CHAPTER – I

INTRODUCTION
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Natural resources especially plants have been used as a supply of therapeutic specialties in medicines for various types of illness since prehistoric time. According to the World Health Organisation (WHO), about 21,000 plant species are being used around the world for healthful functions and medicines. In India, about 2,500 plant varieties belonging to more than 1000 genera are getting used within the native system of medication. India is tenth among the plant-rich countries of the world and fourth among the Asian countries. The northeastern part of India including Assam houses about 220 completely different tribes out of as many as 427 of entire country, having their own ancient material culture and medicinal practices. Several seasonal remedies separately or together are counseled for the cure of various diseases in traditional healthful practices by the tribal communities of northeast India. It is to be noted that, besides several diseases, Tuberculosis, a major infectious disease of the third world countries, is additionally being treated historically using several raw plant extracts (WHO, 2013).

Tuberculosis (TB) is one of the major health threats particularly in Asia and Africa, and the Mycobacterium tuberculosis \((M_{tb})\) is the causal pathogenic microbe for this infectious disease. As reported by Zang (2005), annually about 8.6 million people develop tuberculosis and about 1.3 million people die from the disease in the world. The disease caught the attention of the scientific community during the 19th century when Robert Koch discovered the causal organism of TB (Koch, 1882). The discovery of Mycobacterium tuberculosis - a Gram-positive bacterium led subsequently to the discovery of the first generation drugs against this deadly pathogen during the 1940s and 1960s. Most of the effective drugs against \(M. \, \text{tuberculosis}\) namely streptomycin, rifampicin, isoniazid, ethambutol and ethionamide, kanamycin, etc., were developed during this period. These drugs were found to interact mainly with the ribosomal subunits, RNA polymerase, InhA and DNA gyrase, by inhibiting different vital cellular processes of the mycobacterium like biosynthesis of bacterial cell wall etc. (David, 1970).
While initially, these drugs proved to be very effective as anti-TB chemotherapeutic agents, inappropriate prescription (drugs, dosing intervals, duration) or suboptimal use of these medicines gave rise to drug-resistant mutants which replicate and eventually replace drug-susceptible *M. tuberculosis* populations (Dye *et al.*, 1999). Along with that, the emergence of HIV infection during the late 1980s has brought in its alarming wake changes in the world TB scenario as *M. tuberculosis* is one of the most important opportunistic infections associated with HIV (Corbett *et al.*, 2003). According to a recent WHO report *M. tuberculosis* infects eight million people annually with active tuberculosis, killing about two million of them (WHO, 2015). Emergence of Multi-drug resistant (MDR) TB, that may be a kind of infectious disease immune to a minimum of two first-line medication, namely rifampicin and isoniazid. The intractability of persistent infectious disease infections to treatment with standard anti-TB medication and multiplied status of AIDS patients to *Mycobacterium tuberculosis* infections have been identified as responsible for the changing situation. The situation is further aggravated by the emergence of extensively drug-resistant TB (Hassam *et al.*, 2006).

There is a great urgency in developing new anti-TB drug molecules in the context of the multiple realities briefly outlined above. The conventional drugs work against this bacteria through their inhibitory effects mostly on cell wall synthesis (David, 1970). The other inhibitory effects of these drugs relate to nucleic acid synthesis, peptide and protein synthesis, and membrane energy metabolism (Sacchettini *et al.*, 2008). It is obvious that these drugs are effective only in situations where DNA replication and cell wall biogenesis are in progress in actively dividing pathogens. Therefore, new drug molecules are needed to control the recalcitrant persistent infections (Singh *et al.*, 2007). Also, the advent of MDR and ‘extensively drug-resistant’ (XDR) TB demands identification of new drug target interactions (Johnson, 1998). The XDR TB form is more dangerous than MDR one as this form is resistant not only to rifampicin and isoniazid, but additionally to sure shot second-line medication (at least one fluoroquinolone and amongst the three injectable medication antibiotic drug, amikacin or capreomycin).
It is considered as important and demand of the time to develop new molecules to a successful cure of MDR and XDR varieties of tuberculosis. In this process, computer-aided drug discovery (CADD) is currently being used as a tool for identification of new lead molecules from a group of compounds through in silico approaches (Gomez and McKinney, 2004). In the early period of drug discovery before 1970, new drug candidates were proposed only through time-consuming laboratory synthesis or extraction from nature and by performing enzyme essay and co-crystallization or similar binding analysis studies. CADD has gained momentum in the 1980s with the development in the processes like X-ray crystallography and Nuclear Magnetic Resonance analysis and computer-aided molecular modeling (Jackowski, 1996). These processes of computer modeling and analysis aided in the dramatic progress in the designing of new drug molecules rather than using trial and error methods and manual databases studies which were very exhaustive. The accuracy, fastness and novelty required in the designing and development of new molecules to combat MDR and XDR TB also gained many benefits from the CADD approaches across different leading laboratories of the world. Many potential drug targets were discovered by the researchers against which many natural molecules were modeled to select out the best possible inhibitor of the particular drug target (Moerman, 1986).

A few derivatives of 3,5-dinitrobenzylsulfanyl-1,3,4-oxadiazoles and thiadiazole have already been reported having activity against both replicating and non replicating Mycobacterium. Another series of compounds of 3,5-dinitrophenyl 1,3,4-oxadiazole-2-thiols and tetrazole-5-thiols as prospecting anti-tubercular compounds. This series of compounds were found to be promising against seven different varieties of MDR and XDR strains with MIC90 values below 0.5μM. There are several such examples of series of compounds which were synthesized and phenotypic screening was successfully conducted against MDR and XDR strains preceded by in silico studies (CDC, 2006). In all these complex processes, particularly in the initial stages of the drug discovery process, one of the most important steps is to determine physiochemical properties of the compounds thought to be potentially active. It is accepted that the bioactive compounds having adequate physicochemical properties should be given priority than the highly active
compounds. Therefore, the rules of drug discovery which are based on physicochemical properties of a compound, like ‘Lipinski’s rule’ is to be followed during the initial studies of structure-based drug designing (Smith et al., 2003).

Medicinal plants are an important source of valuable molecules with curative properties and they are an essential pool for the detection of novel medication leads even in the recent situation (Banerjee et al., 1994). Out of many natural compounds, Calanolide A, a compound isolated from a flowering plant in Malaysian Rainforest is a promising drug candidate for AIDS therapy, can be mentioned as a good example (Corbett et al., 2003). However, transformation of the plant derived compounds to a ‘marketed drug’ is associated with increasingly challenging demands for compound amount for change assortment, which often cannot be met by re-separation from the plant resources (Quemard et al., 1995). On the other hand, necessities in the area of product based medicine recognition and development in the area of top class interdisciplinary approaches, well controlled development ideas, technological advancements and research trends strongly indicate that the natural products will be among the most important sources of new drugs also in the future (Jhonson, 1999).

Nature originated products have taken a major part in the discovery and development of compounds for clinical use in the case of tuberculosis. Isoniazid and rifampicin, the two major frontline synthetic drugs used currently for the treatment of tuberculosis were derived from natural compound nicotinamide and rifamycin respectively (Schroeder et al., 2002). Similarly, some of the second line drugs such as kanamycin and amikacin and the peptide antibiotic capreomycin were also derived from natural products. Although after having valid examples of historical success of nature origin compounds as drug lead for TB treatment, no new class of anti-TB compounds have been launched since the discovery of rifampin in the 1960 (Maas, 1960). New, effective and affordable molecules are in urgent demand due to immense burden of the disease across the world, long treatment regimens, incremental number of drug-resistant strains and co-infections associated with HIV infection (Vilchèze et al., 2006).
New molecules to clinic can only be possible through time and resources consuming process which can be collectively termed as drug discovery and development. To streamline the process of drug discovery and development, there is always an increasing demand and need of use of computational processing power. This helps in streamlining of process of lead optimization in drug discovery (Lei et al., 2000). The in silico processes are being used to identify hits, selection hit-to-lead selection, absorption, distribution, metabolism, excretion and toxicity prediction. Generally used computational approaches include

a) Ligand based drug design (Pharmacophore modeling)

b) Structure-based drug design (Drug-target docking)

c) QSAR (Quantitative structure-activity relationships and Quantitative structure-property relationships) and QSPR (quantitative structure-property relationships).

In case of screening of combinatorial library, most frequently used approach is structure-based drug design. Bioinformatics and computational pharmaceutical industry are working hard to develop computational tools or software to improve the efficiency of the process of drug discovery and development (Tomioka, 1998).

Chemical libraries prepared from naturally available plant species or other sources consist of compounds with known chemical formulas. Many of these libraries of compounds are available in the public recourses. Natural compounds from different plants are also available as chemical libraries or databases (Sevrin and Reboli, 2009).

Development of new drug molecules is a long-term project that proceeds through several phases involving in silico, chemical, in vivo and clinical research. The aim of the present investigation was to screen few plant based active compounds along with known antibacterial compounds to identify few potential lead molecules against multiple established drug targets of M. tuberculosis through in silico approach with the following objectives.
Objectives:

a. To create a combinatorial library from plant based natural compounds and their derivatives with antibacterial activity through *in silico* approaches.

b. To select potential molecules from the created combinatorial library for determining anti-mycobacterium activity through *in silico* drug target interaction.

c. To perform computational screening of the molecules using ADME-TOX (Absorption, Dissociation, Metabolism Excretion and Toxicity) determination software.

d. To study of Drug likeliness of the selected molecules to establish themselves as potential lead molecule for discovery of new anti-tubercular drug.