3. REVIEW OF LITERATURE

Despite being a curable infectious disease, tuberculosis still remains as the second highest killer of mankind next to Acquired immunodeficiency Syndrome (AIDS) (Sharma and Mohan, 2013). Efforts laid to curtail this disease were robust detection methodologies, point of care tests and treatment using effective antituberculosis drugs.

More than sixty years ago, on November 20, 1944, achievement came in when the first anti-TB drug streptomycin was administered against TB patients. The effect was almost immediately impressive, as disease was noticeably detained; the bacteria disappeared from the sputum leading to a rapid recovery. But within a few months resistant mutants appeared, endangering the success of chemotherapy of tuberculosis.

Following streptomycin, para aminosalicylic acid, (1949), isoniazid (1952), pyrazinamide (1954), cycloserine (1955), ethambutol (1962), and rifampicin (1963) were introduced as anti-TB agents. To overcome the problem of STR resistance problem, combination therapy using two or three drugs was introduced. After the introduction of DOTS by WHO which includes the first line drugs namely INH, EMB, RIF and PZA for a span of six to eight months depending upon the patient’s smear status, improvement in the cure rate has increased. Enhanced case detection was achieved with the implementation of rapid testing methodologies in the national TB control programs. However, since 1980s, the number of TB cases throughout the world has been increasing rapidly due to the emergence of multi-drug resistant M. tuberculosis (MDR-TB) (Chan, 2002).
WHO defines MDR-TB as the strains of *M. tuberculosis* exhibiting resistance to isoniazid and rifampicin with or without resistance to other first line drugs. These forms of the disease are more often fatal and are difficult and expensive to treat requiring hospitalization and continued care (Basso, 1998). The situation turned more complicated with association of HIV especially in sub-Saharan Africa and in many developing countries (Corbett *et al.*, 2003). WHO has formulated treatment guidelines for the management of MDR-TB patients where the treatment includes the usage of less effective broad spectrum drugs that are bacteriostatic or bactericidal. These second line drugs act on the cell wall, DNA and protein synthesis. Adverse reactions to these drugs are challenging in treatment monitoring (PMDT Guidelines, 2012).

### 3.1. Vaccine for tuberculosis

In the year 1920, the French bacteriologist Calmette together with Guerin, by using a specific culture media to reduce the virulence of the bovine TB bacterium, creating the basis for the *Mycobacterium bovis* Bacille Calmette-Guérin, the BCG vaccine. It is the only vaccine introduced against *M. tuberculosis*. Over the past 50 years, the BCG vaccine against tuberculosis had been the world’s most widely used vaccine, despite showing highly variable efficacy (0-80%) in different trials. The efficacy of BCG in adults is particularly poor in tropical and subtropical regions. Studies in animal models of tuberculosis, supported by data from clinical BCG trials in humans, indicate that this failure is connected to pre-existing immune responses to antigens that are common to environmental mycobacteria and *M. tuberculosis* (Andersen, 2005). Even though relatively ineffective, BCG vaccine is still widely used for infants in developing countries.
3.2. Drugs and mode of action

First Line Drugs

3.2.1. Rifampicin

Rifampicin is highly active against *M. tuberculosis*. This lipophilic drug penetrates cell membranes and kills intracellular actively replicating bacteria (Nau *et al.*, 1992). Mechanism of action is exerted by inhibition of DNA-dependent RNA polymerase in bacterial cells by binding to its β-subunit, thus preventing transcription of RNA and subsequently preventing translation of proteins (Tomioka *et al.*, 2006; Aristoff *et al.*, 2010) Rate of mutation development is $10^{-8}$ which is the highest for any anti-tuberculosis drug currently available. Thus, with the least likelihood for developing resistance, it is an ideal choice in TB treatment. Rifampicin is also considered as surrogate marker for MDR-TB. It is also observed that RIF interacts with anti-retroviral drugs there by posing a hindrance in treating HIV-TB population. Presence of cross resistance within the rifampicin structural group like rifapentine, rifamycin is observed. Rise in the intermediary resistance isolates for RIF has raised an alarm among the researchers.

Most of the *M. tuberculosis* clinical isolates resistant to rifampicin show mutations in the rpoB gene that encodes the β-subunit of RNA polymerase. These mutations cause conformational changes in the polymerase that result in a low affinity for the drug rendering it ineffective (Telenti *et al.*, 1993).

The adverse or side effects include hepatitis with elevation of bile and bilirubin, anaemia, leukopenia, thrombocytopenia, bleeding, fever, eosinophilia, purpura, haemolysis and nephrotoxicity (www.inchem.org).
3.2.2. Isoniazid

Isoniazid (INH) was discovered in 1952. It is bacteriostatic to slow-growing or non-dividing mycobacteria like *M. tuberculosis*. Isoniazid is actually a prodrug and is activated by the mycobacterial enzyme catalase-peroxidase (KatG), which catalyses the formation of the isonicotinic acyl-NADH complex. Subsequently, this complex binds to the enoyl-acyl carrier protein reductase InhA, and then blocks the natural substrate enoyl-AcpM and fatty acid synthase. This results in inhibition of mycolic acidsynthesis which is an essential component in the formation of the mycobacterial cell wall (Timmins, 2006; Suarez et al., 2009).

Resistance to isoniazid occurs due to mutations in several genes, including *katG*, *ahpC*, *inhA*, *kasA* and *ndh*. Point mutations in *katG* are more commonly observed than other types of mutations, and a single point mutation resulting in substitution of threonine for serine at residue 315 (S315T) accounts for the majority of INH resistance among clinical isolates (Abate and Hoffner, 2001). INH is metabolized in the liver and its metabolites are excreted in the urine (Ellard, 1976).

INH affects the liver, hematologic- and peripheral nervous systems resulting in acute hepatitis, peripheral neuropathy and haemolytic anaemia (www.inchem.org).

3.2.3. Pyrazinamide

Pyrazinamide (PZA) was discovered in 1952. PZA is a structural analogue of nicotinamide and kills semi-dormant tubercle bacilli under acidic
The bacterial enzymes convert PZA to pyrazinoic acid, the active derivative. This drug disrupts the membrane function and energy metabolism and possibly also inhibits fatty acid synthesis (Mitchison, 2012).

Mutations in the \textit{pncA} gene are responsible for resistance to this drug (Scorpio, 1996). Pyrazinamide is metabolized by the liver and the metabolic products are excreted by the kidneys (Lacroix \textit{et al.}, 1989).

Some common side effects of PZA include skin rash, nausea, vomiting, hepatotoxicity, anorexia, hyperuricemia, sideroblastic anemia, dysuria, joint pains (arthralgia), urticaria, pruritus, malaise, interstitial nephritis, porphyria and fever (Forget, 2006).

### 3.2.4. Ethambutol

Ethambutol was discovered in 1961. It interferes with the biosynthesis of cell wall arabinogalactan by inhibiting the enzyme arabinosyltransferase. Arabinogalactan is an essential component in the formation of the mycolyl-arabinogalactan-peptidoglycan complex of the \textit{M. tuberculosis} cell wall (Belanger \textit{et al.}, 1996). It enhances the effect of other combination of drugs including aminoglycosides, rifampicin and quinolones.

Mutation in gene \textit{embB} is responsible for resistance to ethambutol (Telenti \textit{et al.}, 1997). Ethambutol is well absorbed in the gastrointestinal tract, and is efficiently distributed in body tissues and fluids. Adverse effects of EMB include peripheral neuropathy, blurred vision, nausea, vomiting, and itching (Lim, 2006; RNTCP-PMDT guidelines, 2012)
3.2.5. Streptomycin

Streptomycin was the first drug introduced in the treatment of TB, in 1948. SM kills actively growing tubercle bacilli, but it is inactive against non-growing or intracellular bacilli (Mitchison, 1985). The drug binds to the 16S rRNA, interferes with translation proof reading, and thereby inhibits protein synthesis (Gale et al., 1981). The adverse effect includes vestibular dysfunction, ototoxicity and nephrotoxicity.

3.2.6. Second Line Drugs

The fluoroquinolones (moxifloxacin, gatifloxacin, sparfloxacin, levofloxacin, ofloxacin, and ciprofloxacin), are currently used as second-line drugs in TB treatment and are particularly used in the treatment of MDR-TB.

Mode of action of fluoroquinolones is by trapping gyrase and topoisomerase IV on DNA as ternary complexes, thereby blocking the movement of replication forks and transcription complexes (Drlica, 2003). Resistance to fluoroquinolones in M. tuberculosis is associated with mutations in the conserved quinolone resistance-determining region (QRDR) of gyrA and gyrB involved in the interaction between the drug and DNA gyrase (Ginsburg et al., 2003). However, fluoroquinolones such as ofloxacin, norfloxacin can be used which are safer than other second-line drugs but have the disadvantage of expenses and its side effect includes gastrointestinal intolerance, rashes, dizziness and head ache (Tripathi et al., 2005; RNTCP-PMDT Guidelines, 2012).
3.2.7. Injectable Drugs

Next to the fluoroquinolones, the injectable aminoglycosides and capreomycin are effective second line drugs for treating MDR-TB. The mode of action of the aminoglycosides against mycobacterial species is through their binding to the 30S ribosomal subunit, which affects polypeptide synthesis, resulting in inhibition of translation.

Capreomycin is a macrocyclic polypeptide. It is like streptomycin and kanamycin and inhibits protein synthesis through modification of ribosomal structures at the 16S rRNA (Wade and Zhang, 2004). Recent studies using site-directed mutagenesis have identified the binding site of Capreomycin on 16S rRNA helix 44 (Akbergenov et al., 2011). In M. tuberculosis, resistance to Capreomycin and Kanamycin has been associated with mutations in the rrs gene encoding 16S rRNA (Taniguchi et al., 1997; Alangaden et al., 1998).

3.3. Need for New TB drugs

The challenge faced with the current chemotherapy is that the treatment duration is prolonged. The combination of drugs is used for six to 24 months depending upon the drug resistance allowing opportunities for interruptions in drug intake resulting in incompliance creating a serious burden for both patients and clinics. The present anti-TB drugs produce lots of side-effects (Yee et al., 2003). TB infection in immune-compromised population leads to extra-pulmonary TB affecting other parts of the body, such as the pleura, meninges, the lymphatic system, the genitourinary system, and the bones (Rivers and Mancera, 2008).
In the case of patients suffering from HIV and TB, chemotherapy may reduce significance of the concentrations of anti-retroviral drugs which may lead to treatment failure or resistance.

Moreover, there have been no new class of anti-TB drugs introduced in past 30 years. So there is an urgent need of new drug that should either shorten the time of treatment, be potent, inexpensive, and safe to tackle resistant strains and to provide effective treatment for non-replicative latent forms not interfering with the antiretroviral therapy at the same time.

3.4. Natural products as a source of drugs

For thousands of years, natural products have played an important role throughout the world in treating and preventing human diseases. Natural product medicines have come from various source materials including terrestrial plants, terrestrial microorganisms, marine organisms, and terrestrial Vertebrates and invertebrates. More than 80% of drug substances were natural products or those inspired by a natural compound (Harvey, 2008).

Over 50 years, humans are benefited from the astonishing property of renowned drugs such as aspirin, morphine, tropine, penicillins, cephalosporins, tetracyclines, aminoglycosides, chloramphenicol, and macrolides which are inspired from natural sources.

In the year 2011 it has been reported that 38 natural product-derived drugs were approved for various indications including 15 for infectious diseases, 7 each for oncology, neurological diseases and cardiovascular disorders, 4 for metabolic disorders and 1 for diabetes (Brahmachari, 2011).
The key function of natural products is to interact with a wide variety of proteins and other biological targets, and also acting as modulators of cellular processes (Barker et al., 2012). Natural products are the biggest single source of anti-cancer drugs as evidenced by the historical data (Newman and Cragg, 2007).

Over a 100 natural-product-derived compounds are currently undergoing clinical trials and at least 100 similar projects are in preclinical development (Table 1). The lead structures for most of the above compounds are derived from plants and microbial sources (Butler, 2008).

**Table 1.** Drug based on Natural Products at different stages of development*

<table>
<thead>
<tr>
<th>Development state</th>
<th>Plant</th>
<th>Bacterial</th>
<th>Fungal</th>
<th>Animal</th>
<th>Semi-synthetic</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-clinical</td>
<td>46</td>
<td>12</td>
<td>7</td>
<td>7</td>
<td>27</td>
<td>99</td>
</tr>
<tr>
<td>Phase I</td>
<td>14</td>
<td>5</td>
<td>0</td>
<td>3</td>
<td>8</td>
<td>30</td>
</tr>
<tr>
<td>Phase II</td>
<td>41</td>
<td>4</td>
<td>0</td>
<td>10</td>
<td>11</td>
<td>66</td>
</tr>
<tr>
<td>Phase III</td>
<td>5</td>
<td>4</td>
<td>0</td>
<td>4</td>
<td>13</td>
<td>26</td>
</tr>
<tr>
<td>Preregistration</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Total</td>
<td>108</td>
<td>25</td>
<td>7</td>
<td>24</td>
<td>61</td>
<td>225</td>
</tr>
</tbody>
</table>

*Source Pharmaprojects database (March 2008)

**3.5 Indian traditional medicine**

India has a gold mine of well-recorded and traditionally well-practised knowledge of herbal medicine. It is the largest producer of medicinal herbs and representing India as “Botanical garden of the World”. 
India is blessed with three (Indo Burma, Himalaya and Western Ghats) of the 34 worlds hotspots of biodiversity (www.conservation.org) and is 7th among the 16 mega diverse countries where 70% of the world’s species occur collectively. India is rich in flora that includes 5,725 species of Angiosperms, 10 species of Gymnosperms, 193 species of Pteridophytes, 678 Bryophytes, 260 liverworts, 466 lichens, 3500 fungi and 1924 algae are reported (Sanjappa, 2005).

India has the second largest tribal population in the world after Africa (Jagtap et al., 2006). Indian Materia Medica reported about 2000 natural based drugs from different folklore practices (Narayana et al., 1998). WHO has listed 21,000 plant species which are used for medicinal purposes around the world. Among these 2,500 species are in India, out of which 150 species are commercially used.

Even today, tribal and certain local communities in India are collecting and preserving locally available wild and cultivated plant species for their day today use (Mahishi et al., 2005). Tamil Nadu is endowed with rich biodiversity, right from marine coastal systems in the Gulf of Mannar to terrestrial evergreen forests in the Western Ghats. It ranks first among all the states in the country with nearly one third of the total flora of India (www.tnenvis.nic.in).

The Kani and Paliyar tribal people in Western Ghats of Tamil Nadu, have a broad acquaintance of medicinal plants. It includes 33 taluks in eight districts viz., Coimbatore, Erode, Dindigul, Theni, Kanyakumari, Virudhunagar, Madurai and Tirunelveli. They use medicinal plants as remedies to treat cough, cold, fever, headache, poisonous bites and some other simple ailments (Ignacimuthu, 2008; Ayyanar and Ignacimuthu et al., 2011).
3.6. Plant as source of drugs

The first generation of plant drugs were usually simple botanicals employed in more or less crude form. According to the World Health Organization, over 80% of the world’s population relies on such traditional plant-based systems of medicine to provide them with primary healthcare (Calixto, 2005). The value of medicinal plants to human livelihood is essentially infinite. India and China are the leading countries with more than 40% plant species marketed as medicinal plant. The importance of medicinal plants is demonstrated by the fact that even in developed countries, about 35% of prescribed drugs are of natural origin and 50% of drugs are of plant origin (Nyiredy, 2004).

The primary benefits of using plant derived medicines are: they are relatively safer than synthetic derivatives, offering profound therapeutic benefits and more affordable treatment. The chemical constituents present in them are a part of the physiological functions of living flora and hence they are believed to have better compatibility with the human body. It gives safe, effective and inexpensive indigenous remedies gaining popularity among the people of both urban and rural areas (Kamboj, 2000).
3.6.1. Plants used to treat other diseases

A number of medicinal plants have been used traditionally in the treatment of malaria. Active compounds isolated from these plants have been reported for antimalarial activity. Biflavonoids such as bilobetin and heveaflavone from *Selaginella bryopteris* (Kunert, *et al.*, 2008), nimbolide from neem plant (Rochanakij *et al.*, 1985), quinoline, alkaloids isolated from leaves of *Ancistrocladus heyneanus* (Bringmann *et al.*, 2004).

Numerous natural products predominantly alkaloids, phenolics and terpenes have shown a promising activity against anti HIV activity. A phenolic compound Theasinensin D isolated from *Thea sinensis* exhibit a good anti-HIV activity. The common phytosterols, ursolic acid and oleanolic acid found in many plants has shown excellent anti-HIV activity (Singh *et al.*, 2005).

The four major structural classes of plant derived cancer treatments include Vinca alkaloids, Epipodophyllotoxin lignans, Taxane diterpenoids and Camptotectin quinolone alkaloid derivatives. Approximately 30 plant derived anti-cancer compounds have been reported to be clinically active against various types of tumors and are currently used in clinical trials (Nirmala *et al.*, 2011).

3.6.2. Plants with Antimycobacterial activity

Newton reviewed plant-derived antimycobacterial natural products, and described the activity for extracts and compounds from 123 plant species (Newton *et al.*, 2000). A review article by Copp covering natural products
reported that antitubercular activity of 352 compounds isolated from both terrestrial and marine sources between 1990 and 2002 (Copp, 2003).

The literature survey indicated that a limited work has been performed on antimycobacterial herbal drugs in India. The antitubercular activity of six medicinal plants, viz., *Desmodium umbellatum* Benth., *Piper longum*, *Plectronia travancorica*, *Scaevola taccada*, *Syncarpia laurifolia* and *Vaccinium vacciniaceum* were evaluated against *M. tuberculosis* H$\text{$_{37}$}$Rv (Goel et al., 2002). In addition a few reports on the antimycobacterial activity of selected plant species have been published by Indian researchers. Thus, considering the rich biodiversity and traditional ethnomedicinal knowledge in India there is a huge potential to initiate a dedicated antimycobacterial screening programme.

Januario et al. (2002) examined antimycobacterial activity of crude extracts and fractions from aerial parts of *Physalis angulata*. Fraction A1-29-12 containing physalins B, F and D exhibited antimycobacterial activity with a MIC of 32 $\mu$g/ml against *M. tuberculosis* H$\text{$_{37}$}$Rv. Purified physalin B and physalin D were shown to have antimycobacterial activity with an MIC of >128 $\mu$g/ml and 32 $\mu$g/ml respectively. Structural elucidation of both physalins D and B was based on detailed 13C and 1H NMR spectral analysis.

A crude ethanol extract and hexane fraction from *Morinda citrifolia* showed antitubercular activity. The major constituents of the hexane fraction are E-phytol, cycloartenol, stigmasterol, beta-sitosterol, campesta-5, 7, 22-trien-3beta-ol and the ketosteroids stigmasta-4-en-3-one and stigmasta-4-22-dien-3-one. E-Phytol, a mixture of the two ketosteroids, and the epidioxysterol
derived from campesta-5, 7, 22-trien-3beta-ol all showed pronounced antitubercular activity (Saludes et al., 2002).

Twenty-two plant extracts in different solvents were screened for activity against *M. tuberculosis* H$_{37}$Rv and *M. avium* at concentrations from 50 to 200 µg/ml. Hexane extracts from *Artemisia ludoviciana*, *Chamaedora tepejilote*, *Lantana hispida*, *Juniperus communis* and *Malva parviflora*, and methanol extracts from *Artemisia ludoviciana* and *Juniperus communis* inhibited the growth of *M. tuberculosis*. The hexane extract and fractions (FVI) of *L. hispida* inhibited the growth of all the MDR-TB clinical isolates at concentrations up to 25µg/ml (Jimenez-Arellanes et al., 2003).

Twenty plants used in traditional medicine for the treatment of symptoms related to tuberculosis (cough, fever or inflammation) were screened for antimycobacterial activity. In total, 55 extracts were evaluated for inhibitory activity against *M. bovis* BCG strain at a concentration of 100 µg/ml. Methanolic and dichloromethane extracts of *Amborella trichopoda*, *Codiaeum peltatum*, *Myristica fatua* and essential oils *Myoporum crassifolium* showed activity. Methanolic extract of *Amborella trichopoda* fruits presented a significant activity with a minimal inhibitory concentration included between 1 and 2.5 µg/ml (Billo et al., 2005).

Molina-Salinas et al. (2006) screened extracts from aerial parts of *Artemisia ludoviciana*, *Chenopodium ambrosioides*, *Marrubium vulgare*, *Mentha spicata* and *Flourensia cernua* against multidrug-resistant *M. tuberculosis*. These five plant species are used to treat respiratory disorders in Mexico. Hexane and acetone extracts of *Flourensia cernua* was uniquely
active among those evaluated. The hexane and acetone extract showed a MIC of 50 and 25 µg/ml against *M. tuberculosis*.

Gautam *et al.* (2007) reviewed that, 255 species were found to be antimycobacterial in preliminary *in vitro* screening and most of these species are mention in traditional systems of medicine. Of the 255 species, 149 plant species showed a positive correlation with ethno medicinal uses of TB related diseases like pulmonary affections, bronchitis, asthma, cough, whooping cough and infectious diseases of chest.

Louis *et al.* (2009) reported the ethyl acetate extract of bark from *Evodia elleryana* produced significant growth inhibition of *M. tuberculosis* at concentrations only minimally inhibitory to human T cells. The crude extract yielded 95% inhibition of TB at 50 µg/ml.

Mmushi *et al.* (2009) evaluated antimycobacterial activity of fifteen medicinal plants in South Africa. Hexane, dichloromethane (DCM), acetone and methanolic extracts were screened for antymycobacterial activity against *Mycobacterium smegmatis*. The acetone extract of *Milletia stulhimannii* was the most active, showing activity against *Mycobacterium smegmatis* with minimum inhibitory concentration (MIC) value of 0.13 mg/ml. Acetone extracts for all plants had lower MIC values ranging between 0.11-1.25 mg/ml against *M. smegmatis*. *Milletia stulhimannii, Albizia gummiifera, Xanthocercis zambesiaca* and *Barringtonia racemosa* have shown great potential as anti-tuberculosis agents. They were active against *M. smegmatis* with average MIC values of acetone extracts of 0.13 mg/ml.
Meilak and Palombo (2008) screened five Mexico plant species, Artemisia ludoviciana, Chamaedora tepejilote, Lantana hispida, Juniperus communis and Malva parviflora against H_{37}Rv, MDR M. tuberculosis and sensitive strains. Among the five plants the n-hexane extract of L. hispida inhibited the growth of MDR TB bacillus at 25µg/ml.

Gupta et al. (2010) examined antimycobacterial activity of aqueous extracts of leaves of A. indica, A. vasica, bulbs of A. cepa, cloves of A. sativum. A pure gel of A. vera leaves in different concentrations was tested *in vitro* for their activity against two MDR isolates using Lowenstein Jensen (LJ) medium and colorimetric method. Among these A. indica and A. cepa exhibited promising activities.

Suresh et al. (2010) screened Centella asiatica against M. tuberculosis by micro dilution bioassay in LJ media and Versa-Trek rapid culture system. The leaf extract showed significant inhibitory activity against MTB in both the methods. The major bioactive compounds present were octadectrienoic acid and n-Hexadecanoic acid by GC-MS analysis.

Green et al. (2010) evaluated antimycobacterial activity of crude acetone, methanol, hexane and ethanol extracts of 21 selected medicinal plants obtained in Venda, South Africa against M. tuberculosis H_{37}Ra and a clinical strain resistant to first-line drugs and one second-line drug using tetrazolium microplate assay to determine the minimum inhibitory concentration (MIC). Few acetone extracts were active against MTB with MIC under 100 µg/ml. Four plants showed lower MIC values; Berchemia discolor 12, 5 µg/ml on H37Ra and 10.5 µg/ml on the clinical isolate, Bridelia micrantha (25 µg/ml), Warbugia salutaris (25 µg/ml), and Terminalia sericea (25 µg/ml) on both
H$_{37}$Ra and clinical isolate. The acetone extracts of *Berchemia discolor*, *Bridelia micrantha*, *Terminalia sericea* and *Warbugia salutaris* could be important sources of mycobactericidal compounds against multidrug-resistant MTB.

Navarro-Garcia *et al.* (2011) evaluated the dichloromethane extract from *Aristolochia brevipes* (Rhizoma) and the compounds isolated from this extract were tested against several mycobacterial strains, sensitive, resistant (mono resistant), and clinical isolates (multidrug-resistant), using the alamar Blue microassay. The extract was fractionated by column chromatography, yielding the following eight major compounds. The structures of these compounds were elucidated by 1H and 13C (1D and 2D) Nuclear Magnetic Resonance (NMR) spectroscopy. This study demonstrates that the dichloromethane extract (rhizome) of *A. brevipes* possesses strong *in vitro* antimycobacterial activity against *M. tuberculosis* H$_{37}$Rv. The most active compound against all mycobacterial strains tested was the compound aristolactam I with MIC values ranging between 12.5 and 25µg/ml.

Shivakumar *et al.* (2012) screened the antimycobacterial activity of *Barlaria buxifolia* against *M. tuberculosis* H$_{37}$Rv using the micro-plate alamar blue dye assay. This study shows that ethanol extract of the stem and leaves was found to be active at minimum inhibitory concentrations (MIC) of 25 and 50µg/ml.

Patilaya (2012) investigated antimycobacterial activity of *Piper nigrum* L. that has been used to treat asthma, bronchitis and tuberculosis. The antimycobacterial activity of the standardized fractions of *Piper nigrum* leaf was tested against *M. tuberculosis* H$_{37}$Rv strain using tetrazolium microplate assay method to determine the MIC. Exposure of the mycobacterial cells to
the active fraction in liquid medium was also carried out to investigate the effects on the growth of the cells using colony count method and scanning electron microscopy. The ethylacetate, n-hexane, and the water fractions of *Piper nigrum* showed antimycobacterial activity against *M. tuberculosis* with MIC values of 25, 50, and 100 µg/ml. Exposure of the mycobacterial cells to the ethylacetate fraction resulted a significant decrease in the number of colony counts.

Sandoval-Montemayor *et al.* (2012) isolated and characterized the active compounds from the hexane extract of the fruit peels of *Citrus aurantiifolia*, that showed activity against one sensitive and three monoresistant (isoniazid, streptomycin or ethambutol) strains of *M. tuberculosis* H$_{37}$Rv. The active extract was fractionated by column chromatography, yielding the five major compounds. The structures of these compounds were elucidated by 1D and 2D NMR spectroscopy. In addition, GC-MS analysis of the hexane extract allowed the identification of 44 volatile compounds. Four isolated coumarins and 16 commercial compounds identified by GC-MS were tested against *M. tuberculosis* H$_{37}$Rv and three multidrug-resistant *M. tuberculosis* strains using the Microplate Alamar Blue Assay. The antimycobacterial activity of the hexane extract of *C. aurantifolia* could be attributed to these compounds.

**3.7. Bioassays for evaluation of anti-tubercular activity**

Bioassay-directed fractionation is an important process in the identification of active principles in natural product extracts. In a drug discovery programme from natural products, two steps are generally followed, viz., development of novel phyto chemical methods and a suitable bioassay in
order to provide valid guidance with respect to ultimate endpoint that is the antibiotic activity against virulent *M. tuberculosis* in vivo.

A well characterized virulent strain, *M. tuberculosis* H$_{37}$Rv has a drug susceptibility profile which fairly represents the majority of drug susceptible clinical isolates. Several natural products researchers have chosen to work with rapidly growing, a virulent, saprophytic mycobacteria. One such species is *M. smegmatis* (ATCC 607), which has been used extensively. This organism, however, only possess a limited degree of similarity to *M. tuberculosis*.

Another alternative to working with virulent strains of *M. tuberculosis* is to use the slow growing, avirulent strain known as *M. tuberculosis* H$_{37}$Ra (ATCC 25177) or the commonly used vaccine strain, *M. bovis* BCG (ATCC 35743). These organisms are more closely related to *M. tuberculosis* H37Rv than the rapid-growing mycobacteria with respect to both drug susceptibility profile and genetic composition (Gautam *et al.*, 2007).

Screening of plant extracts for antimycobacterial activity is conventionally carried out using mycobacteria cultured in various types of broth and agar based media. *M. tuberculosis* has the disadvantage of being slow growing and so it takes several weeks for the availability of drug sensitivity results. Prolonged incubation may be detrimental to the stability of the compounds. Hence, a rapid test should ideally have a minimal time requirement for growth of the organism. The methodology should be simple to perform and easy to learn.
3.7.1. Agar diffusion

The common disc or well-diffusion assays employed in many antimicrobial assays of natural products are not quantitative when used to evaluate extracts or new compounds, but are merely an indication that there is growth inhibition at some unknown concentration along the concentration gradient. The major disadvantage of using diffusion assays is that the mycobacteria, having a very lipid-rich, hydrophobic cell wall, are often more susceptible to less-polar compounds (Connell and Nikaido, 1994). The main disadvantage with such assays is the requirement for at least 18 days to visibly detect growth of the colonies.

3.7.2. Micro broth dilution

The growth of mycobacteria can be quantitated by turbidity in a liquid medium; the tendency of mycobacteria to clump makes this test difficult. In addition, crude extracts may also impart some turbidity to the medium, making interpretation of results difficult. The use of alamar blue (an oxidation/reduction indicator dye) makes this test a rapid sensitive assay.

Microplate alamar blue assay (MABA) results can be read visually without the use of instrumentation (Franzblau et al., 1998). The reduced form of alamar blue can be quantitated colorimetrically by measuring absorbance at 570 nm or fluorimetrically by exciting at 530 nm and detecting emission at 590 nm (Collins and Franzblau, 1997).

Non-fluorometric readouts can also be performed by using non-proprietary resazurin (Martin et al., 2003) or tetrazolium dyes (Foongladda et
al., 2002). Thus, it is possible to perform high throughput anti-TB assays in micro plates using a micro plate spectrophotometer or micro plate fluorimeter.

3.7.3. Radiorespirometry

The growth or inhibition of mycobacteria can be determined within 1 week by the extent of oxidation of $[^{1-14}\text{C}]$ palmitic acid in a liquid Middlebrook 7H12 medium to $^{14}\text{CO}_2$ which is measured in the BACTEC 460 instrument (Collins and Franzblau, 1997). Because of the quantitative nature of the data obtained in this assay, the relative activity of various samples can be compared by testing at only 1 or 2 concentrations and determining a percentage inhibition of $14\text{CO}_2$ production relative to drug-free controls (Cantrell et al., 1998a). Alternatively, multiple concentrations can be tested and MIC can be calculated (Rajab et al., 1998). Newer non-radiometric clinical automated systems use indicators of oxygen consumption (Sanders et al., 2004), carbon dioxide production (Diaz-Infantes et al., 2000) or head space pressure to determine growth/inhibition.

BACTEC method is faster but it is costly, cumbersome and requires the use of radioactive reagents. Owing to its procedural hazards the BACTEC 460 system has been withdrawn and replaced by fluorimetric method namely MGIT 960. Over the past decade, Luciferase Reporter Mycobacteriophage (LRP) have been developed that show great promise for diagnostic mycobacteriology (Jacobs et al., 1993). Phage based assay is a rapid, inexpensive and less laborious method for high throughput screening of compounds for their antimycobacterial activity.
3.7.4. Phage based Assay

Mycobacteriophage based methods have been developed for the rapid detection and drug sensitivity testing of *M. tuberculosis*. Among phage based assays, luciferase reporter phage assay has the potential for the detection, identification and drug sensitivity testing of *M. tuberculosis* (Banaiee *et al.*, 2001).

Firefly luciferase *FFlux* has been one of the most widely used biological reporter molecule for measurement of adenosine tri phosphate (ATP). The enzyme catalyses the oxidation of luciferin to oxy luciferin in the presence of ATP and magnesium ions with light being produced as a by-product that can be measured using a luminometer at 560 nm (Billard and DuBOW, 1998) (figure 1). The quantum yield is 0.85 photons per molecule of substrate reacted (Hastings *et al.*, 1978).

The LRP assay utilizes recombinant mycobacteriophages carrying the luciferase gene *fflux* from the American firefly *Photinus pyralis* cloned in to its genome using the novel strategy of ‘shuttle phasmids’ (Jacobs *et al.*, 1987). Cells are infected with the recombinant phages and light is measured after a specific time of incubation to allow sufficient expression of the enzyme to a detectable limit.

Jacobs *et al.* (1987) developed an efficient transformation system for mycobacteria and using this system, succeeded in developing shuttle phasmids from mycobacteriophages TM4. These entities are chimeras containing the mycobacteriophage DNA in to the non-essential region of which an excisable *E. coli* cosmid is introduced. They are capable of replicating as
plasmids in *E. coli* and phages in mycobacteria and thereby transfer DNA across these genera.

**Figure 1:** An overview of the luciferase reporter phage assay

![Luciferase reporter phage assay diagram](image)

 Luciferase reporter phage assay had been recognized for the rapid assessment of drug susceptibility of *M. tuberculosis*. The time required for the identification of antibiotics sensitivity pattern of *M. tuberculosis* was reduced from weeks to days using LRP assay (*Jacobs et al.*, 1993). Being a viability based test, LRP was also utilized for the rapid screening of new antituberculosis drugs (*Kumar et al.*, 2011).

 A temperate phage Che12, first of its kind, able to infect and lysogenize *M. tuberculosis*, was isolated from soil samples from Chennai, India. A
luciferase reporter phage, phAETRC16, was constructed by cloning firefly luciferase gene into Che12. Infection of viable *M. tuberculosis* cells by phAETRC16 resulted in expression of luciferase leading to sustained light output. Che12, a true temperate phage infecting *M. tuberculosis*, is thus ideally suited for developing a diagnostic tool facilitating rapid diagnosis of *M. tuberculosis* (Kumar et al., 2008).

Sarkis *et al.* (1995) constructed mycobacteriophage L5 recombinants carrying the firefly luciferase gene inserted into the phage genome. Infection of *M. smegmatis* by these phages results in expression of the luciferase gene and light emission. Synthesis of luciferase from a mycobacterial promoter created by cloning enables the detection of extremely small numbers of *M. smegmatis* cells. These reporter phages can be used to discriminate between drug-sensitive and drug-resistant strains of *M. smegmatis*, and may provide tools for the rapid identification and classification of antimycobacterial agents.

Shawar *et al.* (1997) reported that the luciferase assay is rapid and accurate and has the potential to greatly accelerate the evaluation of antimycobacterial activity in plant extracts *in vitro*. A total of 480 plant extracts were tested. Sixteen extracts were found to be active against rBCG, and of those, seven were also active against recombinant *M. intracellulare*. With this method, it is possible to screen a large number of samples in a short period of time.

Prabuseenivasan (2006) reported that *in vitro* screening of 21 selected plant essential oils against *M. tuberculosis* by LRP assay. Among the tested volatile oils, cinnamon oil showed significant activity at a concentration of 50 µg/ml followed by clove oil.
Sivakumar et al. (2007) reported Antitubercular activity of Triterpenoids from *Chrysanthemum morifolium* flowers. Flavonoids are also potent antitubercular agents.

Radhakrishnan et al. (2007) investigated actinomycetes from less explored ecosystems and screened for antimycobacterial activity by luciferase reporter phage assay against drug sensitive and drug resistant clinical isolates. Among 49 actinomycetes tested, 6 strains showed more than 90% reduction in RLU by LRP assay.

Muthuswamy et al. (2013) screened methanol extract of 32 medicinal plants collected from Western Ghats of Tamil Nadu against *M. tuberculosis H₃₇Rv*, multi-drug resistant (MDR) and sensitive strains by LRP assay. Two concentrations viz. 100μg and 500μg were used for this study. Among the thirty two plants tested, *Ruta graveolens* extract exhibited good antimycobacterial activity against all the tested strains *M. tuberculosis H₃₇Rv* (76.60%), MDR, (87.25%) and Sensitive strains and (94.32 %) at a concentration of 100μg/ml. Remaining plants showed moderate or no activity.

### 3.8. Phytochemical screening

The word, phytochemical means the plant chemical that have a major positive impact on human health. The phytochemicals can range from medicinally useful agents to deadly poisons.

Plants are often taxonomically distinct. This is in contrast to primary products, such as carbohydrates, lipids, proteins, chlorophyll, and nucleic acids, which are common to all plants and are involved in the primary metabolic processes of building and maintaining plant cells (Wink, 1999).
Although plant secondary products have historically been defined as chemicals that do not appear to have a vital biochemical role in the process of building and maintaining plant cells, recent research has shown a pivotal role of these chemicals in the ecophysiology of plants. Accordingly, secondary products have both a defensive role against herbivore, pathogen attack, and inter-plant competition and an attractant role toward beneficial organisms such as pollinators (Wink and Schimmer, 1999; Doughari et al., 2009). The most important secondary metabolites include alkaloids, saponins, tannins, phenolic compounds, terpenes, flavonoids and anthraquinones (Mbaebie et al., 2012).

### 3.8.1. Alkaloids

It is a colourless heterocyclic nitrogen compound. The first isolated medically useful alkaloid was morphine in 1805 from *Papaver somniferum*. Leguminosae, papaveraceae, Ranunculaceae, Rubiaceae, Solanaceae and Berberidaceae are excellent plants yielding alkaloids (Pelletier and Page, 1986). The pharmacological action of alkaloids includes analgesics, central nervous stimulants, myotics etc. Solamargine, aglycoalkaloid from the berries of *Solanum Khasianum* is useful against HIV infection as well as intestinal infections associated with AIDS. The alkaloids are highly reactive substances with biological activity in low doses.

### 3.8.2. Flavonoids

Flavonols are colorless or yellow flavonoids found in leaves and many flowers. It is a phenolic compound and occurs as aglycones, glycosides and methylated derivates. More than 4000 varieties of flavonoids have been isolated (Hollmann and Batan, 1997). They are characterized into flavonols,
flavones, catechins, flavanones, anthocyanidins and isoflavonoids. They exhibit antimicrobial, cardioprotective, anti-diabetic, anti-aging and antiplatelet activities

3.8.3. Terpenoids

They are unsaturated compounds having one or more double bonds. Terpenoids are mostly colourless liquid lighter than water and highly refractive. They are categorized into monoterpenoids, sesquiterpenoids, diterpenoids, triterpenoids, tetra and poly terpenoids. The mono and sesquiterpenoids are chief constituents of essential oils. The di and tri-terpenoids are obtained from plant, tree gums and resins. Many sesquiterpenoids and diterpenoids are used as antibiotics (Duke, 1992).

3.8.4. Phenolic compounds

It is composed of one or two aromatic benzene rings with one or more hydroxyl groups (C-OH). It includes numerous plant compounds that chemically distinct from terpenes. Many of the volatile compounds, such as eucalyptol from *Eucalytus globulus*, citronellal from *E. Citriodora* and clove oil from *Syzygium aromaticum*.

3.9. Identification of active principle

The identification of biologically active compounds is an essential requirement for quality control and dose determination of plant-based drugs. A medicinal herb can be viewed as a synthetic laboratory as it produces and contains a number of chemical compounds. These compounds, responsible for medicinal activity of the herb, are secondary metabolites. For example,
alkaloids which are nitrogenous principles of organic compounds combine with acids to form crystalline salts. In addition, herbs may contain saponins, resins, oleoresins, lactones and volatile oils. Complete phytochemical investigations of most of the medicinally important herbs of India have not been carried out so far. This would be beneficial in standardization and dose determination of herbal drugs (Dubey et al., 2004).

**Phytochemicals in the plant can be screened by the sequential procedure that includes:**

**3.9.1. Solvent Extraction**

Plant based natural constituents can be derived from any part of the plant like bark, leaves, flowers, roots, fruits, seeds etc. The basic principle is to grind the plant material (dry or wet) finer, which increases the surface area for extraction thereby increasing the rate of extraction. The different techniques of medicinal plant extraction include maceration, infusion, percolation, digestion, decoction, hot continuous extraction (Soxhlet), aqueous-alcoholic extraction by fermentation, counter-current extraction, microwave-assisted extraction, ultrasound extraction (sonication), supercritical fluid extraction, and phytonic extraction (with hydrofluorocarbon solvents).

For aromatic plants, hydrodistillation techniques (water distillation, steam distillation, water and steam distillation), hydrolytic maceration followed by distillation, expression and enfleurage (cold fat extraction) were employed. The latest extraction methods for aromatic plants include headspace trapping, solid phase micro-extraction, protoplast extraction, microdistillation, thermo microdistillation and molecular distillation (Handa et al., 2008).
3.9.2. Choice of solvents

The successful extraction of active compounds largely depends on the solvent selection, because the active constituent of the plants have an affinity for solvents and the end product will contain traces of residual solvent. The solvent selected should be inert, nontoxic, not easily flammable, and easy of evaporation at low heat. Some of the chief solvents used in the extraction are: water, hexane, acetone, alcohol, chloroform, and dichloromethanol etc. when compared with other solvents, alcoholic solvent such as methanol and 70% ethanol is widely used as it can efficiently penetrate cell membrane and extract high amounts of endocellular components (methanol ref).

3.9.3. Chromatographic methods

Chromatography is an analytical technique used to separate molecules based on their size, shape, or charge. It is used to analyze and isolate a variety of macromolecules. Three well known types of chromatography include: paper chromatography, thin-layer chromatography and column chromatography.

3.9.4. Thin Layer Chromatography

TLC was the common method of choice for herbal analysis before instrumental chromatography methods like GC and HPTLC is used. It is an easier method of initial screening with a semiquantitative evaluation together with other chromatographic techniques. The advantages of using TLC to construct the fingerprints of herbal medicines are its simplicity, versatility, high velocity, specific sensitivity and simple sample preparation. Thus, TLC is a
convenient method to determine characteristic component-patterns of plant extracts and biochemical preparations (Touchstone, 1992).

3.8.5. Column Chromatography

Column chromatography is one of the most useful methods for the separation and purification of both solids and liquids. This is a solid-liquid technique in which the stationary phase is a solid & mobile phase is a liquid. The principle of column chromatography is based on differential adsorption of substance by the adsorbent.

3.9.6. High performance Thin Layer Chromatography

HPTLC is a sophisticated and automated form of TLC. It is the fastest of all chromatography methods. The separation of the sample components is achieved on high performance layers with detection and data acquisition, using advanced work station. These high performance layers are pre-coated with a sorbet of particle size 5-7 microns and a layer thickness of 150-200 microns. The reduction in the thickness of the layer and the particle size are results in increasing the plate efficiency as well as nature of the separation. Separations using high performance thin layer plates give sharper and more compact bands with shorter distances of migration. It is suitable for qualitative, quantitative and micro-preparative chromatography (Touchstone, 1992).

After isolation and purification of the compound, the class of the compound and the specific compound within the class should be determined. It can be identified by measuring physical properties and spectral measurements. The physical properties include melting point (for solids),
boiling point (for liquids), optical rotation (for optically active compounds) and Rf. The spectral measurements include Ultra Violet (UV), Infra-Red (IR), Mass Spectrum (MS) and Nuclear Magnetic Resonance (NMR).

Ultraviolet and visible absorption spectroscopy is the measurement of the attenuation of a beam of light after it passes through a sample or after reflection from a sample surface. It is useful to characterize the absorption, transmission, and reflectivity of a variety of important materials, such as pigments and compounds from plants.

IR is the measurement of the wavelength and intensity of the absorption of mid-infrared light by a sample. It is used to confirm the identity of a particular compound and it is an important tool to help to determine the structure of a newly synthesized molecule with NMR and MS.

Nuclear Magnetic Resonance (NMR) spectroscopy is an analytical chemistry technique used in quality control and research for determining the content and purity of a sample as well as its molecular structure. NMR can quantitatively analyze mixtures containing known compounds. For unknown compounds, NMR can either be used to match against spectral libraries or to infer the basic structure directly. Once the basic structure is known, NMR can be used to determine molecular conformation in solution as well as studying physical properties at the molecular level such as conformational exchange, phase changes, solubility, and diffusion.
3.10. Minimum Inhibitory Concentration

Minimum Inhibitory Concentration (MIC) is defined as the lowest concentration of an antimicrobial that will inhibit the visible growth of microorganism. In some cases, the minimum bactericidal concentration (MBC) or the bacteriostatic concentration is stated, both terms agreeing closely with the MIC (Andrews, 2001). MIC is considered to be the ‘gold standard’ for determining the susceptibility of organism to antimicrobials. It is used to judge the performance of other methods of susceptibility testing.

This method uses standardized inoculums grown on drug-free media and media containing graded concentrations of the drugs to be tested. Resistance is expressed in terms of the lowest concentration of the drug that inhibits growth (less than 20 colonies). It is affected by inoculums size and viability of the organisms (Lab Manual, NIRT, 2006).

Mativandela, et al. (2008) reported the ethanolic extract of five plants Acacia nilotica, Syzygium aromaticum, Cinnamomum zeylanicum, Terminalia arjuna. Eucalyptus globules were tested against ATCC and MDR strains of E. coli, K. pneumoniae, S. bovis, P. aeruginosa and Candida albicans. Among the five plant, A.nilotica proved to be a potent plant with a MIC range of 9.75-31.3 µg/ml.

Gemechu et al. (2013) studied the in vitro activity of root of Calpurnia aurea, seeds of Ocimum basilicum, leaves of Artemisia abyssinica, Croton crostachyus, and Eucalyptus camaldulensisagainst M. tuberculosis and M. bovis strains using resazurin microtiter assay. Themethanolic extracts of the root of C. aurea, seeds of O. basilicum, and leaves of A. abyssinica, C.
**3.11. Cytotoxic activity**

This assay is mainly for evaluating the safety of the separated plant components for determining the maximal toxic free concentration of the test drugs on the cell lines and Cell viability was monitored by MTT colorimetric assay.

Garg *et al.* (2007) studied the twenty one plant extracts of different species used by Indian traditional healers for the treatment of ulcers, cancers, tumors, warts, and other diseases, were tested in vitro for their potential anticancer activity. The ethanolic extracts were tested against six human cancer cell lines using MTT 3-(4,5-dimethylthiazol–2-yl)-2,5-diphenyltetrazolium bromide. Seven out of the 21 extracts (33%) showed remarkable cytotoxic potential. The highest activity was found in the leaf/stem ethanol extracts from *Plectranthus urticoides* and *Garcinea morella* against all the six human cancer cell lines screened.

Ramos *et al.* (2008) investigated thirty-six plant extracts from the brazilian Atlantic Forest and tested for their antimycobacterial activity against *M. tuberculosis* H37Rv and *M. kansasii*, using the method REMA at 100 to 0.20 μg/ml. Among the thirty six extracts tested, five were active against *M.*
tuberculosis, and three of these extracts also showed activity against *M. kansasii*. A cytotoxicity test with VERO cell was performed with the five extracts active against *M. tuberculosis*. Only the extract of *Peschiera affinis* was identified as non-toxic in the concentration of 100μg/ml.

Sahranavard *et al.* (2009) investigated the cytotoxic activity of 15 Iranian traditional medicinal plants against three cancer cell lines and one normal cell line. Some plant’s methanol extracts showed low or no cytotoxicity against the cell lines, whereas *F. szowitsiana* showed the most potent cytotoxicity against all of them.

Webster *et al.* (2010) evaluated the antimycobacterial activity of 5 medicinal plants traditionally used as general therapeutics for pulmonary illnesses and specifically as treatments for tuberculosis. Aqueous extracts of *Aralia nudicaulis*, *Symplocarpus foetidus*, *Heracleum maximum*, *Juniperus communis*, and *Acorus calamus* were screened for antimycobacterial activity against *Bacillus Calmette-Guérin*, *Mycobacterium avium*, and *M. tuberculosis* H₃₇Rv using the colorimetric microplate resazurin assay. Extracts of *Acorus calamus* and *H. maximum* root demonstrated significant antimycobacterial activity comparable to that of the rifampin control (2 μg/ml). Evaluation of the cytotoxicity of these 2 extracts using the MTT assay also showed that the extracts were less toxic to 3 human cell lines than was the DMSO positive control. This study demonstrates that aqueous extracts of the roots of *H. maximum* and *Acorus calamus* possess strong in vitro antimycobacterial activity, validates traditional knowledge, and provides potential for the development of urgently needed novel antituberculous therapeutics.
Tawde et al. (2012) studied the Indian traditional folk medicinal plants against *M. tuberculosis* and their cytotoxic activity and the result showed that the hexane extract of *A. catechu* root showed promising activity against *M. tuberculosis* while remaining extracts showed moderate anti TB activity. The samples were found to possess considerable DPPH and OH radical scavenging activities with no demonstrable cytotoxicity against Chang liver cells.

### 3.12. In silico analysis for molecular docking

In biological process molecular interactions plays a vital role in signal transduction, cell regulation and other macromolecular assemblies. Experimentally it is very difficult to understand these interaction mechanisms, and to design the therapeutic intervention. To overcome the situations the application of computational methods, has been subjected intensively to the research during the last decade.

Docking is an important computational tool to identify the potential leads in drug discovery. Docking is widely used to predict the binding orientation of small molecule drug candidates to their protein targets in order to predict the affinity and activity of the small molecule. Hence docking plays an important role in the rational design of drugs (Kitchen et al., 2004). Especially, protein–ligand docking occupies a very special place because of its applications in medicine (Muegge and Rarey, 2001).

To carry out docking calculations a number of powerful software programs, e.g, AutoDock, HEX, GOLD, FlexX, DOCK, Glide, Surflex, LigandFit, have been developed over the past several decades.
Ramesh et al. (2011) reported the docking of homology modeled Ketoayl-acpeductase (KR) domain with isoniazid and epigallocatechin (EGC) from green tea. The result suggested that EGC had a higher binding affinity to the protein than the synthetic drug isoniazid.

Dhivya et al. (2011) carried an in silico analysis through Accelrys Discovery studio 2.5 software on an active compound arteether isolated from the medicinal plant *Artemisia annua* and the result showed arteether with more binding affinity towards Enoyl acyl carrier protein reductase, and the analogy 14, 15 arteether showed hydrogen bond interactions with least internal energy and high dock score, recommending arteether based analogue as an efficient drug candidate against tuberculosis.

Daisy et al. (2012) investigated the docking of ten phyto-ligands from *Gloriosa superba* to design an inhibitor for the Biotin protein ligase of *M. tuberculosis* using Autodock Vina software. The drugs were calculated by ADMET (absorption, distribution, metabolism, excretion, and toxicity) calculations. Based on the results and ADME values *Gloriosa* was confirmed as a promising lead compound.

Devi (2012) reported that three ligand molecules commonly present in plant secondary metabolites were docked with the selected mycobacterial receptors AccD5 and PKS18. Among them Phytol had a significant inhibitory activity with the receptor AccD5, binding to the pocket (GLY 241–GLY 242) forming hydrogen bonds at a very low energy value, thus forming a stable complex. The active compounds were found to be diterpene alcohol and isomers of diterpene alcohol. These molecules had a good number of conformations showing the flexible behavior of the ligand.
Santhi and Aishwarya et al. (2011) described the docking of 26 withanferin and 14 withanolides from Withania somnifera into the three dimensional structure of Protein kinase G (PknG) of M. tuberculosis using GLIDE. The inhibitor binding positions and affinity were evaluated using scoring functions of Glidescore. The withanolide E, F and D and Withaferin diacetate 2 phenoxy ethyl carbonate were identified as potential inhibitors of PknG. The available drug molecules and the ligand AX20017 showed hydrogen bond interaction with the aminoacid residues Glu233 and Val235. In addition to Val235 the other amino acids, Gly237, Gln238 and Ser239 are important for anolide inhibitor recognition via hydrogen bonding mechanism.

3.12.1. GOLD SOFTWARE

Gold uses genetic algorithm to provide docking of flexible ligand and a protein with flexible hydroxyl groups. Otherwise the protein is considered to be rigid. This makes it a good choice when the binding pocket contains amino acids, which form hydrogen bonds with the ligand. Gold has won a lot of new users during the last few years because of its good results in impartial tests. It has a good hit rate overall. GOLD has one of the most comprehensive validation test sets and from the entire plethora of docking programs, comparing 100 different protein complexes has achieved 71% success rate in identifying the experimental binding mode (Kellenberger et al., 2004).

The above said information clearly shows that, there are several plant species having the potential compounds to control infections yet to identify. Hence, the present study was designed to screen selected medicinal plants and to assess their antimycobacterial activity to identify the promising one.
using LRP assay. The related investigations were carried out to identify the active principle using bioassay-guided fractionation.