulinic acid, tannic acid and Ellagic acid were the most growth inhibitory phenolics of *T. chebula* (Bag et al., 2013).

10. **Triphala**

'Triphala' is a well-known powdered preparation in the Indian system of medicine. Triphala consists of equal parts of the *E. officinalis*, *T. chebula*, and *T. belerica*. Currently, Triphala is being extensively used for its various therapeutic effects including its anti-caries, antioxidant, anti-collagenase, and anti-microbial activities. Triphala has several applications in dentistry. Triphala is useful for gastrointestinal system and also to the whole body. It also acts as a
detoxifier and anti-inflammatory (Prakash et al., 2014). Triphala possess free radical scavenging, antioxidant, antiinflammatory, antipyretic, analgesic, antibacterial, antimutagenic, wound healing, anticariogenic, antistress, adaptogenic, hypoglycaemic, anticancer, chemoprotective, radioprotective and chemopreventive effects. Clinical studies have also shown that Triphala possesses good laxative property, to improve appetite and reduce gastric hyperacidity. Studies have also shown that Triphala is equally effective to that of chlorhexidine in preventing dental caries (Baliga et al., 2012).

Cultivation & Growing Methods

*T. chebula* (Harad) is a medicinal plant widely distributed throughout India, Burma and Srilanka. *T. chebula* is a medium- to large-sized tree distributed throughout tropical and subtropical areas (Rathinamoorthy & Thilagavathi, 2014). In Himachal Pradesh, Harad is found up to 1100 m elevation in Sirmour, Bilaspur, Hamirpur Kangra, Mandi and Una districts. Harad can grow in different environmental conditions. Soil supporting Harad vary widely in depth and composition. The mean maximum temperature in for it varies from 37°C to 48°C, absolute minimum temperature from 1°C to 15°C and annual rainfall varies from 750 to 3250 mm (Sharma et al., 2012).

2.6.31: *Mentha piperita*

Scientific name: *Mentha piperita*

Common name: Peppermint

Family: Lamiaceae

Parts used: Leaves

History

*Mentha piperita* is the oldest known medicinal herb in Eastern & Western traditions. It is used for folk medicine (Rita & Animesh, 2011).

Herbal Properties and Use
It is useful in the treatment of common cold. It is used to treat headaches. It can be used as an astringent, antiseptic, antipruritic, antispasmodic, antiemetic, carminative, diaphoretic, mild bitter, analgesic, antihemorrhagic, antimicrobial, rubefacient, stimulant, and emmenagogue (Rita & Animesh, 2011).

**Cultivation & Growing Methods**

Peppermint generally grows in moist, shaded locations and grows best with a good supply of water. *Mentha piperita* is a perennial herb, propagating through stolons & yield peppermint oil. The plant is native to Europe & is cultivated throughout the world (Rita & Animesh, 2011).

**Synergistic activity**

As reported by Toroglu, 2011; essential oils of *Mentha piperita* have shown synergism with standard antibiotics against different bacteria.