Evaluation of anti-tuberculosis activity in selected vegetable crops: Garlic, Onion and Drumstick

by

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(2009-09-119)

THESIS

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KERALA, INDIA

2019

DECLARATION

I hereby declare that this thesis entitled **"Evaluation of anti-tuberculosis activity in selected vegetable crops: Garlic, Onion and Drumstick"** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award of any degree, diploma, associate ship, fellowship or other similar title, of any other university or society.

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CERTIFICATE

This is to certify that this thesis entitled **"Evaluation of anti-tuberculosis activity in selected vegetable crops: Garlic, Onion and Drumstick"** is a record of research work done by **Mr. Nandu Mohan** (2009-09-119) under my guidance and supervision and that this has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to him.

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Nandu Mohan

CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS AND SYMBOLS USED

%	Per cent
3D	Three dimensional
DprE1	Decaprenylphosphoryl-β-D-ribose epimerase
DST	Dempster-Shafer Theory
et al.	And others
Fig.	Figure
h.	Hours
HTS	high-throughput screening
Kcal	Kilo calorie
LD	Lethal Dose
LRP	Luciferase reporter phage
LTBI	latent tuberculosis infection
M	Mol
MDR-TB	Multidrug-resistant TB
mL	Micro litre
Mm	Milli meter
mM	Milli molar
Mol^{-1}	Per mol
MTB	*Mycobacterium tuberculosis*
NCBI	National Center for Biotechnology Information
NIH	National Institutes of Health

NMR	Nuclear Magnetic Resonance
°C	Degree Celsius
OD	Optical density
PDB	Protein Data Bank
pH	Potential of Hydrogen
RLU	Relative lights units
TB	Tuberculosis
Viz.	Namely
WHO	World Health Organization
XDR-TB	Extensively drug-resistant TB
µg	Micro gram
µL	Micro litre
Via.	By way of

LIST OF APPENDICES

INTRODUCTION

13

1. INTRODUCTION

TB, an acronym of tuberculosis, is the most common contagious disease caused by one of the oldest known human pathogen *Mycobacterium tuberculosis* (MTB). It stands ninth in the mortality rate of a disease caused by single etiological agent worldwide. Two million people die each year from its aliment. Researchers are on the verge of finding new anti-tubercular agents by utilizing the information obtained from the complete genome of *M. tuberculosis*. Identification of novel vaccines and drugs and more specific and rapid diagnostics are the major requirements for TB. According to WHO Global Tuberculosis Report (2017), an estimated 10.4 million people developed active TB in 2016. An increased threat is represented by multidrug resistant (MDR) and extensively drug-resistant (XDR) strains of MTB that are not susceptible to first-line (isoniazid and rifampicin) and second-line injectable drugs (kanamycin, amikacin, capreomycin, and any fluoroquinolone). Typically, the spontaneous development of such strains is attributed to a poor adherence to the treatment protocols, causing the bacteria to build a resistance towards the first-line and second-line drugs. The genus of *Mycobacterium* has originated 150 million years ago (Daniel 2006). It was believed that, three million years ago, both the early progenitor of *M. tuberculosis* and the early hominids in East Africa were co-evolved at the same time. A common progenitor was shared by the modern members of *M. tuberculosis* complex and was originated 15,000 – 35,000 years ago (Gutierrz 2005). TB was first identified 4,000 years ago during the Egyptian civilization and the disease was common among the populations of Neolithic sites such as Italy, Denmark, and countries in the Middle East. The Genus *Mycobacterium* was initially found in soil and that some species evolved to live in mammals. The domestication of cattle, thought to have occurred between 10,000 and 25,000 years ago, would have allowed the passage of a mycobacterial pathogen from domesticated livestock to humans, and in this adaptation to a new host, the bacterium would have evolved to the closely related *M. tuberculosis*. Most of the anti-tubercular drugs target essential enzymes involved in cell wall synthesis, energy metabolism, protein synthesis, phosphate transport, and the metabolism of key molecules and

cofactors. A number of experimental approaches have been utilised for the identification of the MTB genes over a period of time. The technique such as high-throughput and big data analysis has been emerged as a powerful tool for the identification of MTB genes later in 1990s.

Chemical and genetic target validation in animals is performed usually by exorbitant and time consuming in-*vitro* analysis and gene silencing respectively. An alternative practical approach is by incorporating bioinformatics and computational modelling of biological pathways there by predicting potential candidate targets which can then experimentally validated. Although these methods are effective, the current scenario has changed with the introduction of high throughput technology where screening off potential targets is hectic because of the sheer amount of data size. Big data has stepped in to the world of genomics and the basic screening or reductionist approach will not be practical anymore. More robust and efficient computing tools are necessary to get the job done. The precise aid in interpreting the results from such comprehensive analysis of big genomic data files or more precisely networks. Although we have advanced computational power, the integration step still needs the human touch along with bioinformatics and computational tools for collecting experimental and literature data for the construction and analysis of biological network models. In drug discovery many new computational methodologies have developed to aid various stages of drug development which includes target identification, drug design and optimization of lead.

Traditional healers always use natural materials particularly plants to cure diseases since the beginning of human existence. Soon it found its way to industrial purposes too. The medicinal plants so paved the way to a majority of life saving drugs which saved millions of people worldwide. The World Health Organization (WHO) has estimated that 80% of the world's population in developing countries depend on plant derived medicines for basic health care. May it be in Ayurveda or Chinese Medicine; it has always marked as a key aspect in a civilization. Even today we rely on plants more than synthetic drugs as they are safe to use and cost effective. There are no adverse effects if used correctly.

There is a trending shift from synthetic to natural medicine recently. Discover candidate molecule of drugs from active principle of medicinal herbs is a modern approach by pharmaceutical industries but it started since the beginning of 19th century. The first pharmacologically active compound morphine was isolated from the opium plant. Subsequently, countless active compounds have been separated from plants and are not replaced even now. According to WHO, eleven percent of 252 basic and essential drugs are exclusively derived from flowering plants. In this scenario developing an effective stable medicine from plants for TB would recognised as a suitable research problem. In the present study, three indigenous vegetable crops *viz. Allium cepa, Allium sativum* and *Moringa oleifera* which are traditionally been using for the treatment of pulmonary diseases in India, were selected for the phytochemical screening to sought out suitable drug candidates through *in silico* analysis.

Objectives of the study

To evaluate the anti-tuberculosis activity in selected vegetable crops: Garlic, Onion and Drumstick using bioinformatics tools.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

2.1 TUBERCULOSIS

Tuberculosis (TB) is a primordial infectious disease caused by Mycobacterium tuberculosis that mainly affects the lungs. The bacteria often transfer from person to person through the release of droplet nuclei of 1– 5 microns in diameter in to air via coughs and sneezes. The favourable environmental conditions allow the tiny droplets to remain suspended in the air for several hours. Transmission occurs when the person inhales the droplet containing *M. tuberculosis*. Through the mouth and the nasal passages it transverses upper respiratory tract and bronchi to reach the alveoli of the lungs. Coughing, fever, night sweats, weight loss are the major symptoms of TB and it will be mild for months. Those who are having close contact with the infected ones can also affect TB over the course of a year. Compared with other diseases caused by a single infectious agent, tuberculosis is the second biggest killer, globally (WHO, 2013). The historical background of TB, and its impacts in global and national level are well reviewed by several authors (Chakraborty, 2004; Sandhu, 2011). During the 18^{th} and 19^{th} centuries TB has widespread affected throughout Europe and North America. Later Robert Koch, the German microbiologist identified the causative agent of TB in 1882. There was a time people believed that the disease was almost defeated by the invention of new vaccines and rapid diagnosis. Indeed, US predicted that TB would be eliminated by 2025. However, the worldwide rise of TB again began in the mid-1980s. Thus for the first time a disease has been declared as a global emergency by World Health Organisation in 1993. According to them over 9 million are susceptible to TB every year, out of these 3 million are missed by health systems. TB is among the top three causes of death for women aged 15 to 44. One-quarter of the world's population is with latent TB, which means people have been infected by TB bacteria but are not ill with the disease and cannot transmit the disease. People infected with TB bacteria have a 5–15% lifetime risk of falling ill with TB. However, persons with compromised immune systems, such as people living with

HIV, malnutrition or diabetes, or people who use tobacco, have a much higher risk of falling ill. One third of the HIV persons are infected with latent TB. Without proper treatment, 45% of HIV-negative people with TB on average and nearly all HIV-positive people with TB will die. India is the highest TB burden country with World Health Organization (WHO) statistics for 2011 giving an estimated incidence figure of 2.2 million cases of TB for India out of a global incidence of 9.6 million cases. Between 2006 and 2014, the disease cost Indian economy USD 340 billion.

2.2 TREATMENT SYSTEM

The inability of immune system to stop bacteria from growing when the bacteria were active is called TB disease. People who are infected with TB are to be treated with the prescribed drugs within the course period. Discontinuance of the drugs can make the person sick again; and an incorrect intake of the drug will make the TB bacteria resistant to those drugs. TB that is resistant to drugs is harder and more expensive to treat. The World Health Organization (WHO) has recently promoted the DOTS strategy as an effective intervention that will lead to reduce tuberculosis transmission and decreasing numbers of tuberculosis cases (Raviglione and Pio, 2002). This strategy has been shown to be among the most cost-effective global health interventions available today (Murray *et al.,* 1990). Duration of the course period is 6-9 months. A good percentage of TB cases can be cured when the medicines are provided and taken properly. 54 million peoples were saved through the proper diagnosis and treatment between 2000 and 2017. U.S. Food and Drug Administration (FDA) had approved 10 drugs that were treating for TB. Among them the first-line anti-TB agents that form the core of treatment regimens are isoniazid (INH), rifampin (RIF), ethambutol (EMB) and pyrazinamide (PZA). The only vaccine developed against TB was Live Bacille Calmette-Guerin (BCG) in the year 1921.

2.3 PROBLEMS RELATED TO PRESENT TREATMENT SYSTEM

Conventional TB treatment has several problems and is well reviewed on new Drugs against Tuberculosis: problems, progress, and evaluation of agents in clinical development (Jossy van den Boogaard, 2009). The duration and complexity will result in non-adherence to treatment. This leads to suboptimal response, the emergence of resistance, and continuous spread of the disease. *M. tuberculosis* can persists in the host for decades after infection even when confronted with an intact immune response and most anti-tuberculosis drugs efficiently kill actively growing tuberculosis bacilli but are less effective against slow replicating or non-replicating bacilli (Betts *et al.*, 2002; Hu *et al.*, 2000). Second, adverse events in response to anti-TB drugs are common and contribute to the problem of non-adherence. Third, the increasing incidence of multidrug-resistant (MDR; resistance to at least rifampin and isoniazid) and extensively drug-resistant (XDR; MDR resistance plus resistance to a fluoroquinolone and an aminoglycoside) TB is a serious concern. Resistant TB occurs in the presence of partially suppressive drug concentrations that enable replication of bacteria, the formation of mutants, and overgrowth of wild-type strains by mutants (selective pressure). The prevalence of MDR TB in new TB cases ranged from 0% in some Western European countries to more than 22% in some other parts of the world (WHO, 2008); 14 of 72 participating countries reported an MDR TB prevalence of more than 5%. Second-line drugs for drug-resistant TB are not available everywhere and are less effective, more toxic, and require longer use than first-line drugs. Fourth, co-infection of TB and HIV is a problem by itself. Collective management of TB and HIV encompasses a high medication with related problems (Narita et al., 1998). Fifth, prophylactic therapy of latent TB (TB infection without symptoms) with isoniazid is also associated with problems of non-adherence. Attempts to shorten treatment with alternative drugs resulted in severe adverse events.

Directly Observed Therapy Short course (DOTS) strategy to optimize response and adherence to TB treatment introduced by WHO is labor-intensive

and expensive. TB diagnosis in the DOTS strategy is based on sputum microscopy, rather than sputum culture. Only advanced pulmonary TB can be detected by sputum microscopy, and it requires qualified technicians. Consequently, TB detection rates are suboptimal and resistant *M. tuberculosis* strains are not detected (Iseman, 2002). Obviously, the development of new drugs and improve treatment strategies are essential. Potential new agents should reduce treatment duration, have an acceptable tolerability profile, be active against MDR/XDR TB, be of use in HIV-infected patients with TB, and be active against latent TB.

2.4 CHANLLELNGES FOR DEVELOPING NEW ANTI-TB DRUGS

The development of new anti-TB drugs has been affected by several problems. The first and the main challenge is regarding the profit and return of investment in the TB market. 115 to 240 million dollar is required to develop a new drug against TB. To make it profitable, market prices of new drugs should be relatively high, whereas the cost of the standard drug is only about $11 per patient. Governments and other NGOs have started to invest in TB drug research and development prior to the pharmaceutical industries. In the 1990s, the United States Centres for Disease Control and Prevention (CDC) established the Tuberculosis Trials Consortium (TBTC). Private and public sector partnerships formed the Global Alliance for TB Drug Development (GATB) in 2000. It is a non-profit venture that helps in the development of cost-effective new drugs. Various other research consortia are testing new drugs in preclinical and clinical trials. Large funding agencies, such as the European & Developing Countries Clinical Trials Partnership (EDCTP) and the Bill & Melinda Gates foundation are supporting these initiatives.

Another challenge is with the identification of new compounds having activity against *M. tuberculosis*. The compound must have both bactericidal activity and sterilizing activity. The molecular mechanisms responsible for mycobacterial dormancy (Mycobacteria in a state of low metabolic activity and not forming colonies), persistence (drug-susceptible mycobacteria that manage to

survive despite continuous exposure to TB drugs), and drug resistance are not yet fully understood. The deciphering of the mycobacterial genome in 1998 has been of help in elucidating regulatory mechanisms of metabolic pathways and thereby revealing new drug targets. Next challenge is associated with the clinical trials. There are currently no animal models available to determine the accuracy of the newly identified compounds. The guinea pig model is being used as an alternative for the mouse model since it resembles TB pathology in humans more closely.

Time consumption and the scarcity of trial sites are the other challenge with the drug development. In phase II clinical trials, the sputum culture conversion rate after three months of treatment is used as a surrogate marker for relapse rate, but the value of this surrogate marker is controversial. Several other surrogate markers are under evaluation. Large sample sizes are needed in phase III clinical trials to compare the effective standard regimen to a new regimen, even in trials that use a non-inferiority design. This contributes to the length of the TB drug development process. Trials should be performed in countries where the TB burden is highest, but the human and infrastructural capacity for performing large, high-quality phase III clinical trials is usually limited in these settings. Despite the challenges of TB drug development, studies are being conducted with higher doses of the rifamycins and several new drug candidates have reached the phase of clinical testing.

2.5 NEW DRUG DEVELOPMENT CURRENT STATUS

New TB drugs are needed because of the complexity and toxicity of the current TB drug regimes. There is also the major problem of TB drug resistance. This together with the problem of the interactions of the current TB drugs with the ARVs taken by HIV positive people, means that there is an urgent need for new TB drugs. The following questions should be asked about new medications. Can they replace medications lost to resistance? Can they improve the performance of conventional regimens *versus* drug-susceptible disease? New TB drugs need to provide shorter and simpler, but still affordable, multi drug regimens for drug sensitive TB. It should be Shorter, more effective, less toxic, and less expensive

regimens for drug resistant TB, also short, simple, easily tolerable and safe regimes for latent TB. Drugs with few drug-drug interactions can be safely provided for people with HIV. Over 480000 cases of multidrug-resistant (MDR) tuberculosis (TB) occur every year globally, 9% of them being affected by extensively drug-resistant (XDR) strains of *Mycobacterium tuberculosis*. The treatment of MDR/XDR-TB is unfortunately long, toxic and expensive, and the success rate largely unsatisfactory (<20% among cases with resistance patterns beyond XDR).

Several small scale biotech companies and many large pharmaceutical companies, such as Glaxo Smith Kline (Brentford, United Kingdom), Astra Zeneca (London, United Kingdom), and Novartis (Basel, Switzerland), have launched programs that direct to the discovery and development of new tuberculosis drugs. At the same time, the clinical trials infrastructure, which had been greatly eroded in the early 1980s, was being re-established with the formation of groups such as the United States Tuberculosis Trials Consortium (TBTC) (Tuberculosis Trials Consortium, 2001). The Global Alliance for TB Drug Development (TB Alliance), a recently established organization that is forging public-private partnerships with the objective of building a portfolio of new tuberculosis drugs and bringing a major new tuberculosis drug to market in the next decade (TB Alliance). The already available drugs for TB and their approval dates are as follows, BCG (1921), Gold Therapy (1925, abandoned 1934), Actinomycin, Streptothricin (1940), Streptomycin (1943), p-aminosalicyclic acid (1949), Isoniazid (1952), Pyrazinamide (1954), Cycloserine (1955), Ethambutol (1962), Rifampin (1963), Rifapentine (1998), TMC207 (2012), PA824, OPC67683, PNU100480, SQ109, AZD587 (2013) (Susan, 2012).

Non-systematic review based on historical trial results as well as on recent literature and World Health Organization (WHO) guidelines has been performed, with special focus on the approach to managing MDR/XDR-TB.The new, innovative global public health interventions, recently approved by WHO and

known as the "End TB Strategy", support the vision of a TB-free world with zero death, disease and suffering due to TB. Adequate, universally accessed treatment is a pre-requisite to reach TB elimination. New shorter, cheap, safe and effective anti-TB regimens are necessary to boost TB elimination.

2.6 PLANTS AS A SOURCE OF MEDICINE

Plants are major segment of biodiversity. Change in the environmental conditions and the genetic variability makes the plants different from each other. In deferent climatic environment plants shows a remarkable adaptability, which helps them to register their presence in different ecological zones. Human had been using plants for its therapeutic values from the ancient civilisation itself and for a long period minerals, plants and animal products were the sources of drugs (Pasquale, 1984). Other characteristics such as growth phase, seed dormancy and diversity, viability and germination potential make plant a different species. It may be also variable in two individuals of the same species affected by several biotic and abiotic factors in natural habitat. About 250,000-300,000 species can be seen in a hugely diverse plant kingdom. It continues to evolve and adapt to a multiplicity of environmental conditions and to protect from pathogens and predators. Many plant derived chemicals such as the opioid and the more recently discovered cannabinoid receptors has the ability to activate human endogenous receptors and can took part in many physiological functions. A number of plant molecules yet to be characterised which are having pharmacological and physiological significance. Currently about twenty five percent of the prescribe drugs across the world are derived from plants, 121 such active compounds being in current use. There are 252 drugs are considered to be basic and essential by WHO, among them 11 percent are exclusively from plant sources and some significant figures are synthetic drugs obtained from natural precursors. Recently there has been growing interest in alternative therapies and the therapeutic use of natural products, especially those derived from plants (Goldfrank *et al.*, 1982; Vulto and Smet, 1988; Mentz and Schenkel, 1989).

Natural products will definitely continue to be important as sources of medicinal agents. Other than natural products many few molecules can serve as chemical models for the synthesis and semi synthesis of novel substances for treating diseases. Combinatorial chemistry and computer-based molecular modelling design are some new approaches to drug discovery. Most drugs are made by synthetic chemistry and none of them can replace importance of natural products in drug discovery. Plant as a source of medicines has a long history in the treatment of various ailments and the plant-derived compounds have a long clinical use history, better patient tolerance and also acceptance. Payne *et al.*, 1991 states that about 5000 species have been studied for its medical use. Plants which are having ethno-pharmacological uses are the primary sources of medicine for early drug discovery. Fabricant and Farnsworth, (2001) reported that, 80% of 122 plant derived drugs were related to their original ethno-pharmacological purposes. An estimation of 60% anti-tumour and anti-infectious drugs available on the market or under clinical trial is of natural origin (Yue-Zhong Shu, 1998).Natural compounds can be lead compounds, allowing the design and rational planning of new drugs, biomimetic synthesis development and the discovery of new therapeutic properties not yet attributed to known compounds (Hamburger and Hostettmann, 1991). In addition, compounds such as yohimbine, physostigmine, forskolin, colchicines, cannabinoids, muscarine and phorbol esters all obtained from plants, are important tools used in physiological, pharmacological and biochemical studies (Williamson et al., 1996). Present drug discovery from plants have mostly relied on bioactivity guided fractionation and led to isolation of many important anticancer drugs such as camptothecin, paclitaxel etc.

India has been known to be rich repository of medicinal plants among ancient civilisations. The forests in India are the major repository of large number of aromatic and medicinal and, which are largely collected as raw materials for perfumery products and manufacture of drugs. An estimated number of 8,000 herbal remedies have been codified in AYUSH systems in India. Ayurveda, Unani, Siddha and Folk medicines are the major systems of indigenous medicines.

Among them, Ayurveda and Unani Medicine are the most developed and widely practised systems in India. Recently, WHO estimated that 80 percent of people worldwide rely on herbal medicines for some aspect of their primary health care needs. According to WHO, about 21,000 plant species have the potential for being used as medicinal plants. The National Cancer Institute, USA (NCI) has tested more than 50,000 plant samples for anti-HIV activity and 33,000 samples for anti-tumour activity. In 1993, the International Program of Co-operation for Biodiversity (IPCB) was launched in order to promote natural products in Latin America and Africa, linking universities, industries and Governments in a multidisciplinary programme for the sustainable development and preservation of the environment (Rouhi, 1997). Large pharmaceutical companies, such as Merck, CIBA, Glaxo, Boehringer and Syntex, now have specific departments dedicated to the study of new drugs from natural sources (Reid *et al.*, 1993). However, the potential use of higher plants as a source of new drugs is still poorly explored.

Secondary metabolites of the plants are performing role to treat disorders such as alkaloids, flavonoids, saponins, steroids, and terpenoids etc. Plants are also a source of aroma due to presence of some essential compounds in their body. Its types and concentration is variable among the plants and are useful in preparation of perfume. This aroma is also used in the treatment of many disorders based on their effectiveness and potential application.

Plants are a major source of timber, fuel, medicines for human beings. Due to above utility plants should be utilized in sustainable manner following better conservation strategies for their long term presence in nature. As our lifestyle is now getting techno savvy, we are moving away from nature. We cannot escape from nature because we are part of nature. As herbs are natural products they are free from side effects, they are comparatively safe, eco-friendly and locally available. Traditionally there are lot of herbs used for the ailments related to different seasons. There is a need to promote them to save the human lives.

There are about 250,000 to 500,000 species of plants on earth (Borris, 1996). Less than 10 percent are explored as food and fodder. There will be even

more plants are used for medicinal purposes (Moerman, 1996.). In the late 5th century, Hippocrates summed up 300 to 400 medicinal plants (Schultes, R. E. 1978). *De Materia Medica* (Dioscorides in the 1stcentuary), the prototype for modern pharmacopoeias catalogued and described approximately 30 healing plants. A large share of the documentation of the medicinal plant details being destroyed or lost with the intervention of Western advances in the understanding of medicinal plants during the ancient civilisations (Stockwell, C. 1988). The history of medicinal plant use by North America bifurcated into two, first indicates the use of plants by indigenous cultures in the pre-historical period (Weiner, 1980.) and the other signifies the use by Americans of European origin as alternative medicine in the 19th century. The use of plants as medicine by the native Americans were extensively reviewed and documented that 1625 species are used as food while 2564 plants have found use as drug (Moerman, D. E. 1996.). Marjorie Murphy Cowan, (1999) revised the use of plants and their products used as antimicrobial activities. The rapid rate of species extinction in the past two decades (Lewis, W. H., and M. P. Elvin-Lewis. 1995) demands the exploration of their medicinal value and development of conservative measures to protect them before extinction. Natural-products chemists and microbiologists believed that the multitude of potentially useful phytochemical structures which could be synthesized chemically is at risk of being lost irretrievably (Borris, R. P. 1996). Ethnobotany is the scientific discipline developed with the goal to utilise the knowledge assembled by ethnic people regarding the natural product used for their health care management in various level (Georges, M., and K. M. Pandelai. 1949, Rojas, A *et al,*. 1992, Silva, O *et al,*. 1996, Vanden Berghe, D. A., et al. 1986).

2.7 MEDICINAL PLANTS AND TRADITIONAL SYSTEM OF TREATMENT IN INDIA

Traditional healthcare system in India co evolved with the evolution of human being. Ayurveda have developed and slowly in practice between 2500 and 500 BC. The Ayurveda concept appeared and developed between 2500 and

500 BC in India (Subhose et al. 2005). It is based on the concept that balanced diets through the balanced food which is the major source for serving the nutritional needs. Modern life style gives up some traditional methods and adopts different food habit that affects the balanced nutrition. About 60 % of the global population are being using the alternative traditional medicine. It is not only prevalent in rural masses for their primary health care but also in developed countries where modern medicines dominate (B. Ballabh and O. P. Chaurasia, 2007). India is known for its vast diversity of life forms especially plants that are used in traditional medical treatments. Alternative health care systems are evolved from plants, minerals, organic matter, materials derived from animals and so on. Rural people of the country largely depend, about 70 percent on the traditional Ayurveda system of medicine. Different traditional healers prepare their own formulations and dispense to the ailing aspirants. The traditional medication is being popularised due to the attention of governmental agencies and NGO's with the intention that medicine derived from natural products are less harmful, not produce adverse drug reactions, cost effective, etc. that are the draw backs of modern medicines.

India is the largest producer of medicinal plants. There are currently about 250,000 registered medical practitioners of the Ayurveda system, as compared to about 700,000 of the modern medicine. In India, around 20,000 medicinal plants have been recorded; however, traditional practitioners use only 7,000–7,500 plants for curing different diseases. The proportion of use of plants in the different Indian systems of medicine is Ayurveda 2000, Siddha 1300, Unani 1000, Homeopathy 800, Tibetan 500, Modern 200, and folk 4500. In India, around 25,000 effective plant-based formulations are used in traditional and folk medicine. More than 1.5 million practitioners are using the traditional medicinal system for health care in India. It is estimated that more than 7800 manufacturing units are involved in the production of natural health products and traditional plant-based formulations in India, which requires more than 2000 tons of medicinal plant raw material annually (Pandey *et al.*, 2008). More than 1500 herbals are sold as dietary supplements or ethnic traditional medicines.

2.8 VALIDATION OF TRADITIONAL KNOWLEDGE

Traditional knowledge is an important element of the intellectual and cultural heritage of indigenous peoples. Many cultures throughout the world still rely on indigenous medicinal plants for their primary health care needs (Farnsworth *et al.,* 1985). Harshberger in 1895 coined the term ethno-botany to indicate plants used by the aboriginals. It included the study and evaluation of plant-human relations in all phases and the effect of plant environment on human society. Subsequently Schultes (1962) defined ethno-botany as "the study of the relationship which exists between people of primitive societies and their plant environment". Developing countries have long advocated for international protection of traditional knowledge while developed countries have resisted movement on the issues. Most of the international dialogue about the traditional knowledge has been taken place within the concept of intellectual property framework. As most of the diseases of modern society are life style disease and the use of herbal medicines can overcome such problems (Kumar *et al.,* 2008). Protections of the Traditional Knowledge of the local and indigenous groups appear to be a standout amongst the most argumentative and muddled issue. The chronicled advancement of the protection of intellectual property in the wake of individual private property rights, nudged, the traditional knowledge and the innovative practice focused around it outside the domain of the formal intellectual property protection system. Importance of the integration of modern medicine with the best of indigenous medicine is supported and advocated by the great visionaries, Jawaharlal Nehru (1950) and Indira Ghandhi (1973) when they were the Prime ministers of the India. For ensuring the standards of education and practice in the traditional systems of health care the government established a statutory body, Central Council of Indian Medicine in 1971.

The Arab physicians were influenced by the Ayurveda System and local Indian practitioners (Borins, 1987). As in other countries, the Arab physicians absorbed the best from the natural healing practices of the country. They learned about the various herbs and naturally occurring substances, and subjected them to

their own experiments and tests. It is striking to note that the Indian traditional knowledge especially related to indigenous treatment system is still not properly documented and scientifically evaluated. Most of this knowledge is conformed among local traditional levels and transferred only through mouth-to-mouth conversation. Due to the lack of proper documentation multinational companies in technically advanced developed countries are making profit by the way of introducing novel drugs, which have been developed based on our traditional knowledge. The story of neem is the best example for the bio-piracy. Three-fourths of the biologically active plant-derived compounds currently in use have been discovered through follow-up research to verify authenticity of data derived from traditional sources (Farnsworth, *et al.,* 1985). Current research continues to validate the importance of an ethno-botanically targeted approach to the initial discovery of therapeutics (Lewis *et al.,* 1999; Schuster, 2001). As many as three-quarters of plant-derived drugs used today are of traditional origin; such discoveries could generate a total value of $110 billion (Mendelsohn and Balick, 1995).

2.9 METHODS FOR SCREENING DRUG ACTIVITIES IN PLANT

Medicines derived from plants have played a pivotal role in the health care of many cultures, both ancient and modern (Newman *et al.,* 2003; Butler 2004; Balunas and Kinghorn 2005; Gurib-Fakim 2006; Newman and Cragg 2007). Different screening techniques have to be carried out to scientifically validate drug activities of medicinal plants.

2.9.1 High throughput screening

High-throughput screening has as its first objective the identification of a few 'Validated hits' within large compound libraries. The decision as to whether a particular hit is worth pursuing as a chemical lead in a drug discovery project depends on several factors, important ones being its chemical characteristics and its pharmacodynamic and pharmacokinetic properties. High-throughput screening (HTS) is a well-established process in lead discovery for pharma and biotech

companies and is now also being set up for basic and applied research in academia and some research hospitals (Mayr and Fuerst, 2008). The technology involved in miniaturization, automation and assay readouts required for HTS has developed rapidly and continues to do so. As this technology evolves, the laboratory set-ups installed in HTS facilities are steadily broadening their capabilities beyond their primary function of identifying hits to apply HTS techniques to more diverse compound profiling assays relating not only to the target selectivity of compound libraries, but also to their pharmacokinetic characteristics. HTS was first introduced by pharmaceutical companies in the 1990s and is now a routine process for identifying chemistry starting point for drug discovery programmes (Hill and Rang, 2009). Increasingly, therefore, early compound profiling tasks on 'hit' compounds are being carried out in HTS laboratories where the necessary technological expertise is concentrated. Such assays are also very helpful in the 'lead identification' stage of a project, where focused synthetic compound libraries based on the initial hits need to be assessed. As this work generally involves testing small compound libraries, usually fewer than 1000 compounds at a time, in several different assays, small dedicated robotic workstations are needed, rather than the fast but inflexible factory-style robotic assemblies used for large-scale HTS.

In addition, HTS laboratories are continually evaluating new technologies as they struggle to increase their success rate for finding drug candidates (Sandra *et al.*, 2006). This extension of the work of HTS laboratories beyond the primary task of finding hits is a clear and continuing trend, for which the term 'high-throughput profiling' (HTP) has been coined. It brings the work of HTS laboratories into a close and healthy relationship with drug discovery teams. The highly disciplined approach to assay formats and data logging that is essential for HTS, but not second nature to many laboratory scientists, brings the advantage that profiling data collected over a wide range of projects and drug targets is logged in standard database formats, and is therefore a valuable company-wide tool for analysing structure–activity relationships. This is necessity to handle and visualize such data has driven the development software packages such as

Spotfire (spotfire.tibco.com).In summary, it is clear that pharmacological profiling will be an increasing activity of HTS units in the future, and will help to add further value in the drug discovery chain.

2.9.2 *In silico* screening

Imagine evaluating the efficacy and safety of new drugs without having to lift a pipette. Advances in *in silico* drug development are beginning to make this possible. *In silico* drug design can play a significant role in all stages of drug development from the preclinical discovery stage to late stage clinical development. It helps in selecting only a potent lead molecule and may thus prevent the late stage clinical failures; thereby a significant reduction in cost can be achieved (Bharath*et al.,* 2011). Ekins *et al.* (2006) has briefly described the history and development of *in silico* pharmacology. The applicability of computational approaches to ligand and target space in which a lead molecule against one gene family member is used for another similar target (termed chemogenomics) has been reviewed by Morphy *et al.* (2004) and Sharom *et al.* (2004). Briefly the types of proteins that have been modelled and the methods used were reviewed by Ekins *et al.* (2006). The selection process on the virtual (*in silico*) screening was well reviewed by Hill and Rang, (2009). They can also be used to analyze the target structures for possible binding or active sites, generate candidate molecules, and check for their drug likeness. The advantages and disadvantages of *in silico* methods with respect to *in vitro* and *in vivo* methods for pharmacology research have already reported by Ekins *et al.* (2006). A new advanced protein-modelling module predicts the structure of proteins of unknown structure using related proteins of known structure as the models. "It rapidly and accurately provides a valuable protein structure to be used for *in silico* docking experiments," says Kathleen Mensler, vice-president of marketing and corporate development at Tripos. Another new product, Surflex-Sim, helps find new classes of compounds that are structurally similar to already known, suboptimal, lead compounds, but that may be more active or safer.

A pioneer of *in silico* modelling, *De Novo* Pharmaceuticals in Cambridge, UK, recently announced a new focus for its drug-discovery programme - finding drug targets in various metabolic diseases. *De Novo* has upgraded SkelGen, its proprietary *in silico* drug-design platform, with the addition of Reflex, which takes into account the rotational flexibility of amino acids in the target's active site. This better evaluates the shape of the active site and its interaction with small-molecule ligands, so that the modelling more closely resembles the dynamics of real protein -ligand interactions.

2.9.3 In vitro screening

In vitro screening systems and high-throughput screening are critical aspects of the modern drug discovery process. In order to make a safety assessment in the early stage of drug discovery, there is a hurdle to jump over the existing traditional toxicological studies (Horii and Yamada, 2007). Prior to the advent of these technologies, screening candidate compounds for biological activity was a major bottleneck in the identification of novel therapeutics. By the turn of the twenty-first century, however, *In vitro* screening methods had become capable of generating data on hundreds to thousands of compounds per day, greatly enhancing the acquisition of biological data. Detecting the presence of radiolabel material, as we are monitoring changes in fluorescence or absorbance can be used in conjunction with microtiter plates, advanced robotics, and sophisticated software to determine the biological activity of a candidate compound. "Label-free" systems have also been developed as a means of identifying potential useful therapeutic agents without the need to perturb the biological systems of interest.

2.9.4 *In vivo* screening

In *in vivo* studies the effects of various biological entities are tested on whole, living organisms. *In vivo* screening in drug discovery was well reviewed by (Wienkers and Heath' 2005) *In vivo* screening of crude extract of plants and pure secondary metabolites/constituent chemical molecules is essential for the validation of the efficacy of the medicinal property of a herb or its preparations.

2.10 VIRTUAL SCREENING

Virtual screening (VS) is a computational technique used to identify from a large library of compounds those that bind to a specific target, usually an enzyme or receptor. It uses computer-based methods to discover new ligands on the basis of biological structures. Virtual screening is usually approached hierarchically in the form of a workflow, sequentially incorporating different methods, which act as filters that discard undesirable compounds. This makes it possible to take advantage of strengths and avoid limitations of the individual methods. In virtual screening, compounds are docked into a 3D model of structurally defined biological target and the binding energy of the resulting complex is estimated, allowing compounds to be rank-ordered. This technique has provided most successful where the target structure has been determined at high resolution (e.g. By X-ray crystallography). Virtual screening does not, need physical test samples, or even previously synthesised compounds. Information about the phytochemicals (chemical libraries) and their structural & pharmacological details are well documented in various online databases such as PubChem, Chemspider, Dictionary of Natural Products etc. Various bioinformatics tools for creating, editing and analysing the structure of chemical molecules *viz.* ChemSketch, CORINA etc. are also freely available on net. Structure based and Ligand based Virtual screening approaches are in practice. Virtual screening strategies in drug discovery was well described by Jain (2004);Walters *et al,* (1998); Kitchen *et al.* (2004); Reddy *et al.* (2007); Schneider (2010); Lavecchia.

2.10.1 Docking

Docking is a term used for computational schemes that attempt to predict the structure of the intermolecular complex formed between two or more constituent molecules: a receptor and a ligand (Sousa *et al.*, 2013). According to Kitchen *et al.,* (2004) docking is frequently used to predict the binding orientation of small molecule drug candidates to their protein targets in order to in turn predict the affinity and activity of the small molecule. A protein molecule from

Mycobacterium tuberculosis is selected here as the target and phytochemicals obtained from the selected plants were used as ligands in the present study. Docking of receptor with ligand and the formation of its complex was well explained by Mihasan, (2012). The protein structure and a database of potential small molecules of drug value (ligands) serve as inputs to a docking program. The success of a docking program depends on two components: the search algorithm and the scoring function. The first docking program was developed by the Kuntz group and named DOCK (Kuntz *et al.*, 1982). The commonly used docking software are AutoDock, DOCK, Gold, V Life MDS and Flex X (Mukesh and Rakesh, 2011). Citations towards different docking software were explained by Mihasan (2012).

2.10.2 PubChem

PubChem is an open chemistry database at the National Institutes of Health (NIH) and was first released in 2004 as a large repository of three interlinked databases Substance, Compound and BioAssay. The Substance database contains chemical information (more than 200 million entries, while Compound contains the actual chemical structure data (more than 90 million entries) derived from the Substance database. BioAssay contains all of the biological activity data that has been deposited with PubChem, currently over 1 million entries, with more than 230 million bioactivities. The data is provided to PubChem by well over 500 contributors. PubChem mostly contains small molecules, but also larger molecules such as nucleotides, carbohydrates, lipids, peptides, and chemically-modified macromolecules. PumChem collect information on chemical structures, identifiers, chemical and physical properties, biological activities, patents, health, safety, toxicity data, and many others.
PubChem is available in the URL: https://pubchem.ncbi.nlm.nih.gov

2.10.3 ChemSpider

ChemSpider is a free chemical structure database providing fast text and structure search access to over 67 million structures from hundreds of data

sources. By integrating and linking compounds from hundreds of high-quality data sources, ChemSpider enables researchers to discover the most comprehensive view of freely available chemical data from a single online search. It is owned by the Royal Society of Chemistry. ChemSpider builds on the collected sources by adding additional properties, related information, and links back to original data sources. ChemSpider offers text and structure searching to find compounds of interest and provides unique services to improve this data by curation and annotation, and to integrate it with users' applications. ChemSpider is available on the URL: www.chemspider.com

2.10.4 Dictionary of Natural Products

The Dictionary of Natural Products is a comprehensive source of chemical data on natural products. It provides the busy scientist with fast access to chemical, physical, bibliographic, and structural data on over 139,000 natural products organized into more than 43,000 -virtually every natural product isolated and reported in the literature. Dictionary of Natural Products is available on the URL: https://dnp.chemnetbase.com

2.10.5 ChemSketch

ACD/ChemSketch Freeware is a drawing package that allows you to draw chemical structures including organics, organo-metallics, polymers, and Markush structures. It also includes features such as calculation of molecular properties (e.g., molecular weight, density, molar refractivity etc.), 2D and 3D structure cleaning and viewing, functionality for naming structures (fewer than 50 atoms and 3 rings), and prediction of log*P*. The freeware version of ChemSketch does not include all of the functionality of the commercial version. ChemSketch is available for download from the URL: https://chemsketch.xtremedownload.com

2.10.6 CORINA

Pro. Johann Gasteiger and his research group at the University of Erlangen, Germany developed a computer program for generating three-dimensional structures of molecules by certain coordinates called CORINA. It is a powerful and reliable tool for the conversion of 2D structures to 3D compounds. CORINA is available on the URL: www.molecular-networks.com/onlinedemos/corinademo.

2.11 POST DOCKING ANALYSIS

Post docking analysis is the critical part in the hit/lead identification process. Dampster-Shafer Theory (DST) was used to select the high top ranked compounds (molecules with least free energy of binding) from different docking. The use of DST to select the high ranking top compounds for further analysis and consideration was well reviewed by Rao *et al.* (2013). DST is a mathematical theory of evidence. The theory was first developed by Arthur P. Dempster and Glenn Shafer (Fine, 1977).

2.12 SELECTED PLANTS

Kerala is blessed with a number of globally accepted unique vegetables which have been used in the traditional systems of medicine for curing many ailments particularly diseases affecting human respiratory system.

2.12.1 *Allium cepa* L.

Onion (*Allium cepa* L.) has been valued as a food and a medicinal plant since ancient times. It is a widely cultivated plant second to tomato, and is a vegetable bulb crop known to most cultures and consumed worldwide (FAO, 2012). Onion is a short duration horticultural crop (Brewster, 1990) grown at low latitudes. It is known as "Queen of the kitchen", due to its highly valued flavor, aroma, and unique taste, and the medicinal properties of its flavour compounds (Selvaraj, 1976; Griffiths et al., 2002). Onion is used throughout the year in every home especially in curries in the form of spices, in salads, as a condiment, or

cooked with other vegetables, such as boiled or baked. It is also used in different forms of processed food, e.g. pickles, powder, paste, and flakes, and it is known for its medicinal values. Onion was considered to be the main cuisine in India long centuries ago. Both Ayurveda and Greek system of medicine were demonstrated onion as a promising medicinal plant. *Allium cepa* shows effective results against various antimicrobial, anti-cancer, anti-oxident, antidiabetic, anti-cardiovascular and anti-mutagenic studies (Upadhyay, 2016).

2.12.2 *Allium* sativum L.

Garlic, *Allium sativum* L. is a member of the Alliaceae family, has been widely recognized as a valuable spice and a popular remedy for various ailments and physiological disorders. The name garlic may have originated from the Celtic word 'all' meaning pungent. Cultivated practically throughout the world, garlic appears to have originated in central Asia and then spread to China, the Near East, and the Mediterranean region before moving west to Central and Southern Europe, Northern Africa and Mexico (Singh and Singh 2008). Interest in garlic among researchers, particularly those in medical profession, has stemmed from the search for a drug that has a broad-spectrum therapeutic effect with minimal toxicity. Recent studies indicate that garlic extract has antimicrobial activity against many genera of bacteria, fungi and viruses. Antimicrobial activity of garlic is due to the activity of allicin (allyl 2-propene thiosulfinate); a notable flavonoid in garlic is formed when garlic cloves are crushed (Garba*et al.*, 2013). The role of garlic in preventing cardiovascular disease has been acclaimed by several authors. Chemical constituents of garlic have been investigated for treatment of hyperlipidemia, hypertension, platelet aggregation and blood fibrinolytic activity. Experimental data indicate that garlic may have anti-carcinogenic effect. Recent researches in the area of pest control show that garlic has strong insecticidal, nematicidal, rodenticidal and molluscicidal activity. Despite field trials and laboratory experiments on the pesticidal activity of garlic have been conducted, more studies on the way of delivery in environment and mode of action are still recommended for effective control of pest. Adverse

effects of oral ingestion and topical exposure of garlic include body odor, allergic reactions and acceleration in the effects of anticoagulants and reduction in the efficacy of anti-AIDS drug Saquinavir.

2.12.3 *Moringa oleifera* Lam.

Moringa oleifera is a medium-size fast-growing draught resistant, graceful, deciduous tree. It belongs to the family Moringaceae with the lone genera Moringa. It is commonly known as drumstick tree, horseradish tree or benzoil tree in English. Moringa is the only genus in the family. This plant is native to sub Himalayan regions of India, Pakistan, Bangladesh and Afghanistan (Amit Kumar Dutta, 2017). Moringa is very common in Kerala and is widely cultivated in various subtropical and tropical areas of the world. Moringa is a rich source of vitamins, minerals, and amino acids. It contains significant amounts of vitamin A, C and E; Calcium; Potassium and Protein (Amit Kumar Dutta, 2017). The plant is a rich source of unique secondary metabolites like glucosinolates, flavonoids, phenolic acids, carotenoids, polyunsaturated fatty acids etc. The potent antioxidant activity the plant is attributed to the high concentration of these polyphenols. (Ramesh Kumar Saini et al., 2016). Moringa is used for different purposes as food, fodder, medicine, coagulant and gum, etc. It is also used for fencing and firewood (K. Gandji *et al.* 2018).

2.13 *MYCOBACTERIUM TUBERCULOSIS*

Mycobacterium tuberculosis (Mtb), an etiologic agent of tuberculosis (TB), infects around one-third of world population and kills millions of people annually (WHO, 2011). MTB was first described on 24 March 1882 by Robert Koch, who subsequently received the Nobel Prize in physiology or medicine for this discovery in 1905 (Nobel Foundation, 2008). The high lipid content of this pathogen accounts for many of its unique clinical characteristics (Southwick, 2007). The most frequently used diagnostic methods for TB are the tuberculin skin test, acid-fast stain, and chest radiographs (Kassim and Ray, 2004). Cole et al. (1998) and Camus et al, (2002) were well explained about the genomic

sequencing of MTB. Strain variation and evolution was explained in detailed by Gagneux (2009).

2.14 TARGET PROTEIN

An enzyme, Decaprenyl phosphoryl-β-D-ribose 2-Epimerase-I (DprEI), has shown considerable promise as a drug target due to its vital importance in mycobacterial cell wall biosynthesis (Crellin *et al.*, 2011; Caroll *et al.*, 2012). Previous inhibition of DprE1 has induced cell death ((Neres *et al.*, 2012). The epimerization of Decaprenyl Phosphoryl Ribose (DPR) to Decaprenyl Phosphoryl Arabinose (DPA) is initially catalysed by oxidoreductase DprE1 and subsequently by reductase DprE2. The inhibition of DprE1 prevents the formation of DPA, which is an essential donor substrate for M. tuberculosis's cell wall biosynthesis. There is no known alternative pathway for synthesis of DPA, thereby making DprE1 a promising drug target (Makarov et al., 2009). The enzyme can be partitioned into two distinct domains; an FAD binding domain and substrate binding domain. The two domains are situated face to face to facilitate the interaction between the substrate and FAD.

DprE1 was virtually screened against 4.1 million compounds from several diverse libraries using the molecular docking program, AutoDockVina (Trott and Olson, 2010; Franco et al., 2013). The recently solved co-crystal structure of DprE1 and CT319 (PDB ID: 4FDO) was adopted for the screening process (Batt et al., 2012). This enzyme is emerging as one of the most vulnerable target in M. Tuberculosis (Riccardi *et al.*, 2013). The biosynthesis pathway of decaprenylphosphoryl arabinose in mycobacteria was well explained by Wolucka (2008).

2.15 RELEVANCE OF THE PRESENT STUDY

Following nearly three decades of neglect, there is now renewed interest in the development of new drugs for the treatment and prevention of tuberculosis (O'Brien and Nunn, 2001). Medicinal plant drug discovery continues to provide new and important leads against various pharmacological targets including cancer,

HIV/AIDS, Alzheimer's, malaria, tuberculosis and pain (Balunas and Kinghorn, 2005). Drug discovery from medicinal plants continues to provide an important source of new drug leads, numerous challenges are encountered including the procurement of plant materials, the selection and implementation of appropriate high-throughput screening bioassays, and the scale-up of active compounds (Balunas and Kinghorn, 2005). Molecular docking has become an increasingly important tool for drug discovery (Meng et al., 2011).

MATERIALS AND METHODS

3. MATERIALS AND METHODS

3.1 PLANTS SELECTED FOR THE STUDY

To validate anti-tuberculosis activity and identification of lead compounds most common vegetables with pulmonary protective activity such as *Allium cepa* L., *Allium sativum* L. and *Moringa oleifera* Lam. were selected for the study (Figure-1.a-e).

Figure-1: Plant parts used: a. *Moringa oleifera*, b. *Allium sativum* bulbs, c. *Allium sativum* plants, *d. A. cepa* bulbs, e. *A. cepa* plants

43

3.2 *IN SILICO* SCREENING

3.2.1 Source of phytochemical structure

Information regarding the chemical molecules (phytochemicals) reported in the selected spices were collected through extensive literature survey and from 'Dr. Duke's Phytochemical Database'. The canonical SMILES of these phytochemicals were retrieved from chemical databases such as PubChem, Chemspider and Dictionary of Natural Products. The three dimensional structures (3D) of these phytochemicals were created using the online software CORINA. The structures of phytochemicals which are not available on databases were created using ChemSketch.

3.2.2 Dr. Duke's phytochemical and ethnobotanical databases

The Dr. Duke's Phytochemical and Ethnobotanical Database is an online database developed by James A. Duke at the USDA which provides information on species, phytochemicals, and biological activity, as well as ethnobotanical uses. The current Phytochemical and Ethnobotanical databases facilitate plant, chemical, bioactivity, and ethno-botany searches. A large number of plants and their chemical profiles are covered, and data are structured to support browsing and searching in several user-focused ways.

A) Plant Searches

- Chemicals and activities in a particular plant.
- High concentration chemicals.
- Chemicals with one activity.
- Ethnobotanical uses.

B) Chemical Searches

- Plants with a chosen chemical.
- Activities of a chosen chemical.

C) Activity Searches

- Plants with a specific activity.
- Search for plants with several activities.

- Chemicals with a specific activity.
- Chemicals with a lethal dose (LD) value.

D) Ethnobotany Searches

- Ethnobotanical uses for a particular plant.
- Plants with a particular ethnobotanical use.

E) Database References

- Reference citations.

The database is available on the URL: www.ars-grin.gov/duke

3.3 TARGET MOLECULE SELECTION (PROTEIN)

3.3.1 *Mycobacterium tuberculosis* DprE1 protein

Different proteins and peptides are contributing the physiological properties of *Mycobacterium tuberculosis.* Out of the 40 proteins present 16 are lethal. The selected target molecule for *in silico* screening was Decaprenylphosphoryl-β-D-ribose epimerase (DprE1) (Figure-2) which plays a key role in the synthesis of arabinan, a major component in bacterial cell wall and responsible for virulence in bacteria. The three dimensional structure of DprE1 (4FDO;Riccardi *et al.,* 2013) was retrieved from Protein Data Bank (PDB id 4FDO).

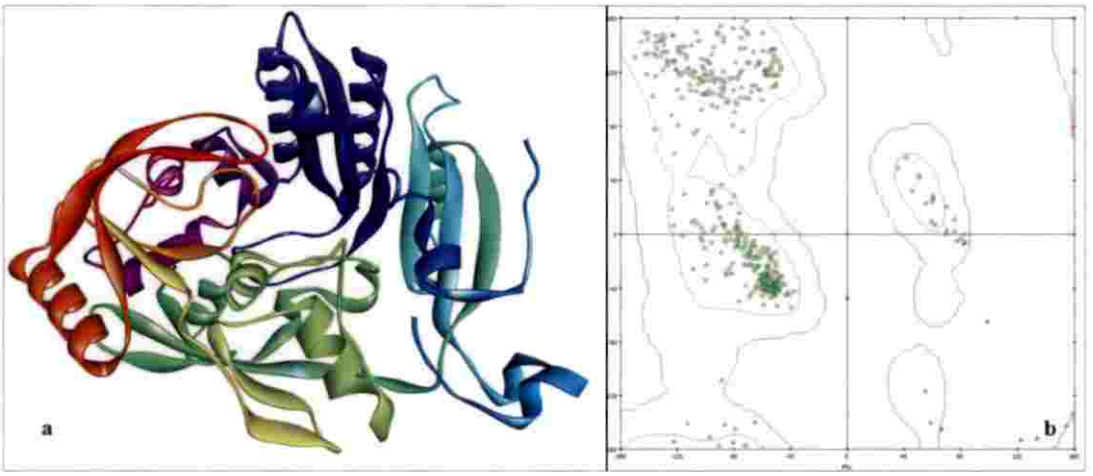


Figure-2: Target protein: DprE1 (PDB ID: 4FDO).

a) 3D structure of the protein.

b) Ramachandran plot of the protein

3.4 SOURCE OF TARGET MOLECULE

3.4.1The Protein Data Bank (PDB)

Protein Data Bank (PDB) is the single worldwide archive of structural data of biological macromolecules. It includes data obtained by X-ray crystallography and nuclear magnetic resonance (NMR) spectrometry submitted by biologists and biochemists from all over the world. Presently, PDB is under the purview of the Worldwide Protein Data Bank (wwPDB), a network of four organizations - Research Collaboratory for Structural Bioinformatics (RCSB) PDB (USA), PDB in Europe (PDBe) (Europe), PDB Japan (PDBj) (Japan), and the Biological Magnetic Resonance Data Bank (BMRB) (USA) – whose mission is to "maintain a single Protein Data Bank Archive of macromolecular structural data that is freely and publicly available to the global community." Currently, more than 83,900 biological macromolecular structures have been deposited in PDB. The database is freely accessible at www.rcsb.org/.

3.4.2 PDBsum

The PDBsum is a pictorial database that provides an at-a-glance overview of the contents of each 3D structure deposited in the Protein Data Bank (PDB).It shows the molecule(s) that make up the structure (*ie* protein chains, DNA, ligands and metal ions) and schematic diagrams of their interactions. Extensive use is made of the freely available RasMol molecular graphics program to view the molecules and their interactions in 3D.URL: http://www.ebi.ac.uk/pdbsum/

3.5 DOCKING TOOLS

Binding affinity and the free energy calculation of the ligand-protein complex is done using AutoDock which helps to dock the selected phytochemicals against the target protein. The phytochemicals having value

greater than 5 kcal $mol^{-1}$of binding affinity were further docked with other docking tools such as iGEMDOCK, HEX Server, FireDock and SwissDock to identify true hit molecule. The scores obtained were statistically analysed following Dempster-Shafer Theory (DST) and identified best leads.

3.5.1 AutoDock (Figure-3)

AutoDock is a suite of automated docking tools. It is designed to predict how small molecules, such as substrates or drug candidates, bind to a receptor of known 3D structure. Current distributions of AutoDock consist of two generations of software: AutoDock-4 and AutoDockVina. AutoDock-4 actually consists of two main programs: *AutoDock* performs the docking of the ligand to a set of grids describing the target protein; *autogrid* pre-calculates these grids. In addition to using them for docking, the atomic affinity grids can be visualised. This can help, for example, to guide organic synthetic chemists design better binders. AutoDockVina does not require choosing atom types and pre-calculating grid maps for them. Instead, it calculates the grids internally, for the atom types that are needed, and it does this virtually instantly. Application of AutoDock ranges from X-ray crystallography; structure-based drug design; lead optimization; virtual screening (HTS); combinatorial library design; protein-protein docking to chemical mechanism studies etc. AutoDock is freely downloadable from the URL: www.autodock.scripps.edu/.

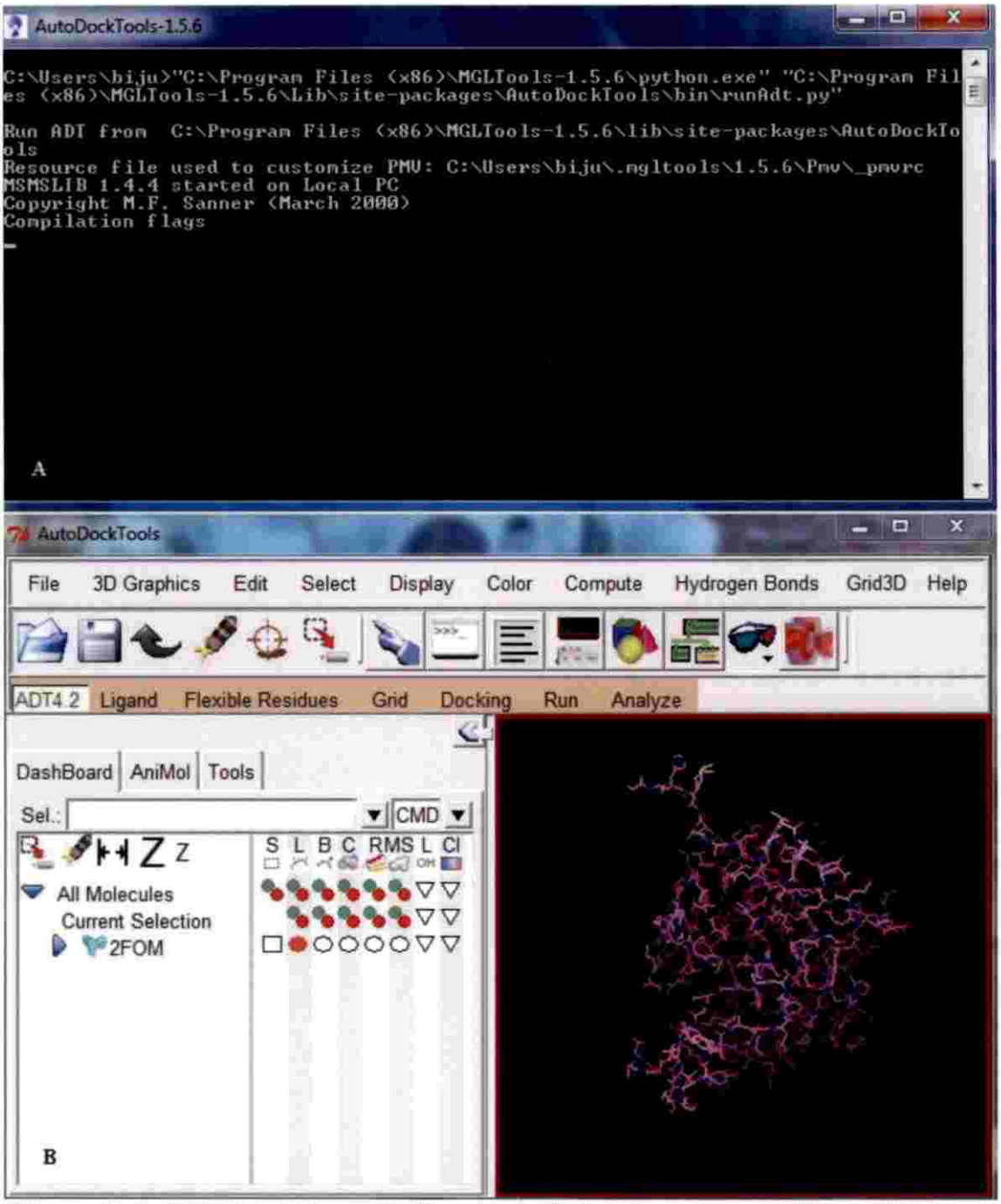


Figure-3: AutoDock windows: A. Terminal window for command line and B. Autodock tool (ADT)

3.5.2 iGEMDOCK

Generic Evolutionary Method for molecular docking iGEMDOCK is a program for computing a ligand conformation and orientation relative to the

active site of target protein. iGEMDOCK is a Graphical Environment for Recognizing Pharmacological Interactions and Virtual Screening. Pharmacological interactions are useful for identifying lead compounds and understanding ligand binding mechanisms for a therapeutic target. iGEMDOCK is available for free on non-commercial researches. iGEMDOCK is freely downloadable from the URL: www.gemdock.life.nctu.edu.tw/dock/igemdock.php

3.5.3 Hex Server

Hex is an interactive protein docking and molecular superposition program. It is an online application and completely free service with no login or registration is required. The docked results will send *via.* E-mail. URL:http://hexserver.loria.fr/

3.5.4 FireDock

The FireDock server addresses the refinement problem of protein-protein docking solutions. The method simultaneously targets the problem of flexibility and scoring of solutions produced by fast rigid-body docking algorithms. Given a set of up to 1000 potential docking candidates, FireDock refines and scores them according to an energy function, spending about 3.5 seconds per candidate solution. To the best of our knowledge, this is the first web server that allows performing large-scale flexible refinement and scoring of docking solutions online. URL: www.bioinfo3d.cs.tau.ac.il/FireDock/

3.5.5 SwissDock

SwissDock is a web service to predict the molecular interactions that may occur between a target protein and a small molecule. It is based on the EADockDSS engine, combined with setup scripts for curating common problems and for preparing both the target protein and the ligand input files. An efficient Ajax/HTML interface was designed and implemented so that scientists can easily submit dockings and retrieve the predicted complexes. URL: www.swissdock.ch

3.5.6 Molecular docking

All selected phytochemicals were docked into the binding site of mycobacterial virulence protein DprE1 using the open access software application tool, AutoDock 4.2. The active sites of the molecules were detected using the database PDBsum. The docking was performed following the AutoDock procedure (Morris *et al.*, 2009). This tool use Monte Carlo Simulated Annealing and Lamarckian genetic algorithm for the generation of possible orientations of ligand at the binding site of target protein. The grid spacing was set to 0.375 Å. The grid was centred on the active site and XYZ-coordinates of the macromolecules were 29.645 Å, 13.35 Å and 13.134 Å respectively. For docking, all the parameters were kept as default including population number. The ligand bound complexes were analyzed for its binding affinity and possible orientations were ranked according to their lowest binding energy through cluster analysis. The top ranked molecules with free energy of binding $\leq$-5 kcal mol^{-1} were considered as hit molecules and they were further analyzed by Lipinski's rule of five.

The result obtained after AutoDock were analyzed based on free energy of binding. Top ranked molecules were selected as hit molecules and were subjected to further docking using iGEMDOCK, Hex server, FireDock and SwissDock. This was to reduce the errors in single scoring scheme and to improve probability of identifying true hits. The results obtained from the five software applications were subjected to statistical analysis using Dampster Shafer Theory (DST) and lead molecules were selected. The orientations of lead molecules at the active site of DprE1 were visualized using PyMOL software.

3.6 BIOACTIVITY AND DRUGLIKENESS PREDICTION

Biological activity of the lead molecules against different class of target molecules GPCR ligand, ion channel modulator, kinase inhibitor, nuclear receptor ligand, protease inhibitor and enzyme inhibitory activity were analyzed using the tool molinspiration. Druglikeness score is predicted using the tool MolSoft. Input

for the analysis was the SMILES notation or CAS Number/ IUPAC name/InChI/InChIKey.

3.7 ADMET ANALYSIS OF HIT MOLECULES

The Bioinformatics tool pkCSM was used to predict the Absorption, Distribution, Metabolism, Excretion and Toxicity (ADMET) properties, where the input files are the SMILES notation. For studying the absorption of the ligand, CaCO2 permeability, skin permeability, P-glycoprotein substrate inhibitors were checked. Regarding distribution, volume of distribution in humans, BBB (Blood Brain Barrier) & CNS (Central Nervous System) permeability are determined. The metabolic parameters include cytochrome P450 substrate and inhibitors. Renal OCT2 substrate and total clearance are predicted as excretion parameters. AMES toxicity for analysis of mutation, skin sensitization, hepato toxicity and oral rat acute toxicity were checked.

RESULTS

4. **RESULTS**

To validate the anti-tubercular activity of the plants phytochemicals present in each plant were allowed to interact with an essential biological target Decaprenylphosphoryl-beta-D-ribose epimerase (DprE1) through *in silico* methods followed by *in silico* analysis of the activity of the plant *in toto* using crude extracts.

4.1 *IN SILICO* SCREENING

4.1.1 Selection of target molecule

Decaprenylphosphoryl-beta-D-ribose epimerase (DprE1), an enzyme responsible for the synthesis of arabinan, the virulent factor in *Mycobacterium tuberculosis* was selected as the target molecule. The 3-D structure of the molecule was retrieved from PDB (PDB id 4FDO, Figure-2). It consists of 481 amino acids, 26 β sheets, 20 α helices, 293 H-bonds. It involves the biosynthesis of decaprenylphosphoryl-β-D-arabinose from decaprenylphosphoryl-β-D-ribose (DPR). It is an FAD dependent oxidation of DPR to decaprenylphosphoryl-2-keto-D-erythro-pentofuranose (DPX) and then converts to decaprenylphosphoryl-β-D-arabinose (DPA) which is the sole donor of arabinose sugars that are essential for cell wall biosynthesis in *M. tuberculosis*. Here the epimerisation of DPR to DPX is catalysed by DprE1.

$$\text{DPR} \xrightarrow[\text{DprE1} + \text{FAD} + \text{H}^+]{} \text{DPX} + \text{FADH}_2$$

4.1.2 Detection of active site

The active site of the target protein Decaprenylphosphoryl-beta-D-ribose epimerase was detected using the tool PDBsum. In the active site following residues were detected Leu 371, Trp 230, Fad 900, Lys 418, Tyr 60, Cys 387, Ans 385, Val 365, Gly `117, Lys 134, Gly 321 and Phe 320 (Figure-4).

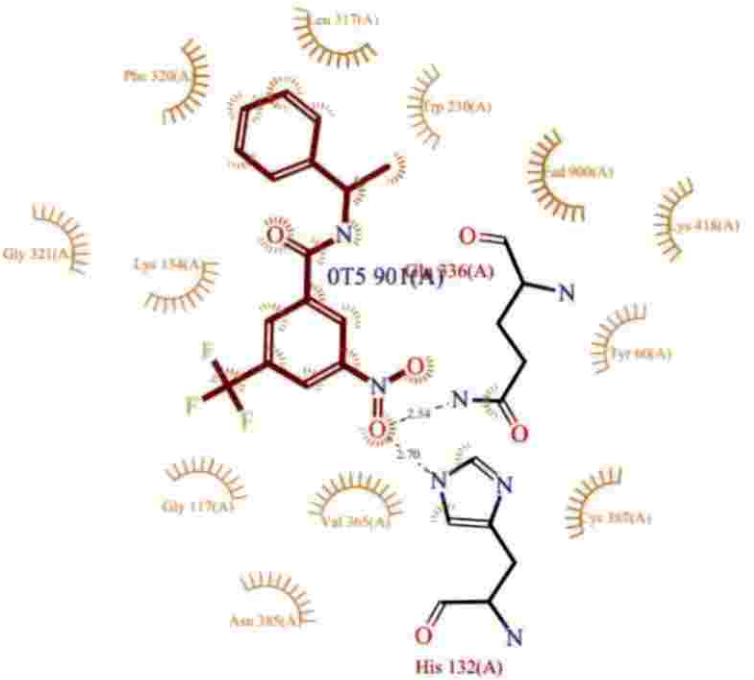


Figure-4: The active site of the target protein (DprE1) detected using the tool PDBsum.

4.1.3 Ligand preparation

After a thorough understanding on number of atoms (< 1280), molecular weight (≤ 500 ± 10g/mol) and number of rotational bonds (< 32) in the small phytomolecules reported from the selected three plants were screened and prepared for docking analysis. The list of selected phytochemicals with their molecular formula, molecular weight and canonical smiles retrieved from the open access databases for each plant were depicted in Appendix I, II, and III respectively. The structures of phytochemicals that are not in any of the literature sources & databases were created based on canonical smiles using the tool ChemSketch that are shown in Figure 5.

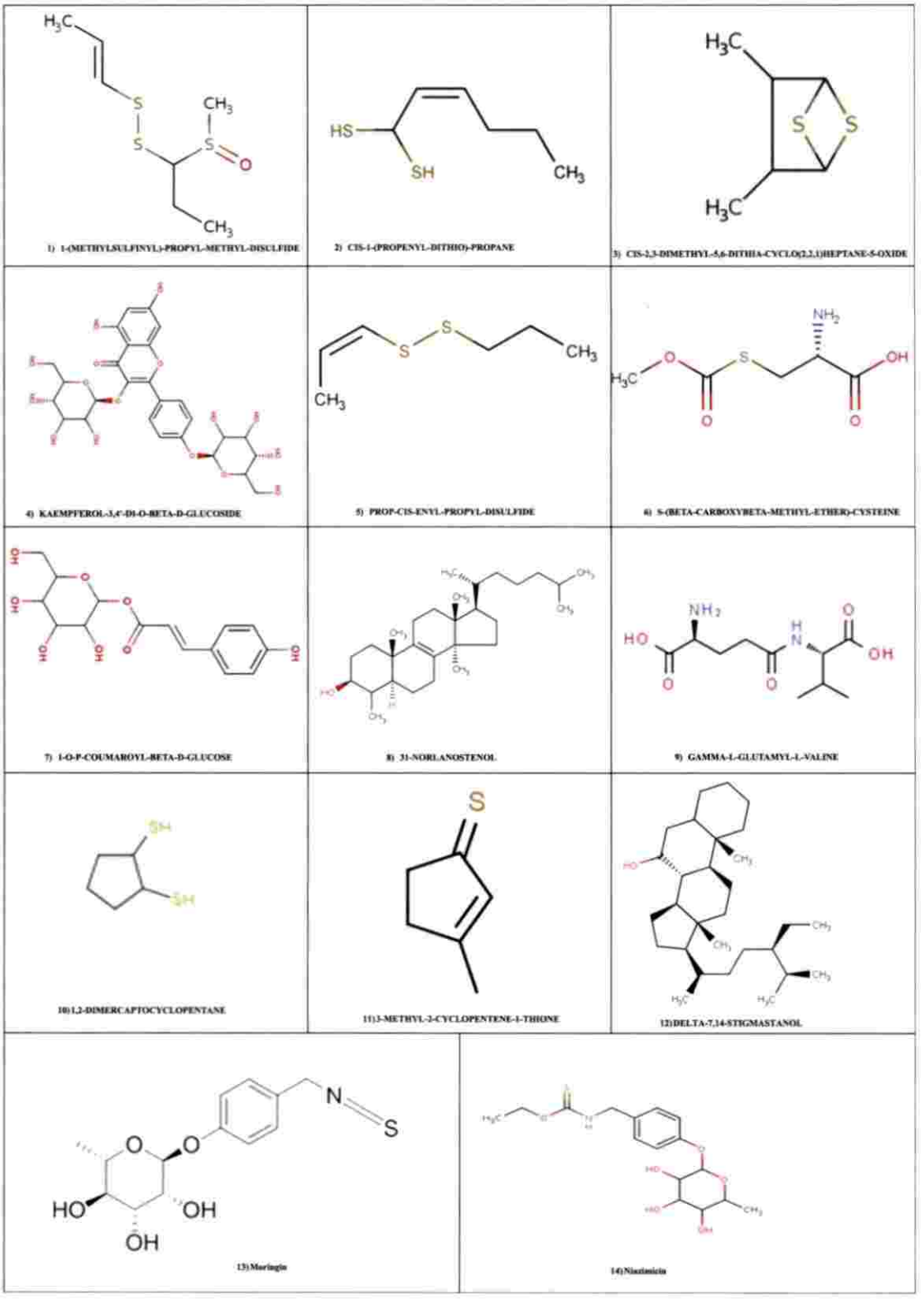


Figure-5: Structure of phytochemicals created using ChemSketch. 1-8 (*Allium cepa*), 9-11 (*Allium sativum*), 12-14 (*Moringa oleifera*)

55

4.2 DOCKING

4.2.1 Phytochemicals from *Allium cepa* and DprE1 protein

In order to find out active hits, competent 81 small molecules from *Allium cepa* were docked with DprE1, the biological target from the bacteriausing the tool AutoDock. The docked molecules having free energy of binding (ΔG_{bind}) $\leq$ -5 kcal/mol were selected as hit molecules. The total docked result is depicated in the table as annexure-4. Seven out of the total 81 molecules showed free energy of binding less <-5 kcal/mol and are considered them as active small molecules against the target. For further analysis to ensure the most suitable hits and nullify the false negative candidates five molecules with least free energy level from the active compounds were selected. The selected hit molecules with free energy of binding, inhibition constant, number of hydrogen bonds, bond type & bond length (Å) are depicted in the table below (Table-1). Interaction between the target with the hit is also depicted in the Figure-6.

Sl. No.	Phytochemical	ΔG_{bind}(kcal/mol)	Inhibition Constant (µm)	H bonds (bond length)	Hydro-phobic residues
1	24-methylene-cycloartenol	-10.81	0.012	No Hbond	16
2	4-alpha-methyl-zymostenol	-9.66	0.0827	No Hbond	16
3	campesterol	-7.12	6.07	No Hbond	16
4	1-o-ferulyoyl-beta-d-glucose	-6.15	30.84	Ligand:O24-Gly117:O(3.05) Ligand:O25-Tyr415:OH(2.94) Ligand:O3- Tyr415:OH(2.72) Ligand:O33-Thr118:OG1(2.76) Ligand:O33-Arg 58:NE(2.82)	9
5	5-octyl-cyclopenta-1,3-dione	-6.14	31.36	Ligand:O1-His132:N(3.14) Ligand:O1-TYR415:OH(2.75) Ligand:O5 –GLY117:N(3.01)	8

Table-1: details of hit molecules obtained from *Allium cepa*

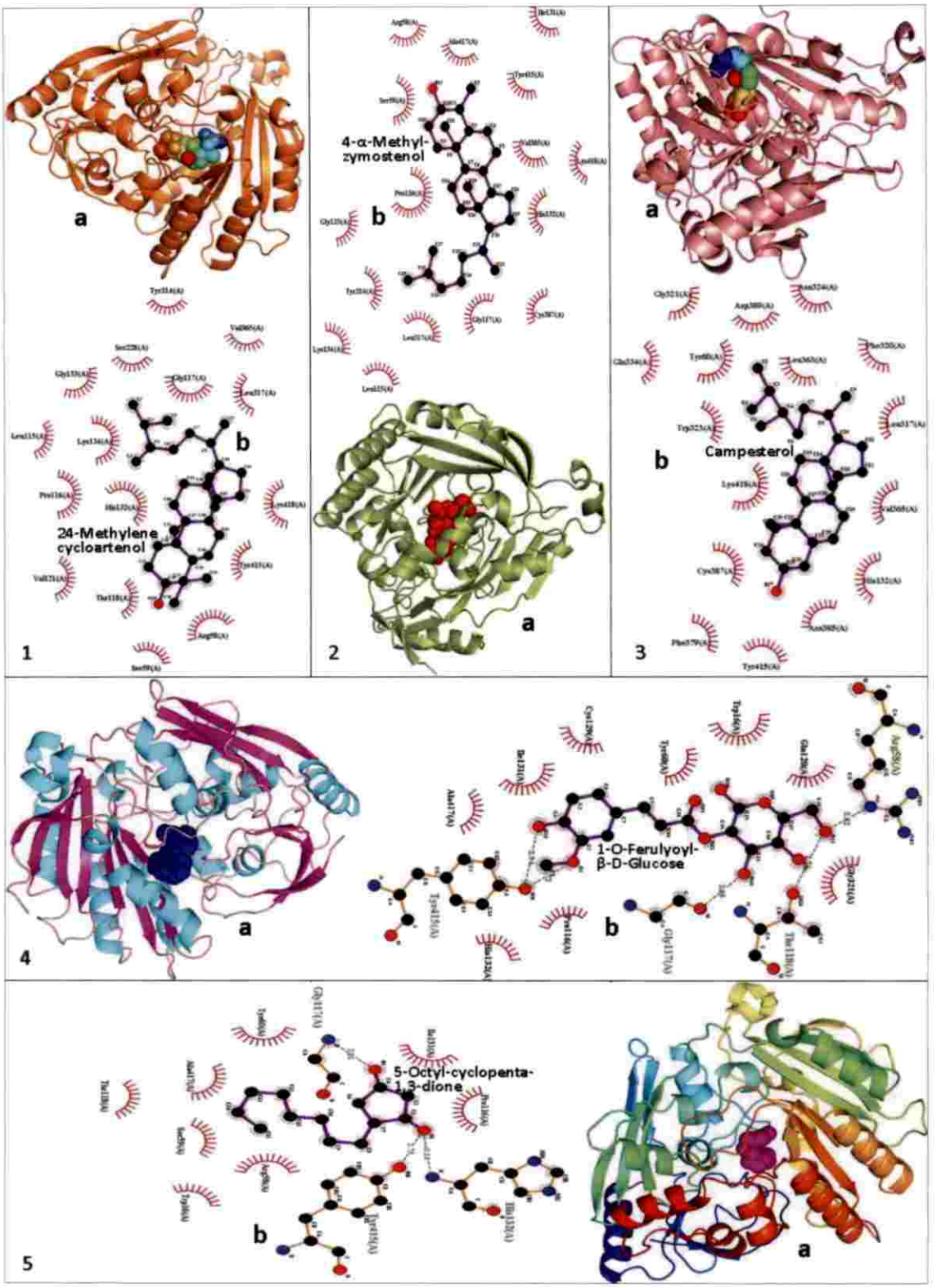


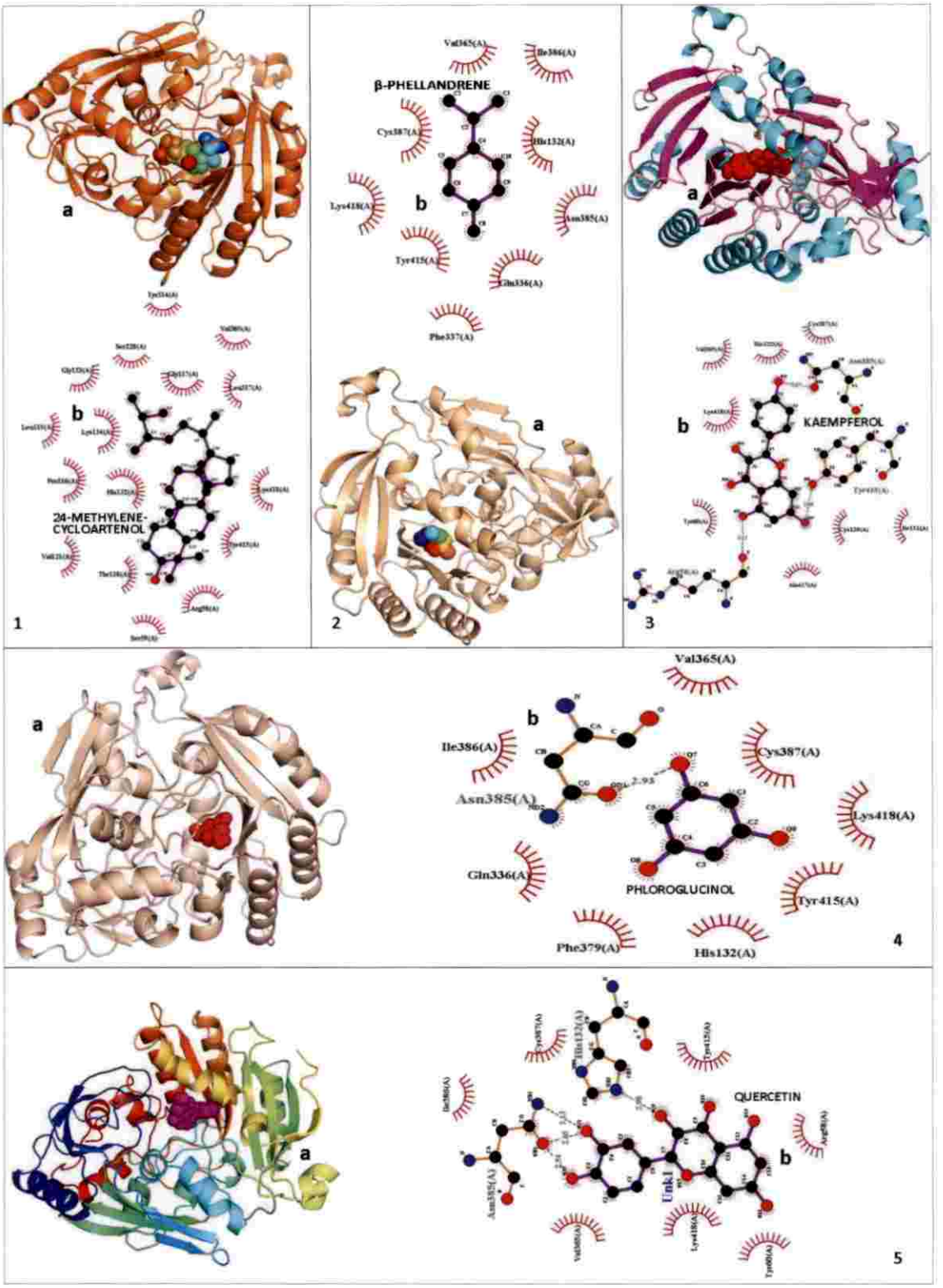
Figure-6: Interaction of the target, DprE1 with the hit molecules obtained from *Allium cepa*- a. Pymol view, b. LigPlot view

4.2.2 Phytochemicals from *Allium sativum* and DprE1 protein

A total of 52 chemical molecules which satisfied basic requirements for virtual screening from *Allium sativum* were docked with the same target DprE1. Three of them showed free energy of binding <-5 kcal/mol and two of them are> -4.5kcal/mol. Therefore the five molecules which showed least free energy of binding were selected as hit molecules for further analysis. The list of hit molecules with binding parameters such as free energy of binding (ΔG_{bind} kcal/mol), inhibition constant (μM), number of hydrogen bonds, bond type and bond length (Å) were shown in the table below (Table-2). Interaction between the target with the hits is also depicted in the figure-7

Sl. No	Phytochemical	ΔG_{bind} (kcal/ mol)	Inhibition Constant (μm)	H bonds (bond length)	Hydro-phobic residues
1	24- Methylene-cycloartenol	-10.81	0.012	No Hbond	16
2	β-Phellandrene	-5.47	98.57	No Hbond	9
3	Kaempferol	-4.64	393.73	Ligand:O21-ASN385:OD1(2.67) Ligand:O18-TYR415:OH(2.98) Ligand:O19-ARG58:O(3.11)	8
4	Phloroglucinol	-4.51	495.83	Ligand:O7-ASN385:OD1(2.93)	8
5	Quercetin	-5.29	131.96	Ligand:O22-ASN385:OD1(2.54) Ligand:O21-ASN385:OD1(2.65) Ligand:O21-ASN385:ND2(3.13) Ligand:O20-HIS132:NE2(2.90)	7

Table-2: Details of hit molecules obtained from *Allium sativum*



Figuire-7: Interaction of the target, DprE1 with the hit molecules obtained from *Allium sativum* - a. Pymol view, b. LigPlot view

4.2.3 Phytochemicals from *Moringa olifera* and DprE1 protein

A total of 42 molecules derived from *Moringa oleifera* were fit for docking against the biological target DprE1.Out of 42 chemical molecules 3 of them showed binding energy less than ˉ5 kcal mol^{-1}. However, five molecules showed top least free energy of binding were selected as hit molecules for further studies. The list of hit molecules with binding parameters such as free energy of binding (kcal mol^{-1}),inhibition constant (μM), number of hydrogen bonds, bond type and bond length (Å) (Table-3)and the docked structures between the target protein and top ranked hit molecules were shown in (Figure-8).

Sl. No.	Phytochemical	ΔG_{bind}(kcal/ mol)	Inhibition Constant (μm)	H bonds (bond length)	Hydro-phobic residues
1	Campe-sterol	-7.12	6.07	Ligand:O10-CYS387:O(2.98) Ligand:O10-LEU363:O(2.38) Ligand:O12-CYS387:SG(3.00) Ligand:O14-TYR60:OH(2.84)	6
2	Quercetin	-5.29	1.51	No Hbond	16
3	4-Hydroxy-mellein	-5.07	191.46	Ligand:O21-ASN385:OD1(2.67) Ligand:O18-TYR415:OH(2.98) Ligand:O19-ARG58:O(3.11)	8
4	Kaempferol	-4.64	948.55	Unk: N1-ASN385:OD1(2.46)	8
5	Moringine	-4.34	660.97	Unk: O20-HIS132:NE2(2.90) Unk: O21-ASN385:ND2(3.13) Unk: O21-ASN385:OD1(2.65) Unk: O22-ASN385:OD1(2.54)	7

Table-3: Details of hit molecules obtained from *Moringa oleifera*

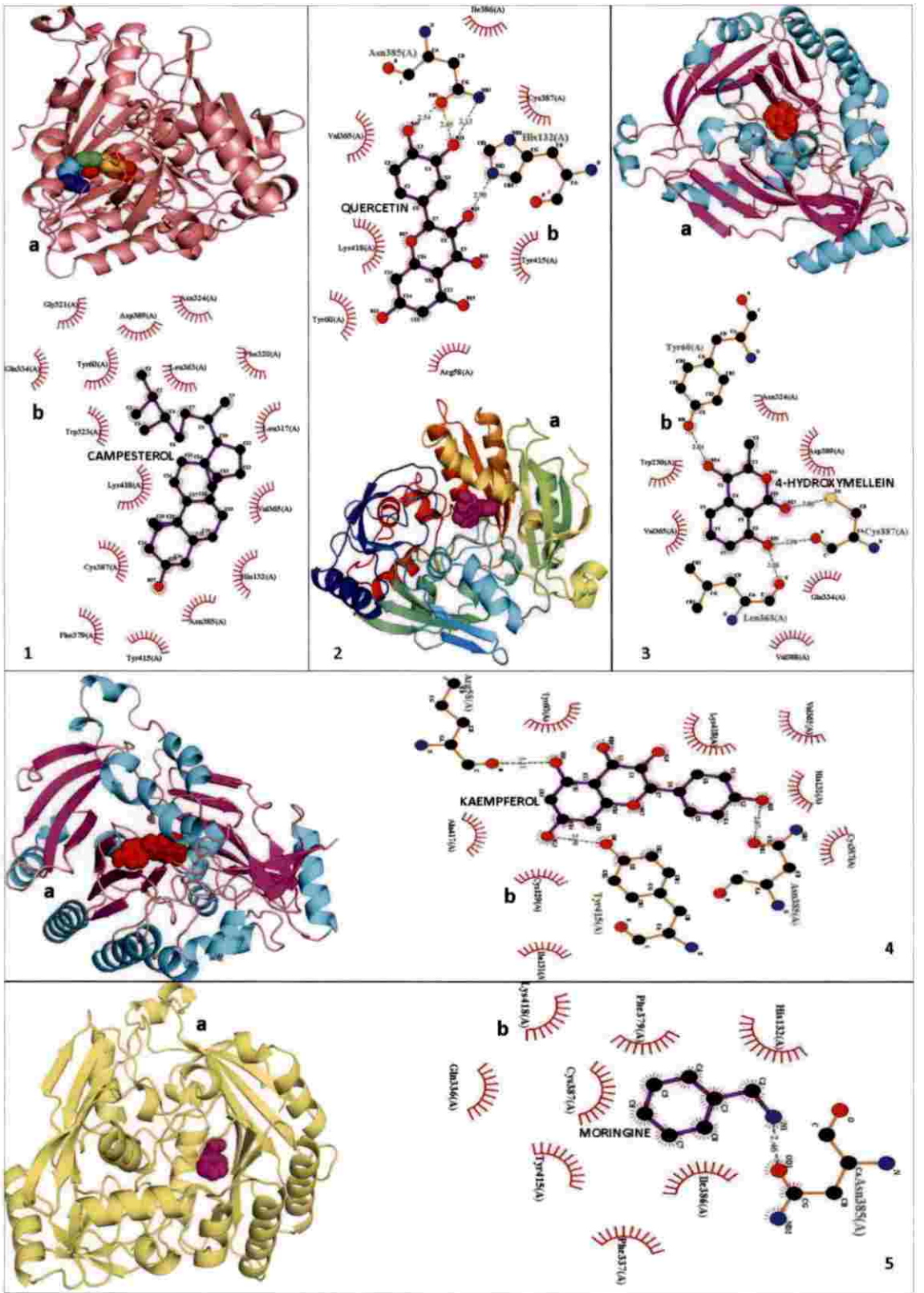


Figure-8: Interaction of the target, DprE1 with the hit molecules obtained from *Moringa oleifera* -a. Pymol view, b. LigPlot view

2-D structures of hit molecules sought out from each plant were illustrated in Figure-9.

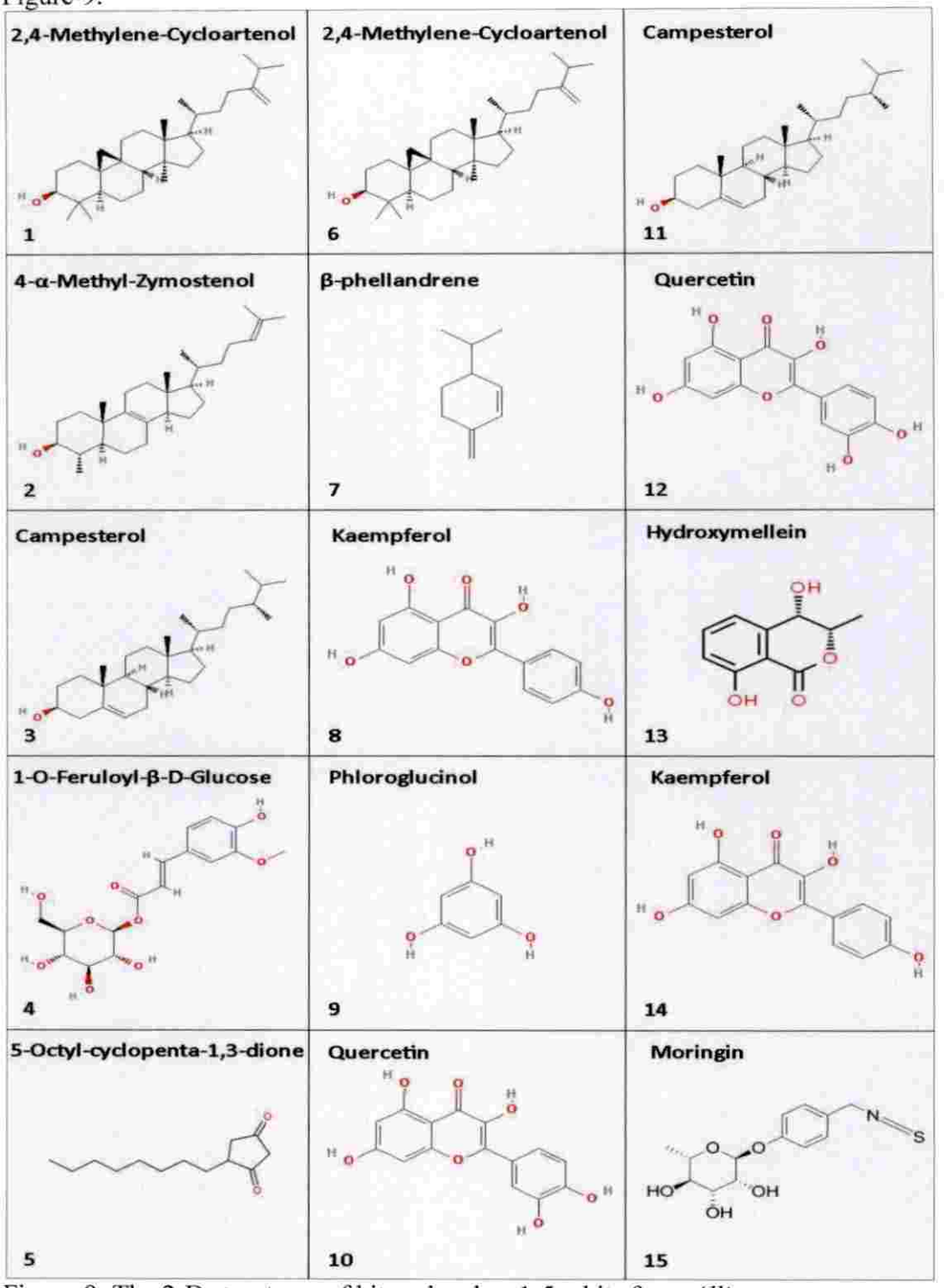


Figure-9: The 2-D structures of hit molecules: 1-5 – hits from *Allium cepa*; 6-10 – hits from *Allium sativum* and 11-15 – hits from *Moringa oleifera*.

4.3 IDENTIFICATION OF LEAD MOLECULES THROUGH RANK SUM TECHNIQUE

Selected hit molecules with least free energy of binding from each plant were again docked with DprE1 using different docking tools such as Hex server, iGEMDOCK, FireDock and SwissDock. Consensus scoring combines information from different scores to balance errors in single scores and improve the probability of identifying 'true' ligands. The results were then statistically analysed using Dempster Shafer Theory (DST). The data were analysed in five step procedure. 1. Class generation (the whole data is divided into four classes), 2. Get result from Rank Sum technique, 3. Get result from DST unweighted, 4. Get result from DST weighted, 5. Get result from Zhang rule. Top ranked molecules from second to fifth were selected as true hits and proposed as lead molecules for further evaluation. The result of consensus scoring is given below (table-4)

Plant	Phytochemicals	Free energy of binding in various docking tools (kcal/mol)					**Rank**
		AutoDock	Hex Server	iGEMDOCK	PatchDock	SwissDock	
Allium cepa (Onion)	1-O-Feruloyl-β-D-Glucose	-6.15	-253.99	-110.578	5166	-8.84	15
	24-Methylene-Cycloartenol	**-10.81**	**-258.44**	**-98.5279**	**6692**	**-5.96**	**16**
	4-Alpha-Methyl-Zymostenol	-9.66	-247.46	-99.3651	5900	-6.6	15
	5-Octyl-Cyclopenta-1,3-Dione	-6.14	-200.36	-91.4327	4424	-7.52	7
	Campesterol	-7.12	-251.14	-75.7682	6284	-9.62	14
Allium sativum (Garlic)	24-Methylene-Cycloartenol	-10.81	-258.44	-98.5279	6692	-5.96	16
	β-Phellandrene	-5.47	-165.56	-56.7378	-27.73	-6.62	12
	Kaempferol	**-5.64**	**-254.58**	**-114.112**	**-48.6**	**-6.12**	**17**
	Phloroglucinol	-5.51	-135.27	-66.5714	-24.68	-6.95	9
	Quercetin	**-5.29**	**-245.58**	**-115.395**	**-52.3**	**-7.37**	**20**
Moringa oleifera (Drumstick)	**4-Hydroxymellein**	**-5.07**	**-164.62**	**-114.531**	**-50.74**	**-7.12**	**18**
	Campesterol	-7.12	-251.14	-75.7682	6284	-9.62	14
	Kaempferol	**-5.64**	**-254.58**	**-114.112**	**-48.6**	**-6.12**	**17**
	Moringine	-5.34	-240.75	-113.324	-45.16	-7.05	11
	Quercetin	**-5.29**	**-245.58**	**-115.395**	**-52.3**	**-7.37**	**20**

Table-4: Docking scores in different tools and Rank obtained after Consensus scoring.

Through Consensus scoring most suitable lead molecule from each plant were identified and they are 24-Methylene cycloartenol, Kaempferol, Quercetin and 4-hydroxymellein (table-4). The result showed that Moringa produce all the three top ranked leads. Garlic produces two top ranked leads while onion produce only one lead molecule which is the lowest ranked molecule among the four leads.

4.3.1 Pharmacokinetics Properties Prediction Results

It has become increasingly apparent that the pharmacokinetic properties of a drug, i.e. absorption, distribution, metabolism and excretion (ADME), are of the utmost importance for clinical success. The most widely used bioinformatics tool pkCSM was used here for the prediction of ADME as well as toxicity of the selected molecules. Besides, MolSoft and Molinspiration tools were also used to analyse the Bioactivity as well as druglikeness of the selected leads. The results were depicted in table-5 and table-4 respectively. Absorption can be conceived in simple terms as the process of movement of a drug from an extravascular site of administration into the systemic circulation. The absorption parameters considered were Caco-2 permeability, Intestinal absorption and Skin permeability. Distribution of drug refers to the distribution of the compound throughout different compartments within the body. Here the distribution parameters considered were volume of distribution (VDss (Human)) and Blood Brain Barrier (BBB) permeability. Metabolism is the biotransformation of drugs and xenobiotic compounds to facilitate their excretion. The metabolism parameters considered here were CYP2D6 substrate and CYP2D6 inhibitors. Excretion is the process whereby a drug molecule is eliminated by liver, kidney and other organs from the body. Arguably, drug toxicity is the most challenging drug property that remains one of the most significant reasons for many drugs failing to reach the market and for many drugs not approved to the market and withdrawal from the market during the late-stage drug development. The toxicity parameters considered here were AMES toxicity, Maximum tolerated dose, hERG-1 inhibitors and hERG 2 inhibitors.

Compound	Molinspiration Drug likeness and Bio activity score												Molsoft
	logp	Mol wt	TPSA	nON	nOHNH	nrotb	GPCRL	ICL	KI	NRL	PI	EI	DLS
24-Methylene cycloartanol	**8.03**	**440.8**	**20.2**	**1**	**1**	**5**	**0.14**	**0.11**	**-0.37**	**0.90**	**0.06**	**0.60**	**-0.51**
4-hydroxymellein	**1.07**	**194.1**	**66.8**	**4**	**2**	**0**	**-0.55**	**-0.27**	**-0.64**	**-0.29**	**-0.94**	**-0.04**	**0.19**
Kaempferol	**2.17**	**286.2**	**111.1**	**6**	**4**	**1**	**-0.10**	**-0.21**	**0.21**	**0.32**	**-0.27**	**0.26**	**0.77**
Quercetin	**1.68**	**302.24**	**131.35**	**7**	**5**	**1**	**-0.06**	**-0.19**	**0.28**	**0.36**	**-0.25**	**0.28**	**0.93**

Table-5: Bioactivity score in Molinspiration and Druglikeness score in MolSoft

Compound	Absorption				Distribution		Metabolism	Excretion	Toxicity			
	logs	**Caco2 permeability**	**HIA (%)**	**logkp**	**VDss (logl/kg)**	**BBB Permeability**	**Cyp450**	**Renal OCT2 Substrate**	**AT**	**HT**	**HERG inhibition**	**Skin sensitisation**
24-Methylenecycloartanol	-5.1	1.22	95.31	-2.741	-0.072	0.845	Non Inhibition	No	No	No	(inhibitor of herg II)	No
4-hydroxymellein	-1.8	0.64	87.88	-2.84	0.385	0.336	Non Inhibition	No	Yes	No	No	No
Kaempferol	-3.0	0.03	74.29	-2.735	1.274	-0.939	Non Inhibition	No	No	No	No	No
Quercetin	-2.9	-0.23	77.21	-2.735	1.559	-1.098	Non Inhibition	No	No	No	No	No

Table-6: ADMET properties of leads generated using the tool PkCSM

In the Bioactivity score analysis using Molinspiration shows that 24-Methylene-cycloartanol has comparatively low oral bioavailability due to less lipid solubility (logp = 8.03) and very low polar surface area (20.23 Å [standard TPSA ranging between 74.03 and 105.65 Å]) (Table-5). The drug likeness score

(-0.51) generated in Molsoft is also not promising for the compound (Figure-10). ADMET properties of the leads studied using PkcSM (Table-6) also showed that 24-Methylenecycloarenol is inhibiting a hERGII protein which has to be avoided during drug development. Kaempferol, Quercetin and 4-hydroxymellein showed acceptable range in both bioactivity analysis and in ADMET property prediction (Table-5 & 6). Therefore the study recommends three compounds in the order of merit Quercetin in the first place, Kaempferol in the second place and 4-hydroxymellein in the third place as leads for the development of drug against TB. The study well substantiated the anti-tubercular activity of the medicinal plants *Allium sativum* and *Moringa oleifera* as it produce phytochemicals that were competent to inhibit the multiplication and cell wall virulence of *Mycobacterium tuberculosis*. Of course *A. cepa* also produce certain phytochemicals that can interact with our target (DprE1) but comparatively better inhibitors are present in other two plants. Moringa stands first in the evaluation as it produce all the three sought out leads followed by garlic with two leads. Theoretical prediction of drug likeliness only gives probability and its activity in a biological system. Therefore, *in vitro* and *in vivo* studies and in-depth clinical trials are necessary for developing a drug out of the lead molecules.

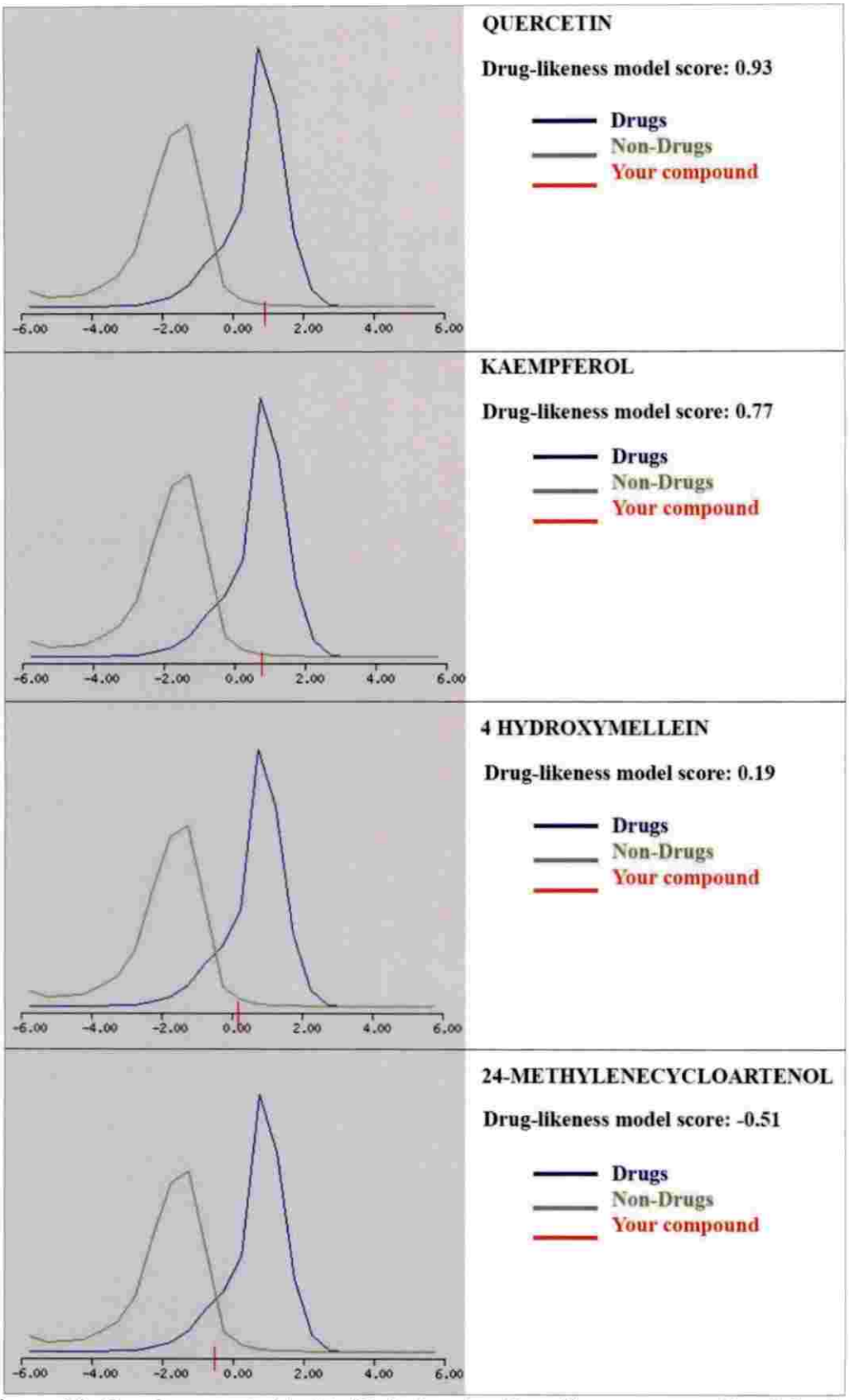


Figure-10: Graph generated in MolSoft showing Druglikenessscore of each leads

DISCUSSION

5. DISCUSSION

Tuberculosis (TB) is one of the ancient diseases in the recorded history going back to over 17,000 years. According to World Health Organization, TB is considered as a worldwide pandemic. It is one of the leading causes of death among HIV-infected people. In India, the fight against TB can be broadly classified into three periods. Early period before the inventions of x-ray and chemotherapy, post-independence period during which the country has initiated and implemented TB control programs and thirdly the current period during which the ongoing WHO assisted TB control program is in place. Today in India DOTS (directly observed treatment short course) is the fastest expanding and the largest program in the world in terms of patients initiated on treatment and the second largest in terms of population coverage. The main reasons for increasing the rates in tuberculosis includes the deterioration of various public health programs aimed at preventing tuberculosis and encouraging completion of therapy for the disease. Another important reason is the incomplete treatment. Increasing in the rates of HIV infection, imprisonment, homelessness, and immigration also contributed to the resurgence of tuberculosis. Depending on region and age groupabout 35 percent of those with tuberculosis also infected with HIV. Emergence of mutated drug resistant varieties such as multi drug resistant (MDR), extensively drug resistant (XDR) and total drug resistant (TDR) strains of the bacteria are the actual burden of the spread and prevention of TB.

Rifampicin was the latest anti-TB drug to be licensed in 1963. Since that time a number of compounds have entered human trials and encouragingly two compounds bedaquiline and delamanid have recently received fast tracked approval for use against MDR-TB. However, both drugs are having side effects and are only recommended for those without other treatment options. Considering the restrictions on bedaquiline use and the fact that XDR and TDR strains cannot be adequately treated with currently available antibiotics, many more compounds must enter the TB drug development 'pipeline' in order to adequately combat the TB problem. The ideal anti-TB drug must have high potency particularly against

drug resistant strains and possess an adequate safety profile. In addition to this drugs should be active against latent and replicating forms of *M. tuberculosis* and have limited drug-drug interactions especially with antiretroviral agents.

Many plant species as well as marine organisms and fungi have been used in various traditional healing systems around the world. Besides the anti-mycobacterial activity of medicinal plants it is useful in adjuvant therapy to improve the efficacy of conventional therapies to decrease their adverse effects and to reverse mycobacterial multi drug resistance due to the genetic plasticity and environmental adaptability of the *Mycobacterium* species. However even if some natural products have still been investigated in preclinical and clinical studies, the validation of their efficacy and safety as anti-tuberculosis agents is far from being reached and therefore according to an evidence based approach more high level randomized clinical trials are urgently needed.

India is one of the 12 mega biodiversity countries in the world and in plant diversity it ranks fourth among Asian countries. The country is blessed with varied agro-climatic and geographical conditions and that led to high rate of (35%) endemism and genetic diversity (Nayar, 1996) that make the country as a gold mine of life forms with high therapeutic activity. The God's own country, Kerala has many unique varieties of vegetable plants having high therapeutic value. The vegetable plants of Kerala have been used in the traditional systems of medicine for curing many ailments particularly diseases affected to human respiratory system. Nutraceuticals are the emerging class of natural products that makes the line between food and drugs to fade (Adelaja and Schilling, 1999). Although the use of nutraceuticals by people has a long history, only recently scientifically supported nutritional and medical evidence has allowed nutraceuticals to emerge as being potentially effective (Dillard and German, 2000). In these backdrops, the present investigation was carried out to scientifically demonstrate the efficacy of anti-tuberculosis activity in the three selected vegetables *viz.* Onion *(Allium cepa)*, Garlic (*Allium sativum)* and Drumstick (*Moringa oleifera)* of Kerala.

The drug discovery process involves the identification of the lead structure followed by the synthesis of its analogues, their screening to get candidate molecules for drug development. The goal of the drug discovery process is to search for new drug molecules which can bind to a specific target known to be involved in causing a disease and change the target's function. In the traditional drug discovery process, identification of the suitable drug target is the first and foremost task. These targets are biomolecules which mainly include DNA, RNA and proteins (such as receptors, transporters, enzymes and ion channels). Identification of right target molecule is the key role in any docking based screening program. Decaprenylphosphoryl-beta-D-ribose epimerase (DprE1) is involved in the epimerization reaction of decaprenylphosphoryl-beta-D-ribose (DPR) to decaprenylphosphoryl-beta-D-arabinofuranose (DPA) which is the sole donor of arabinose sugars that are essential for cell wall biosynthesis in *M. tuberculosis*. Therefore, the present study focused the target DprE1 and their inhibitors. The X-ray crystallographic 3D structure of the protein was downloaded from Protein Data Bank, which is a major source of open access authenticated protein structure database. Ramachandran plot with permissible dihedral rotational bonds and Visualization of the 3 D structure of the protein showed that the structure contains enough hydrogen bonds and other molecular interaction forces, which indicate that the target molecule was structurally stable. As followed by others (Elmazar *et al.*, 2013) the active site/the ensemble of interaction points/residues were detected using the tool PDBsum and the presence of more than 12 amino acid residues as interaction functional group indicate the binding affinity and specificity of the active site.

Preparation of ligand molecules is another important task in docking. Many authors have screened plant derived molecules for demonstrating their therapeutic activities following standard methods (Nisha *et al.*, 2013; Rini *et al.*, 2014). The available structures of the selected plant derived molecules were retrieved from open access chemical databases and the remaining compounds structures were created using the tool ChemSketch. Similarly, CORINA is an open access tool for the generation of 3D structures of small molecules (Nisha *et*

al., 2014). Generally, in order to avoid the unwanted docking exercise the small molecules will be analyzed based on the conventional Lipinski's rule of Five (Lipinski *et al.*, 1997) However many works have proved that natural products are exception to the Lipinski's rule of five (Boldi, 2006; Ganesan, 2008).

A total of 175 compounds derived from *Allium cepa* (81), *Allium sativum* (52) and *Moringa oleifera* (42) were studied and subjected for docking analysis followed by the selection of active ingredients. Five active compounds sought out based on AutoDock analysis from each plants were allowed to dock with other four different docking tools and subjected them for statistical analysis following Dempster-Shafer Method (DST) to find out most suitable compound with minimum false negative and error free result. Through Consensus scoring most suitable lead molecule from each plant were identified and they are 24-Methylene cycloartenol, Kaempferol, Quercetin and 4-hydroxymellein (table-4). The result showed that Moringa produce all the three top ranked leads. Garlic produces two top ranked leads while onion produce only one lead molecule which is the lowest ranked molecule among the four leads.

In the Bioactivity score analysis using Molinspiration shows that 24-Methylene-cycloartanol has comparatively low oral bioavailability due to less lipid solubility (logp = 8.03) and very low polar surface area (20.23 Å [standard TPSA ranging between 74.03 and 105.65 Å]) (Table-5). The drug likeness score (-0.51) generated in Molsoft is also not promising for the compound (Figure-10). ADMET properties of the leads studied using PkcSM (Table-6) also showed that 24-Methylenecycloarenol is inhibiting a hERGII protein which has to be avoided during drug development. Kaempferol, Quercetin and 4-hydroxymellein showed acceptable range in both bioactivity analysis and in ADMET property prediction (Table-5 & 6). Therefore, the study recommends three compounds in the order of merit, Quercetin in the first place, Kaempferol in the second place and 4-hydroxymellein in the third place as leads for the development of drug against TB. The study well substantiated the anti-tubercular activity of the medicinal plants *Allium sativum and Moringa oleifera* as it produces phytochemicals that were

competent to inhibit the multiplication and cell wall virulence of *Mycobacterium tuberculosis*. Of course *A. Cepa* also produce certain phytochemicals that can interact with our target (DprE1) but comparatively better inhibitors are present in other two plants. Moringa stands first in the evaluation as it produces all the three sought out leads followed by garlic with two leads. Theoretical prediction of drug likeliness only gives probability and its activity in a biological system. Therefore, *in vitro* & *in vivo* studies and in-depth clinical trials are necessary for developing a drug out of the lead molecules.

SUMMARY

6. Summary

Tuberculosis (TB) is one of the oldest known human ailments caused by single infectious agent *Mycobacterium tuberculosis,* and the second largest killing disease globally. According to WHO approximately, 10.4 million TB cases are reporting annually worldwide and the rate is still increasing rapidly. In 2017, 1.8 million people died from the disease, with 40,000 in India. Prolonged use of a combination of various antibiotics is the effective treatment of TB in the current scenario. However, the rational use of first- and second-line anti-TB drugs has resulted in the emergence of multidrug-resistant (MDR) and extensively-drug resistant (XDR) TB. On the other hand, traditional treatment system based on herbal medicine against TB shows success stories. Thus the use of new vaccines and novel drugs based on safe and reliable source are required to eradicate the globally affected epidemic of TB. India is a country known for its vast traditional knowledge. A number of plants including vegetables are being used for curing diseases affected to human respiratory system and particularly against tuberculosis by different ethnic communities. Irony in such treatment system is that either the man who treat or the person who receives the treatment doesn't know the method of action or the active ingredient of crude preparations. So such valuable information has to be evaluated scientifically and find out the exact lead candidate from the natural gifts (plants) for the development of harmless, cost effective medicine to treat TB.

In these backdrops, the phytomolecules reported from three vegetable plants of Kerala *viz.* Onion, Garlic and Drumstick (*Allium cepa, Allium sativum* and *Moringa oleifera)* were gathered through end to end literature search and subjected for the screening of phytochemicals using bioinformatics tools. Anti-tuberculosis activities of the crude extracts of all these plants were well demonstrated in various works. Bulbs of *Allium cepa* & *Allium sativum* and leaves of *Moringa oleifera* extracts showed high activity against different strains of *M. tuberculosis*. To identify the active ingredient essentially present in these anti-TB plants, quick and cost effective *in silico* methods were followed in the present

investigation.Decaprenylphosphoryl-beta-D-ribose epimerase (DprE1) produced by *M. Tuberculosis* was selected as the biological target protein for the screening study. DprE1is responsible for the synthesis of the cell wall component arabinan which is essential for the virulence of the bacteria. The 3D structure of the molecule was retrieved from PDB (PDB id 4FDO) and the active site of the target protein (DprE1) was detected using the tool PDBsum.

A total of 175 phytochemicals derived from the selected plants (*Allium cepa* – 81, *Allium sativum* – 52 and *Moringa oleifera* – 42) were subjected for the screening analysis. Out of 175 phytochemicals, structures of 161 (*Allium cepa* - 73, *Allium sativum* -49 and *Moringa oleifera* -39) were retrieved from databases and the remaining structures were created using ChemSketch. The 3Dstructures of all phytochemicals were created using the tool CORINA. The selected phytochemicals then docked against DprE1 using the docking tool Auto Dock 4.2. The docked results having ΔG_{binid}-5kcal mol^{-1} were selected as hit molecules. Top five hit molecules which showed least free energy of binding from each plant were selected for further analysis. In order to get the true hit molecules the top 5 hit molecules were again docked with other 4 docking tools *viz.* Hex server, iGEMDOCK, Fire Dock and SwissDock. The docked results were statistically analyzed following DST and Zhang rule and selected the top ranked molecules from each plant *viz.* 24-Methylenecycloartanol (*A.cepa*), Kaempferol & Quercetin (*A. sativum* and *M. oleifera*) and 4-hydroxymellein from *M. oleifera.* The bioactivity and druglikness score were done in Molinspiration and Molsoft tools and ADMET analysis was done using the tool pkCSM. Thebioactivity and ADMET analysis showed Kaempferol, Quercetin and 4-hydroxymellein were lies in the acceptable range and it can be used as leads for the development of drug against TB. The study well substantiated the anti-tubercular activity of the vegetables as it produce phytochemicals that were competent to inhibit the multiplication and cell wall virulence of *M. tuberculosis*. *A. cepa* also produce certain phytochemicals that can interact with our target (DprE1) but comparatively better inhibitors are present in other two plants. Moringa stands first in the evaluation as it produce all the three sought out leads followed by

garlic with two leads. Theoretical prediction of drug likeliness only gives probability and its activity in a biological system. Therefore, *in vitro* & *in vivo* studies and in-depth clinical trials are necessary for developing a drug out of the lead molecules.

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APPENDICES

APPENDIX -I

Phytochemical derived from *Allium cepa*

Sl. No.	Name of Phytochemical	Mol. formula	Mol. Wt. [g/mol]	Canonical Smiles
1.	1(f)-beta-fructosyl-sucrose	$C_{18}H_{32}O_{16}$	504.43708	C(C1C(C(C(C(O1)OC2(C(C(C(O2)CO)O)O)COC3(C(C(C(O3)CO)O)O)CO)O)O)O)O
2.	1-(methylsulfinyl)-propyl-methyl-disulfide	C5H12OS	120.22	CCCCS(=O)C
3.	1-methylthio-propane	$C_4H_{10}S$	90.188	CCCSC
4.	1-o-caffeoyl-beta-d-glucose	$C_{15}H_{18}O_9$	342.29802	C1=CC(=C(C=C1C=CC(=O)OC2C(C(C(C(O2)CO)O)O)O)O)O
5.	1-o-ferulyoyl-beta-d-glucose	$C_{16}H_{20}O_9$	356.3246	COC1=C(C=CC(=C1)C=CC(=O)OC2C(C(C(OC2O)CO)O)O)O
6.	1-o-p-coumaroyl-beta-d-glucose	$C_{15}H_{18}O_8$	326.2986	OCC1OC(OC(=O)\C=C\C2=CC=C(O)C=C2)C(O)C(O)C1O
7.	2,3-dimethyl thiophene	C_6H_8S	112.19272	CC1=C(SC=C1)C
8.	2,3-dimethyl-5,6-dithia-bicyclo(2,2,1)hexane-5-oxide	$C_6H_{10}S_2$	146.274	CC1C2SC(S2)C1C
9.	2,4-dimethylthiophene	C_6H_8S	112.19	CC1=CC(=CS1)C
10.	2,5-dimethyl thiophene	C6H8S	112.19272	CC1=CC=C(S1)C
11.	2-methyl-but-2-en-1-al	$C_5H_{10}O$	86.13	CC=C(C)CO
12.	2-methyl-butyr-2-aldehyde	C5H10O	86.13	CCC(C)C=O
13.	24-methylene-cycloartenol	$C_{31}H_{52}O$	440.74398	CC(C)C(=C)CCC(C)C1CCC2(C1(CCC34C2CCC5C3(C4)CCC(C5(C)C)O)C)C

14.	28-isofucosterol	C31H50O2	454.7275	CC=C(CCC(C)C1CCC2C1(CCC3C2CC=C4C3(CCC(C4)OC(=O)C)C)C)C(C)C
15.	3,4-dimethyl-2,5-dioxo-2,5-dihydrothiophene	$C_6H_6O_2S$	142.176	CC1=C(C)C(=O)SC1=O
16.	3,4-dimethylthiophene	C_6H_8S	112.19272	CC1=CSC=C1C
17.	31-norcycloartenol	$C_{29}H_{48}O$	412.69082	CC1C2CCC3C4(CCC(C4(CCC35C2(C5)CCC1O)C)C(C)CCC=C(C)C)C
18.	31-norlanostenol	$C_{29}H_{50}O$	414.7	CC1C2CCC3=C(C2(CCC1O)C)CCC4(C3(CCC4C(C)CCCC(C)C)C)C
19.	4-alpha-methyl-zymostenol	$C_{28}H_{46}O$	398.66424	CC1C2CCC3=C(C2(CCC1O)C)CCC4(C3CCC4C(C)CCC=C(C)C)C
20.	5-dehydro-avenasterol	$C_{29}H_{46}O$	410.67494	CC=C(CCC(C)C1CCC2C1(CCC3C2=CC=C4C3(CCC(C4)O)C)C)C(C)C
21.	5-hexyl-cyclopenta-1,3-dione	$C_{11}H_{18}O_2$	182.26	CCCCCCC1CC(=O)CC1=O
22.	5-octyl-cyclopenta-1,3-dione	$C_{13}H_{22}O_2$	210.31	O=C1CC(=O)CC1CCCCCCCC
23.	9,12,13-trihydroxy-octadec-10-enoic-acid	C18H34O5	330.45956	CCCCCC(C(C=CC(CCCCCCCC(=O)O)O)O)O
24.	9,10,13-trihydroxy-octadec-11-enoic-acid	C18H34O5	330.45956	CCCCCC(C=CC(C(CCCCCCCC(=O)O)O)O)O
25.	Alanine	C3H7NO2	89.09318	CC(C(=O)O)N
26.	Alliin	C6H11NO3S	177.22144	CC=CS(=O)CC(C(=O)[O-])[NH3+]
27.	Alliofuroside-a	C44H72O18	889.0317	CC1C2C(CC3C2(CCC4C3CC=C5C4(C(CC(C5)O)OC6C(C(C(CO6)O)O)OC7C(C(C(C(O7)C)O)O)O)C)C)OC1(CCC(C)COC8C(C(C(C(O8)CO)O)

91

				O)O)O
28.	Allyl-propyl-disulphide	$C_6H_{12}S_2$	148.28948	CCCSSCC=C
29.	Alliospiroside-a	$C_{38}H_{60}O_{12}$	708.9	CC1CCC2(C(C3C(O2)CC4C3(CCC5C4CC=C6C5(C(CC(C6)O)OC7C(C(C(CO7)O)O)OC8C(C(C(C(O8)C)O)O)O)C)C)C)OC1
30.	Alpha-amyrin	C30H50O	426.7174	CC1CCC2(CCC3(C(=CCC4C3(CCC5C4(CCC(C5(C)C)O)C)C)C2C1C)C)C
31.	Alpha-sitosirol	C30H50O	426.7174	CC=C(CCC(C)C1CCC2C1(CCC3C2=CCC4C3(CCC(C4C)O)C)C)C(C)C
32.	Campesterol	C28H48O	400.68012	CC(C)C(C)CCC(C)C1CCC2C1(CCC3C2CC=C4C3(CCC(C4)O)C)C
33.	Allicin	$C_6H_{10}OS_2$	162.273	C=CCSS(=O)CC=C
34.	Alpha-tocopherol	$C_{29}H_{50}O_2$	430.7061	CC1=C(C(=C2CCC(OC2=C1C)(C)CCCC(C)CCCC(C)CCCC(C)C)C)O
35.	Beta-carotene	$C_{40}H_{56}$	536.9	CC1=C(C(CCC1)(C)C)C=CC(=CC=CC(=CC=CC=C(C)C=CC=C(C)C=CC2=C(CCCC2(C)C)C)C)C
36.	Beta-sitosterol	$C_{29}H_{50}O$	414.7067	CCC(CCC(C)C1CCC2C1(CCC3C2CC=C4C3(CCC(C4)O)C)C)C(C)C
37.	Brassicasterol	$C_{28}H_{46}O$	398.7	CC(C)C(C)C=CC(C)C1CCC2C1(CCC3C2CC=C4C3(CCC(C4)O)C)C
38.	Catechol	C6H6O2	110.11064	C1=CC=C(C(=C1)O)O
39.	Cholest-7-en-3-beta-ol	C27H46O	386.65354	CC(C)CCCC(C)C1CCC2C1(CCC3C2=CCC4C3(CCC(C4)O)C)C
40.	Choline	C5H14NO	104.17076	C[N+](C)(C)CCO

41.	Cis-1-(propenyl-dithio)-propane	$C_{25}H_{60}O$	185.4523	CCCCCCCCCCCCC=CO
42.	Cis-2,3-dimethyl-5,6-dithia-cyclo(2,2,1)heptane-5-oxide	C6H12S4	191.55	C1=CC(=C(C=C1C2=C(C=C3C(=CC(=CC3=[O+]2)O)O)OC4CO)O)O)OC5C(C(C(C(O5)CO)O
43.	Cis-3,5-diethyl-1,2,4-trithiolane	$C_6H_{12}S_3$	180.4	CCC1SC(SS1)CC
44.	Cyanidin-3-o-beta-d-diglycoside	C21H21O11	449.38484	C1=CC(=C(C=C1C2=C(C=C3C(=CC(=CC3=[O+]2)O)O)OC4C(C(C(C(O4)CO)O)O)O)O)O
45.	Cyanidin-3-o-laminaribioside	C27H31O16	611.52544	C1=CC(=C(C=C1C2=C(C=C3C(=CC(=CC3=[O+]2)O)O)OC4C(C(C(C(O4)CO)O)OC5C(C(C(C(O5)CO)O)O)O)O)O)O
46.	Cyanidin-diglucoside	C27H31O16	611.52544	C1=CC(=C(C=C1C2=C(C=C3C(=CC(=CC3=[O+]2)O)O)OC4C(C(C(C(O4)CO)O)O)O)OC5C(C(C(C(O5)CO)O)O)O)O
47.	Cycloalliin	C6H11NO3S	177.22144	C[C@H]1CS(=O)C(CN1)C(=O)O
48.	Cycloartenol	C30H50O	426.7174	CC(CCC=C(C)C)C1CCC2(C1(CCC34C2CCC5C3(C4)CCC(C5(C)C)O)C)C
49.	Cycloeucalenol	C30H50O	426.7174	CC1C2CCC3C4(CCC(C4(CCC35C2(C5)CCC1O)C)C(C)CCC(=C)C(C)C)C
50.	Diallyl-sulfide	$C_6H_{10}S$	114.21	C=CCSCC=C
51.	Diallyl-trisulfide	C6H10S3	178.3386	C=CCSSSCC=C

52.	Dimethyl-disulfide	C2H6S2	94.19904	CSSC
53.	Dimethyl-sulfide	C2H6OS	78.13344	CS(=O)C
54.	Dimethyl-trisulfide	C2H6S3	126.26404	CSSSC
55.	Diphenylamine	C12H11N	169.22244	C1=CC=C(C=C1)NC2=CC=CC=C2
56.	Dipropyl-disulphide	C6H14S2	150.31	CCCSSCCC
57.	Dipropyl-trisulfide	C6H14S3	182.37036	CCCSSSCCC
58.	Eicosen-1-ol	$C_{20}H_{40}O$	296.531	CCCCCCCCCCCCCCCCCCC=CO
59.	Eo-1428	$C_{20}H_{16}BrClN_2O$	415.71084	CC1=CC=CC=C1C(=O)C2=C(C=C(C=C2)NC3=C(C=C(C=C3)Br)N)Cl
60.	Ferulic-acid	$C_{10}H_{10}O_4$	194.18	COC1=C(C=CC(=C1)C=CC(=O)O)O
61.	Gamma-glutamyl-leucine	$C_{11}H_{20}N_2O_5$	260.2869	CC(C)CC(C(=O)O)NC(=O)CCC(C(=O)O)N
62.	Gamma-glutamyl-phenylalanine	$C_{14}H_{18}N_2O_5$	294.30312	C1=CC=C(C=C1)CC(C(=O)O)NC(=O)CCC(C(=O)O)N
63.	Gamma-l-glutamyl-cysteine	$C_8H_{14}N_2O_5S$	250.27	C(CC(=O)NC(CS)C(=O)O)C(C(=O)O)N
64.	Gamma-l-glutamyl-s-(1-propenyl)l-cysteine-sulfoxide	$C_{11}H_{18}N_2O_6S$	306.335	C\C=C\S(=O)CC(NC(=O)CCC(N)C(O)=O)C(O)=O
65.	Hexadecen-1-ol	$C_{16}H_{32}O$	240.42	CCCCCCCCCCCCCCC=CO
66.	Kaempferol	$C_{15}H_{10}O_6$	286.2363	C1=CC(=CC=C1C2=C(C(=O)C3=C(C=C(C=C3O2)O)O)O)O
67.	Kaempferol-3,4'-di-o-beta-d-glucoside	C21H20O11	448.378	C1=CC(=CC=C1C2=C(C(=O)C3=C(C=C(C=C3O2)O)O
68.	Lophenol	$C_{28}H_{48}O$	400.7	CC1C(CCC2(C1CC=C3C2CCC4(C3CCC4C(C)CCCC(C)

				C)C)C)O
69.	Methyl-cis-propenyl-disulfide	$C_4H_8S_2$	120.2	CC=CSSC
70.	Methionine-sulfone	$C_5H_{11}NO_4S$	181.21	CS(=O)(=O)CCC(C(=O)O)N
71.	Methyl-propenyl-trisulfide	$C_4H_8S_3$	152.30132	CC=CSSSC
72.	N-propyl-mercaptan	C_3H_8S	76.16	CCCS
73.	Phloroglucinol	$C_6H_6O_3$	126.11	C1=C(C=C(C=C1O)O)O
74.	Prop-cis-enyl-propyl-disulfide	$C_7H_{12}O_4S$	256.213	CSCC(C(=O)O)N)C(=O)
75.	Pufa	$C_{60}H_{92}O_6$	909.4	CCC=CCC=CCC=CCCCCC CCC(=O)O.CCC=CCC=CCC =CCC=CCC=CCCCC(=O)O. CCC=CCC=CCC=CCC=CC C=CCC=CCCC(=O)O
76.	Quercetin	$C_{15}H_{10}O_7$	302.2357	C1=CC(=C(C=C1C2=C(C(= O)C3=C(C=C(C=C3O2)O)O) O)O)O
77.	S-(2-carboxy-propyl)-glutathione	$C_7H_{13}NO_4S$	207.24742	CC(CSCC(C(=O)O)N)C(=O) O
78.	S-(beta-carboxybeta-methyl-ether)-cysteine	$C_{23}H_{24}O_8$	428.4	COC1=CC(=CC(=C1OC)OC) C2C3C(CC4=C(C5=C(C=C2 4)OCO5)OC)COC3=O
79.	Stigmast-7-en-3-beta-ol	C29H50O	414.7067	CCC(CCC(C)C1CCC2C1(CC C3C2=CCC4C3(CCC(C4)O) C)C)C(C)C
80.	Stigmasterol	$C_{29}H_{48}O$	412.69082	CCC(C=CC(C)C1CCC2C1(C CC3C2CC=C4C3(CCC(C4)O)C)C)C(C)C
81.	Thiopropanal-s-oxide	C_3H_6OS	90.15	CCC=S=O

APPENDIX -II

Phytochemicals derived from *Allium sativum*

Sl.No	Phytochemical	Mol.formula	Mol. Wt. [g/mol]	Canonical Smiles
1	1,2-dimercaptocyclopentane	$C_5H_{10}S_2$	134.265	SC1CCCC1S
2	1,2-epithiopropane	C_4H_5NS	99.1542	C1C(S1)CC#N
3	1,3-dithiane	$C_4H_8S_2$	120.2	C1CSCSC1
4	1-hexanol	$C_6H_{14}O$	102.17476	CCCCCCO
5	2,3,4-trithiapentane	$C_2H_6S_3$	126.26404	CSCSS
6	2-methyl-benzaldehyde	C_8H_8O	120.14852	CC1=CC=CC=C1C=O
7	2-vinyl-4h-1,3-dithiin	$C_6H_8S_2$	144.25772	C=CC1SCC=CS1
8	3-methyl-hexane	C_7H_{16}	100.2	CCCC(C)CC
9	3-methyl-cis-propenyl-disulfide	$C_4H_8S_2$	120.2	CC=CSSC
10	3-methyl-2-cyclopentene-1-thione	$C_8H_{12}S$	140.24588	CCC1=C(CCC1=S)C
11	3-vinyl-4h-1,2-dithiin	$C_6H_8S_2$	144.25772	C=CC1CC=CSS1
12	4-methyl-5-vinylthiazole	C_6H_7NS	125.19148	CC1=C(SC=N1)C=C
13	5-butyl-cysteine-sulfoxide	C_5H_9NO	99.13106	CCCCN=C=O
14	24-methylene-cycloartenol	$C_{31}H_{52}O$	440.74398	CC(C)C(=C)CCC(C)C1CCC2(C1(CCC34C2CCC5C3(C4)CCC(C5(C)C)O)C)C
15	Ajoene	$C_9H_{14}OS_3$	234.40186	C=CCSSC=CCS(=O)CC=C
16	Alanine	$C_3H_7NO_2$	89.09318	CC(C(=O)O)N
17	Allicin	$C_6H_{10}OS_2$	162.273	C=CCSS(=O)CC=C

18	Alliin	$C_6H_{11}NO_3S$	177.22144	CC=CS(=O)CC(C(=O)[O-])[NH3+]
19	Allixin	$C_{12}H_{18}O_4$	226.26892	CCCCCC1=C(C(=O)C(=C(O1)C)OC)O
20	Allyl-disulfide	$C_4H_8S_2$	120.23632	CSSCC=C
21	Allyl-methyl-disulfide	$C_4H_8S_2$	120.23632	CSSCC=C
22	Allyl-methyl-trisulfide	C_4H_8S	88.17132	CSCC=C
23	Allyl-propyl-disulfide	$C_6H_{12}S_2$	148.28948	CCCSSCC=C
24	Alpha_tocopherol	$C_{29}H_{50}O_2$	430.7061	CC1=C(C(=C2CCC(OC2=C1C)(C)CCCC(C)CCCC(C)CCCC(C)C)C)O
25	Alpha-prostaglandin-f-1	$C_{20}H_{36}O_5$	356.49684	CCCCCC(C=CC1C(CC(C1CCCCCCC(=O)O)O)O)O
26	Alpha-prostaglandin-f-2	$C_{21}H_{36}O_5$	368.50754	CCCCCC(C=CC1C(CC(C1CC=CCCCC(=O)OC)O)O)O
27	Beta-phellandrene	$C_{10}H_{16}$	136.23404	CC(C)C1CCC(=C)C=C1
28	Beta-sitosterol	$C_{29}H_{50}O$	414.7067	CCC(CCC(C)C1CCC2C1(CCC3C2CC=C4C3(CCC(C4)O)C)C)C(C)C
29	Beta-tocopherol	$C_{28}H_{48}O_2$	416.67952	CC1=CC(=C(C2=C1OC(CC2)(C)CCCC(C)CCCC(C)CCCC(C)C)C)O
30	Biotin	$C_{10}H_{16}N_2O_3S$	244.31064	C1C2C(C(S1)CCCCC(=O)O)NC(=O)N2
31	Diallyl-disulfide	$C_6H_{10}S_2$	146.2736	C=CCSSCC=C
32	Diallyl-tetrasulfide	$C_6H_{10}S_4$	210.4036	C=CCSSSSCC=C
33	Diallyl-trisulfide	$C_6H_{10}S_3$	178.3386	C=CCSSSCC=C
34	Eicosapentaenoic-acid	$C_{20}H_{30}O_2$	302.451	CCC=CCC=CCC=CCC=CCC=CCCCC(=O)O
35	Gamma-l-glutamyl-l-valine	$C_{11}H_{20}N_{25}$	260.2869	CCC(C)C(C(=O)O)NC(=O)CCC(C(=O)O)N
36	Geraniol	$C_{10}H_{18}O$	154.24932	CC(=CCC/C(=C/CO)/C)C

37	Isobutyl-isothiocyanate	C_5H_9NS	115.19666	CC(C)CN=C=S
38	Kaempferol	$C_{15}H_{10}O_6$	286.2363	C1=CC(=CC=C1C2=C(C(=O)C3=C(C=C(C=C3O2)O)O)O)O
39	Monogalactosyl-diglyceride	$C_{11}H_{18}O_{10}$	310.25462	C(C1C(C(C(C(O1)OCC(COC=O)OC=O)O)O)O)O
40	Phloroglucinol	$C_6H_6O_3$	126.11004	C1=C(C=C(C=C1O)O)O
41	Quercetin	$C_{15}H_{10}O_7$	302.23	C1=CC(=C(C=C1C2=C(C(=O)C3=C(C=C(C=C3O2)O)O)O)O)O
42	Quercetin-3-o-beta-d-glucoside	$C_{27}H_{30}O_{17}$	626.5169	C1=CC(=C(C=C1C2=C(C(=O)C3=C(C=C(C=C3O2)O)O)OC4C(C(C(C(O4)CO)O)O)OC5C(C(C(C(O5)CO)O)O)O)O)O
43	Rutin	$C_{27}H_{30}O_{16}$	610.5175	CC1C(C(C(C(O1)OCC2C(C(C(C(O2)OC3=C(OC4=CC(=CC(=C4C3=O)O)O)C5=CC(=C(C=C5)O)O)O)O)O)O)O)O
44	S-(2-carboxy-propyl)-glutathione	$C_7H_{13}NO_4S$	207.24742	CC(CSCC(C(=O)O)N)C(=O)O
45	S-allyl-cysteine	$C_6H_{11}NO_2S$	161.22204	C=CCSCC(C(=O)O)N
46	S-allyl-cysteine-sulfoxide	$C_6H_{11}NO_2S$	161.22204	C=CCSCC(C(=O)[O-])[NH3+]
47	S-ethyl-cysteine-sulfoxide	$C_5H_{11}NO_3S$	165.21074	CCS(=O)CC(C(=O)[O-])[NH3+]
48	S-methyl-cysteine	$C_4H_9NO_2S$	135.18476	CSCC(C(=O)O)N
49	S-methyl-cysteine-sulfoxide	$C_4H_9NO_3S$	151.18416	CS(=O)CC(C(=O)O)N
50	S-methyl-l-cysteine-sulfoxide	$C_4H_9NO_3S$	151.18416	CS(=O)CC(C(=O)O)N
51	S-propenyl-cysteine	$C_6H_{10}ClNO_2S$	195.6671	CC(=CCl)SCC(C(=O)O)N
52	S-propyl-cysteine-sulfoxide	$C_6H_{13}NO_3S$	179.23732	CCCS(=O)CC(C(=O)[O-])[NH3+]

APPENDIX -III

Phytochemical derived from *Moringa oleifera*

Sl. No.	Phytochemical	Mol. formula	Mol. Wt. [g/mol]	Canonical Smiles
1.	Kaempferol	$C_{15}H_{10}O_6$	286.2363	C1=CC(=CC=C1C2=C(C(=O)C3=C(C=C(C=C3O2)O)O)O)O
2.	4-(alpha-l-rhamnosyloxy)-benzyl-isothiocyanate	C_8H_7NS	149.21288	C[C@@H]1O[C@@H](OC2=CC=C(C=O)C=C2)[C@H](O)[C@H](O)[C@H]1OC(C)=O
3.	Choline	$C_5H_{14}NO^+$	104.17076	C[N+](C)(C)CCO
4.	4 hydroxymellein	$C_{10}H_{10}O_4$	194.18	CC1C(C2=C(C(=CC=C2)O)C(=O)O1)O
5.	Niazimin	$C_{18}H_{25}NO_8$	383.4	CCOC(=O)NCC1=CC=C(C=C1)OC2C(C(C(C(O2)C)OC(=O)C)O)O
6.	Quercetin	$C_{15}H_{10}O_7$	302.2357	C1=CC(=C(C=C1C2=C(C(=O)C3=C(C=C(C=C3O2)O)O)O)O)O
7.	Tocopherols	$C_{28}H_{48}O_2$	416.70	CC1=C(C=C2CCC(OC2=C1C)(C)CCCC(C)CCCC(C)CCCC(C)C)O
8.	Alpha-tocopherol	$C_{29}H_{50}O_2$	430.70	CC1=C(C2=C(CCC(O2)(C)CCCC(C)CCCC(C)CCCC(C)C)C(=C1O)C)C
9.	Arachidic-acid	$C_{20}H_{40}O_2$	312.5	CCCCCCCCCCCCCCCCCCCC(=O)O
10.	Behenic-acid	$C_{28}H_{58}O_2Si$	454.8	CCCCCCCCCCCCCCCCCCCCCC(=O)O[Si](C)(C)C(C)(C)C
11.	Beta-carotene	$C_{40}H_{56}$	536.9	CC1=C(C(CCC1)(C)C)C=CC(=CC=CC(=CC=CC=C(C)C=CC=C(C)C=CC2=C(CCCC2(C)C)C)

99

				C)C
12.	Beta-sitosterol	$C_{29}H_{50}O$	414.7	CCC(CCC(C)C1CCC2C1(CCC3C2CC=C4C3(CCC(C4)O)C)C)C(C)C
13.	Brassicasterol	C28H46O	398.66424	CC(C)C(C)C=CC(C)C1CCC2C1(CCC3C2CC=C4C3(CCC(C4)O)C)C
14.	Campestanol	$C_{28}H_{50}O$	402.696	CC(C)C(C)CCC(C)C1CCC2C1(CCC3C2CCC4C3(CCC(C4)O)C)C
15.	Campesterol	$C_{28}H_{48}O$	400.7	CC(C)C(C)CCC(C)C1CCC2C1(CCC3C2CC=C4C3(CCC(C4)O)C)C
16.	24-methylene-cholesterol	$C_{28}H_{46}O$	398.7	CC(C)C(=C)CCC(C)C1CCC2C1(CCC3C2CC=C4C3(CCC(C4)O)C)C
17.	28-isoavenasterol	$\underline{C_{32}H_{52}O_2}$	468.8	CC=C(CCC(C)C1CCC2(C1(CCC3C2=CCC4C3(CCC(C4)OC(=O)C)C)C)C)C(C)C
18.	4-(alpha-l-rhamnosyloxy)-benzylglucosinolate	C10H18O6	234.25	CC1C(C(C(C(O1)OCCCC=O)O)O)O
19.	Delta-5-avenasterol	$C_{29}H_{48}O$	412.7	CC=C(CCC(C)C1CCC2C1(CCC3C2CC=C4C3(CCC(C4)O)C)C)C(C)C
20.	Delta-7,14-stigmastanol	$C_{29}H_{48}O$	412.702	[H][C@@]1(CC[C@@]2([H])[C@]3([H])CC=C4C[C@@H](O)CC[C@]4(C)[C@@]3([H])CC[C@]12C)[C@H](C)\C=C\[C@@H](CC)C(C)C
21.	Delta-7-avenasterol	$C_{29}H_{48}O$	412.7	CC=C(CCC(C)C1CCC2C1(CCC3C2=CCC4C3(CCC(C4)O)C)C)C(C)C
22.	Delta-tocopherol	$C_{27}H_{46}O_2$	402.7	CC1=CC(=CC2=C1OC(CC2)(C)CCCC(C)CCCC(C)CCCC(C)C)O

23.	Eicosanic-acid	$C_{20}H_{40}O_2$	312.5	CCCCCCCCCCCCCCCCCCCC(=O)OC
24.	Ergostadienol	$C_{28}H_{46}O$	398.7	CC(C)C(C)C=CC(C)C1CCC2=C3CCC4CC(CCC4(C3CCC12C)C)O
25.	Gadoleic-acid	$C_{20}H_{38}O_2$	310.5	CCCCCCCCCCC=CCCCCCCCC(=O)O
26.	Gamma-tocopherol	$C_{28}H_{48}O_2$	416.7	CC1=C(C=C2CCC(OC2=C1C)(C)CCCC(C)CCCC(C)CCCC(C)C)O
27.	Lignoceric-acid	$C_{24}H_{48}O_2$	368.6	CCCCCCCCCCCCCCCCCCCCCCCCC(=O)O[Si](C)(C)C1=C(C(=C(C(=C1F)F)F)F)F
28.	Myristic-acid	$C_{14}H_{28}O_2$	228.37	CCCCCCCCCCCCCC(=O)O
29.	Moringine	C_7H_9N	107.15306	COC1=CC(=C2C(=C1)OC(=C(C2=O)O)C3=CC(=C(C=C3)O)O)O
30.	Moringinine	$C_{22}H_{18}N_2O_2S_2$	406.5	C1=CC=C(C=C1)CN
31.	Stigmastanol	$C_{29}H_{52}O$	416.7	CCC(CCC(C)C1CCC2C1(CCC3C2CCC4C3(CCC(C4)O)C)C)C(C)C
32.	Stigmasterol	$C_{29}H_{48}O$	412. 702	CCC(C=CC(C)C1CCC2C1(CCC3C2CC=C4C3(CCC(C4)O)C)C)C(C)C
33.	Beta-sitosterol	$C_{29}H_{50}O$	414.7	CCC(CCC(C)C1CCC2C1(CCC3C2CC=C4C3(CCC(C4)O)C)C)C(C)C
34.	Beta-sitosterone	$C_{29}H_{50}O$	414.7	CCC(CCC(C)C1CCC2C1(CCC3C2CC=C4C3(CCC(=O)C4)C)C)C(C)C
35.	Octacosanoic-acid	$C_{28}H_{56}O_2$	424.754	CCCCCCCCCCCCCCCCCCCCCCCCCCCCCC(=O)O
36.	Vanillin	$C_8H_8O_3$	152.15	COC1=C(C=CC(=C1)C=O)O

37.	1-beta-d-glucosyl-2,6-dimethyl-benzoate	$C_{13}H_{16}O_8$	300.26	C1=CC=C(C=C1)C=O
38.	4-(alpha-l-rhamnosyloxy)-benzylglucocyanate	C21H20O11	448.378	C1(=C(C(C2=C(C=C(C=C2O1)O)O)=O)O[C@H]3[C@H](O)[C@H]([C@@H]([C@@H](CO)O3)O)O)C=4C=CC(O)=CC4
39.	Benzyl-isothiocyanate	C_8H_7NS	149.21	C1=CC=C(C=C1)CN=C=S
40.	Glucotropaeolin	$C_{14}H_{19}NO_9S_2$	409.4	[C@H]1(O[C@@H]([C@@H](O)[C@@H]([C@H]1O)O)CO)S/C(=N\OS(O)(=O)=O)/CC=2C=CC=CC2
41.	Phytosterols	C29H50O	414.7067	CCC(CCC(C)C1CCC2C1(CCC3C2CC=C4C3(CCC(C4)O)C)C)C(C)C
42.	Pterygospermin	C22H18N2O2S2	406.52052	C1=CC=C(C=C1)CN2C(=S)OC23C=CC4(C=C3)N(C(=S)O4)CC5=CC=CC=C5

ABSTRACT

ABSTRACT

Tuberculosis (TB) is the second worldwide killer infectious disease caused by *Mycobacterium tuberculosis* (TB). Globally it kills 1.8 million people annually and 40,000 from India. Current TB treatment is based on principles of combination chemotherapy which takes prolonged period. Most of the anti-TB drugs are good old discoveries and now they are resistant to new strains of TB. The emergence of multi drug resistant (MDR) and extensively drug resistant (XDR) mutants and the adverse effect to immune system in patients co-infected with HIV are the major hurdles in the TB treatment. Thus the discovery of new vaccines and novel drugs become the need of the hour. History shows the practice of several herbal combinations that have been using traditionally from the ancient civilizations against tuberculosis. Many indigenous vegetable crops of Kerala are having anti-TB properties but its efficacies and the pharmacological action are not yet scientifically investigated.

In the present study the phytomolecules reported from *Allium cepa, Allium sativum* and *Moringa oleifera* were screened through *in silico* methods. Decaprenylphosphoryl-beta-D-ribose epimerase (DprE1) protein was selected as the target molecule for doing the *in silico* docking analysis. DprE1 is an enzyme responsible for the synthesis of arabinan, the virulent factor in *Mycobacterium, tuberculosis*. The 3-D structure of the molecule was retrieved from PDB (PDB id 4FDO) and the active site DprE1 was detected using the tool PDBsum. Information regarding the chemical molecules reported in the selected spices was collected through literature survey and databases. A total of 175 phytochemicals (*Allium cepa* – 81, *Allium sativum* – 52 and *Moringa oleifera* – 42) were screened.

The selected phytochemicals were docked against DprE1 protein using AutoDock 4.2. The docked structures having $\Delta G_{bind} \leq$ ¯5 kcal/mol^{-1} were selected as best hit molecules. To nullify the errors in lead identification the top ranked hit molecules were again docked using the tools Hex server, iGEMDOCK, FireDockand SwissDock. The docked results were statistically analyzed following DST and Zhang rule and selected the top ranked molecules *viz.* 24-

Methylenecycloartanol (*A.cepa*), Kaempferol & Quercetin (*A. sativum & M. oleifera*) and 4-hydroxymellein from *M. oleifera.* All the four compounds have been subjected for bioactivity and druglikness score using Molinspiration and MolSoft tools. The ADMET property study was done by using the tool pkCSM. Kaempferol, Quercetin and 4-hydroxymellein showed acceptable range in both bioactivity analysis and in ADMET property prediction. Overall study substantiate that Moringa stands first in the evaluation as it produce all the three sought out leads followed by garlic with two leads. Theoretical prediction of drug likeliness only gives probability and its activity in a biological system. Therefore, *in vitro & in vivo* studies and in-depth clinical trials are necessary for developing a drug out of the lead molecules.